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Deliverable 4.2:
Manuscripts on stressor effects at the river basin level

Beneficiaries: D4.2-1 – University of Duisburg-Essen (**UDE**, lead beneficiary), University of Cardiff (**CU**) and University of Lisbon (**ULisboa**)

Beneficiaries: D4.2-2 – Stichting (DELTARES, lead beneficiary), Natural Environmental Research Council (**NERC**), Arhus University (**AU**)

Beneficiary: D4.2-3 – Fundacion AZTI (**AZTI**, lead beneficiary)

Beneficiaries: D4.2-4 – Eesti Maalikool (**EMU**, lead Beneficiary), Natural Environmental Research Council (**NERC**), Institut Nacional de la Recherche Agronomique (INRA, external) and Consiglio Nazionale delle Ricerche de Itália (CNR, external)

Beneficiary: D4.2-5 – Natural Environmental Research Council (**NERC**)

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Overview

In the previous report (D4.1, September 2016) we have developed predictive linkages between indicators of environmental quality and ecosystem services, and different types of pressures, single or multiple, across river basins from all over Europe, in a latitudinal and a west-east gradient, and having very different conditions of climate and land use drivers. Using such predictive linkages resulting both from empirical data treatment and process based modelling, and following a common approach for climatic scenario changes, downscaled to region level, we have studied the future evolution of indicators and services. Furthermore, we have looked into the programs of measures that are being implemented in each country and we attempted to understand how will be the responses of the indicators of quality and services, according to such implementation. The studies performed in these 16 basins have a great potential for common applications and studies, and these are being developed in the moment.

Although the hydrological and nutrient process-based models that were used and calibrated at each basin scale mostly contain liaisons between the different water compartments, these studies were mostly focused in surface waters and in the indicators of quality that were developed for the Water Framework Directive, as also the information from monitoring taking pace for the last decade, or in previous times. Many issues remain to be studied, related with interfaces between water compartments and also with the biotic aspects of the pressures, also important for some communities and in some cases.

This Deliverable (D4.2) is composed of five reports, dealing with stressor effects at the river basin level. These reports cover special aspects of the linkage between ecosystem compartments, multiple pressures and the responses of particular elements, notably the interface between terrestrial and aquatic environments represented by the riparian transitional ecosystems (D4.2-1), stressor propagation between surface waters and groundwaters (D4.2-2) and estuarine waters (D4.2-3), or deal with particular biological stressor effects when such are added to the more common chemical and physical stressors (D4.2-4 and D4.2-5). Most of these reports use different case studies (D4.2-1, D4.2-2 and D4.2-4) from MARS to pool data and experiences for a common target. The other two focus on the stressor propagation in transitional waters in the example of the estuary of Nervion (D4.2-3) and finally two other are related with biotic pressures, i.e. fisheries management and pathogens (D4.2-4 and D4.2-5). All of these studies will be adapted/are being prepared for publication submission.

The Deliverable 4.2. is therefore composed of five reports, with the non-technical summaries following this introduction:

D4.2-1: Riparian-to-catchment management options for stressor reduction and service enhancement

D4.2-2: Stressor propagation through surface-groundwater linkages and its effect on aquatic systems

D4.2-3: Stressor propagation through inland-transitional linkages and management consequences

D4.2-4: Fisheries as a source and target of multiple stressors

D4.2-5: Multiple-stressor risks for pathogens

Summary of D4.2-1

Riparian management is considered a key management option to improve lotic ecosystem status, functioning and services. Vegetated riparian buffer strips can retain nitrogen from sub-surface runoff, and phosphorous and fine sediments from surface runoff. Thus, they can reduce and mitigate the effects of diffuse pollution by agricultural and other land uses. However, the effectiveness of riparian buffers, to a large extent, depends on the location within the stream continuum, as well as on the land use conditions further upstream of the buffered stream sections. Catchment-scale effects can counteract riparian management effects. In the manuscript, we synthesise the evidence of riparian management options in light of catchment-scale pressures. We reviewed 53 management studies addressing both scales and developed a conceptual model to highlight management options with and without conflicts among management scales.

Summary of D4.2-2

The good ecological status of Europe's freshwaters is still lacking. This paper reviews the role of groundwater in these systems and demonstrates that it is an important factor to include in surface water management. Groundwater influences streamflow, water chemistry and water temperature and connects rivers and streams with their catchment and thus functions as a pathway for stressors to reach the surface water. A new 'Groundwater DPS' framework is proposed which shows how groundwater fits in the system of a stressed aquatic ecosystem. The functioning of this framework is demonstrated using examples from four different European lowland catchments: the Thames, Odense, Regge and Dinkel catchments. The importance of groundwater varies between scales, between catchments and within catchments.

The Groundwater DPS will aid water managers in understanding the importance of groundwater in their management areas and promotes the consideration of groundwater in future water management practices.

Summary of D4.2-3

We have used the Nervión estuary case study (north Spain), to investigate: (i) the links between pressures, recovery of the system and provision of cultural ecosystem services (i.e. recreational fishing and bathing waters); (ii) the future climate change scenarios for these services (with three scenarios: economic focus, green focus and survival of the fittest); and (iii) the management implications. For the study, we have used historical data, questionnaires to users and modelling.

Our findings show that, in the last 25 years, the improvement in environmental conditions led to radical structural changes in the estuary and influenced also the practice of recreational fishing, especially in the inner estuary. The abiotic data gathered prove a great improvement on water conditions and consequently a dramatic increase in the abundance and richness of demersal fishes, which has been demonstrated to be linked with the increase of oxygen saturation and decrease in turbidity, resulting from the water treatment (it has been shown here the dramatic decrease in nutrient load and the increase of oxygen). Also, the increase in the number of recreational fishing licences issued in the villages along the estuary suggests a growing interest in this recreational activity. All these data are indicators of better fishing conditions, and could be indicating an increase and an extension of the fishing activity throughout the Nervión estuary. In turn, fishers´ perceptions on changes in catches abundance, variety and size, drawn a less clear picture of the situation; perceptions are more negative than the changes depicted by the environmental data.

Regarding climate change scenarios, the combination of temperature increase, runoff decrease and improvement of the water treatment efficiency in the future scenarios has shown a general improvement of the bathing water quality in the beaches of the estuary, especially in Storyline 2 (green focus) in year 2050. Nonetheless, even in the best scenario, the closest beach to the sources of pollution has shown not sufficient bathing water quality problems during the bathing season. For recreational fishing, the expected changes would infer in a negligible change in fish abundance.

The management consequences of these findings lead us to recommend: (i) removing the discharge from the water treatment plant, diverting it to the coastal zone; (ii) engage end users (i.e. recreational fishers, beach users) in the governance, promoting the sustainable use of the ecosystem services in the estuary; and (iii) apply the recommendations from UN for governance and management of recreational fisheries.

Summary of D4.2-4

Case studies from five European lake basins of differing trophic states were analysed (Lake Võrtsjärv, two basins of Windermere, Lake Geneva and Lake Maggiore) with long-term limnological and fisheries data. Decreasing phosphorus concentrations (re-oligotrophication) and increasing water temperatures have been reported in all five lake basins, while phytoplankton concentration has decreased only slightly or even increased in some cases. To examine possible ecosystem-scale effects of fisheries we analysed correlations between fish and fisheries data, and other food web components and environmental factors. Re-oligotrophication over different ranges of the trophic scale induced different fish responses. Although a general model predicted increasing fish production and biomass with increasing trophic state, parameters other than phosphorus including fisheries pressure and the balance between predatory and non-predatory fish species explained a significant part of the observed variability in fish abundance. In the deeper lakes Geneva and Maggiore, we found a stronger link between phytoplankton and planktivorous fish and thus a more important cascading top-down effect than in other lakes. This connection makes careful ecosystem-based fisheries management extremely important for maintaining high water quality in such systems. We also demonstrated that increasing water temperature may favour piscivores at low phosphorus loading, but suppresses them at high phosphorus loading and may thus either enhance or diminish the cascading top-down control over phytoplankton with strong implications for water quality.

Overall, the assessment of long-term concurrent effects of fisheries, changing trophic state and changing climate upon lake ecosystems in five European lake basins of differing trophic states (Lake Võrtsjärv, two basins of Windermere, Lake Geneva and Lake Maggiore) revealed that decreasing phosphorus concentrations (re-oligotrophication) and increasing water temperatures in all five lake basins have coincided with no or slight decreases in phytoplankton concentrations. Parameters other than phosphorus, including fisheries pressure and the relative abundances of predatory and non-predatory fish species, explained a significant part of the observed overall variability in fish abundance. A stronger link between phytoplankton and planktivorous fish was observed in Lakes Geneva and Maggiore, suggesting a more important cascading top-down effect in these relatively deeper lakes which makes their careful ecosystem-based fisheries management extremely important for maintaining high water quality. Analyses indicated that increasing water temperature may favour piscivores at low phosphorus loadings, but suppress them at high phosphorus loadings and may thus either enhance or diminish the cascading top-down control over phytoplankton with strong implications for water quality.

Summary of D4.2-5

A wide variety of disease-causing pathogenic microorganisms, including bacteria, are spread and transmitted via water. There are many potential sources of waterborne diseases in freshwater environments, although the main recognised routes are via direct contamination of water by faeces from both humans and animals, as well as by pathogens that can survive and are distributed by sewage treatment processes. Understanding the dynamics of both pathogenic bacteria and bacteria associated with faeces and sewage infrastructure can be helpful in determining the sources of bacteria and how they interact with freshwater environments. Many freshwaters are experiencing a high degree of degradation in the form of interacting stressors such as elevated nutrients, chemical pollution, modified morphology and flows, as well as increases in algal bloom frequency and intensity. It is important to understand how these multiple stressors, particularly those associated with climate change, interact to affect pathogen abundance and dynamics. This study used DNA sequencing to investigate the microbial composition of a lowland river, the river Thames, UK, that is experiencing multiple stressors, at a weekly resolution over a two-year period. It aimed to examine whether new molecular approaches, such as high throughput sequencing of the 16S rRNA bacterial gene, could provide more targeted indication of pathogenic bacteria loads rather than proxies, such as Faecal Indicator Organisms (FIOs). Families and genera of bacteria were selected to act as indicators of pollution from faeces, sewage infrastructure and a group of potentially pathogenic bacteria. Strong seasonal dynamics in abundance were observed, with most faecal and sewage indicators increasing in abundance during the winter months. Of the potential pathogen indicators, no confirmed pathogens were identified to species level, but closely related genera were present in low abundance, or if in higher abundance, were more likely associated with pathogens or parasites of natural populations of aquatic wildlife. These results indicate that whilst faecal and sewage indicators show that contamination via these routes is occurring in the river Thames, it is not associated with high loads of pathogen species. Future studies covering a wider range of sites are recommended to identify potential hotspots associated with agriculture or sewage treatment.

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MARS PROJECT

Managing Aquatic ecosystems and water Resources under multiple Stress

Deliverable 4.2-1: Riparian-to-catchment management options for stressor reduction and service enhancement

Lead beneficiary: **UDE**

Contributors: Christian K. Feld, Daniel Hering (**UDE**), Cayetano Gutiérrez Cánovas, Steve Ormerod (**UCardiff**), Maria do Rosário Fernandes, Maria Teresa Ferreira (**ULisboa**)

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Contents

Non-technical summary

Riparian management is considered a key management option to improve lotic ecosystem status, functioning and services. Vegetated riparian buffer strips can retain nitrogen from sub-surface runoff, and phosphorous and fine sediments from surface runoff. Thus, they can reduce and mitigate the effects of diffuse pollution by agricultural and other land uses. However, the effectiveness of riparian buffers, to a large extent, depends on the location within the stream continuum, as well as on the land use conditions further upstream of the buffered stream sections. Catchment-scale effects can counteract riparian management effects. Here, we synthesise the evidence of riparian management options in light of catchment-scale pressures. We reviewed 53 management studies addressing both scales and developed a conceptual model to highlight management options with and without conflicts among management scales.

Riparian-to-catchment management options for stressor reduction and ecological status enhancement in lotic systems – a review

Christian K. Feld, Daniel Hering (UDE), Cayetano Gutiérrez Cánovas, Steve Ormerod (UCardiff), Maria do Rosário Fernandes, Maria Teresa Ferreira (ULisboa)

Introduction

Worldwide, lotic ecosystems are subjected to multiple stressors, imposing serious threats on riverine biology, ecosystem functions and related ecosystem services (Vörösmarty *et al.* 2010, Hering *et al.* 2015). Land use and related diffuse pollution by nutrients and fine sediments are considered major drivers of ecosystem change and thus constitute widespread sources of stressors for lotic ecosystem integrity (Matthaei, Piggott & Townsend 2010). Besides hydromorphological degradation, eutrophication and sediment pollution through diffuse sources are considered the most important pressures on aquatic ecosystems in Europe (European Environment Agency 2012).

Vegetated riparian buffers are commonly considered the main management option to reduce or mitigate nutrient pollution and fine sediment erosion from agricultural fields adjacent to impacted water bodies. There is tremendous evidence that riparian trees and shrubs can effectively reduce nitrogen from the upper groundwater layer (reviewed by Dosskey 2001 and Feld *et al.* 2011), while the organic enrichment in the riparian zone (e.g., through leaf litter) promotes microbial denitrification (Dosskey 2001). Riparian grass strips can effectively reduce sediment pollution by surface runoff (Dosskey 2001) and thus also prevent from phosphorous adhered to the sediment particles to enter the stream network.

However, there is also evidence in the management literature suggesting that riparian management alone does not constitute a universal remedy against diffuse pollution. Too short or too narrow strips have only limited retention capacity (Sweeney *et al.* 2014), while landscape topography (e.g., slope), hydrology and geology can largely influence buffer function (Mayer *et al.* 2005). Gaps in the buffer strip may dramatically reduce buffer efficiency, if gully erosion occurs (Dosskey 2001). And in the drained agricultural landscape, riparian buffers cannot retain nutrients and sediments that enter the stream system through drainage.

Yet, more important than malfunctioning buffers, there is increasing evidence of the role of spatial scaling. More specifically, it is the catchment above a given stream or river reach that determines the ecosystem's status and functioning (Feld *et al.* 2011, Lorenz & Feld 2013). Riparian buffers further downstream cannot manage ongoing intensive land uses upstream in a watershed. This raises the question, whether riparian buffer management is an effective option to improve lotic ecosystem status and functioning in intensively used agricultural regions.

Here, we try to help answer this question, by synthesising the evidence of the effects of riparian buffer management in light of catchment-scale pressures and management options to reduce broad-scale impacts. With the outcome of the synthesis, we aim to highlight riparian 'no regret' management options and distinguish those from options that require concerted efforts at both the riparian and the (sub-)catchment scale.

Material and Methods

Literature Review

Our synthesis focuses on studies that addressed the effects of riparian-scale and catchment-scale management for stressor reduction and ecological status improvement in river systems. This focus on management studies aimed to gather evidence of actual management effects and thus to distinguish actual evidence from mere statistical relationships. The latter is reported in the huge body of 'degradation literature' that relates environmental and biological effects to gradients of ecosystem degradation. However, with regard to the biological response, restoration is not the opposite of degradation (Feld *et al.* 2011), which means that biology does not necessarily respond to restoration as it is often predicted based on data along degradation gradients.

We also focussed mainly on peer-reviewed studies using the Web of Science and Scopus (search terms: (catchment OR watershed OR land use OR riparian OR riparian vegetation OR buffer) AND (management OR enhance) AND (river OR stream); (riparia* AND catchmen* AND management) AND (river* OR strea*); riparia* AND land use AND catchment* AND management AND (river* OR strea*); (rive* OR strea*) AND (land use AND catchmen* AND restoratio* AND managemen*); (river* OR strea*) AND (land us* AND managemen* AND spatial scal*; (riparia* AND catchmen*) AND (stress* AND river*) OR (riparia* AND catchmen* AND stress* AND strea*); (riparia* AND basin* AND stress* AND river*) OR (riparia* AND basin* AND stress* AND strea*).

Depending on the combination of search terms, they resulted in 219–998 hits, which were briefly scanned to exclude unsuited references (711 studies remained). Studies were included in the analysis, if they satisfied the following criteria: i) addressing the management effect at riparian-scale and catchment-scale simultaneously; ii) addressing the management effect at either scale (riparian or catchment) iii) addressing important modelling analysis or reviews about management effects at either scale. Out of the 711 initial manuscripts, the final review database comprises 135 studies, of which 53 core management studies were selected to develop and populate a conceptual model (see below). For all 135 studies, metadata and core results were extracted and entered a database (e.g., origin of studies, geographical attributes, main drivers of change and catchment pressures addressed, riparian and catchment management characteristics, abiotic and biological response to management).

General study characteristics:

Geographical location (country, latitude, longitude, altitude) was either reported in the manuscripts or identified using Google Earth. For the climate information, we adopted the 3rd level of Köppen's classification system, using the Köppen-Geiger climate classification layer (Geiger 1961; available at the ArcGIS Online Resource Center). Concerning the target area of the management actions, studies were classified as: headwaters (catchment area <10 km^2), small (10–100 km^2); medium-sized (100–1,000 km^2) and large (>1,000 km^2). Regarding the position in the river continuum, we distinguished upstream sections (Strahler order 1–2), middle sections (3–4) and downstream sections (>5). Altitude was classified as lowlands (<200 m a.s.l.), uplands (200–500 m a.s.l.), mountainous (>500–800 m a.s.l.) and alpine (>800 m a.s.l.).

Main drivers and catchment pressures

We identified the main drivers of changes at catchment-scale and the related pressures addressed in each study. Agriculture, urban and silviculture land-uses were identified as main drivers promoting nutrient and sediment pollution, waste water pollution, and sediment pollution, respectively. The removal of the riparian vegetation was also classified as a pressure derived from all land-uses activities developed nearby fluvial corridors. Concerning the hierarchical position in river system, pressures were analysed at site, reach, segment, sub- catchment and catchment scale.

Riparian management characteristics

Riparian management actions were categorized as active restoration (riparian planting) or passive restoration (fencing or protection of riparian buffer remnants). Management measures were classified as occurring at site, reach, segment or sub-catchment scale. We also extracted the information concerning the length of the managed section, the width, the density, the height and the age (years after implementation of the management action). Riparian management configurations were classified as single zone or multi-zone and using a combination of single strata or multi-strata (herbaceous/grass, shrubs and trees vegetation). The longitudinal continuity of the managed section was classified as total or partial.

Catchment management characteristics

Catchment management actions were categorized as structural measures and non-structural measures. Structural measures include agricultural management (e.g. crop rotations and tillage), grazing management (e.g. reduction of livestock density), urban management and land-use change (e.g. afforestation). Non-structural measures include nutrient management plans (e.g. reducing of nutrient application), change legal regulation and watershed planning. Catchment management was classified as occurring at sub-catchment or catchment scale.

Instream abiotic and biological responses

Effects of riparian-scale and catchment-scale management for stressor (i.e. abiotic states) reduction and ecological status improvement were addressed via a instream abiotic and biological response analysis. The information concerning abiotic and biological responses were extracted mostly from figures and tables accessible on the selected articles, and include information regarding qualitative variations (increase *vs.* decrease) or/and quantitative variations (% changes) in: Nitrogen (N) and Phosphorous (P), Fine sediments, Light/shade, Water temperature and Instream habitat (Large Woody Debris (LWD) and Coarse Particulate Organic Matter (CPOM). As to the biological attributes, we extracted qualitative and quantitative effects on species richness, diversity, abundance and biomass, for fish and macroinvertebrates (no other biological community were addressed by the 53 core management studies) as well as changes in primary production.

Conceptual Model

Based on the main cause-effect relationships identified in the peer-reviewed literature we developed a conceptual model addressing the effects of riparian-scale and catchment-scale on stressor reduction and ecological status enhancement in rivers systems (Fig 1). The conceptual model graphically describes the linkage between catchment land-use drivers (pink boxes), catchment pressures (purple diamonds) and abiotic and biological states (grey boxes and yellow ellipses, respectively). Blue boxes represent the riparian buffer characteristics impacted by pressures and influential on the abiotic states (grey) that act as stressors of biological states (yellow).

Each cause-effect relationship is represented in the conceptual model by an arrow. Red arrows indicate positive relations, that is, the effect variable increase if the cause variable increases, while blue arrows indicate negative relations and grey arrows indicate indifferent relations. The strength of each relationship was then estimate based on the number of supporting references (i.e., individual articles) that (statistically-proven) support the relationship. Arrow thickness was then graphically classified in the conceptual model in four classes: class 1 (1 reference), class 2 (2–5), class 3 (6–10, class 4 (>10).

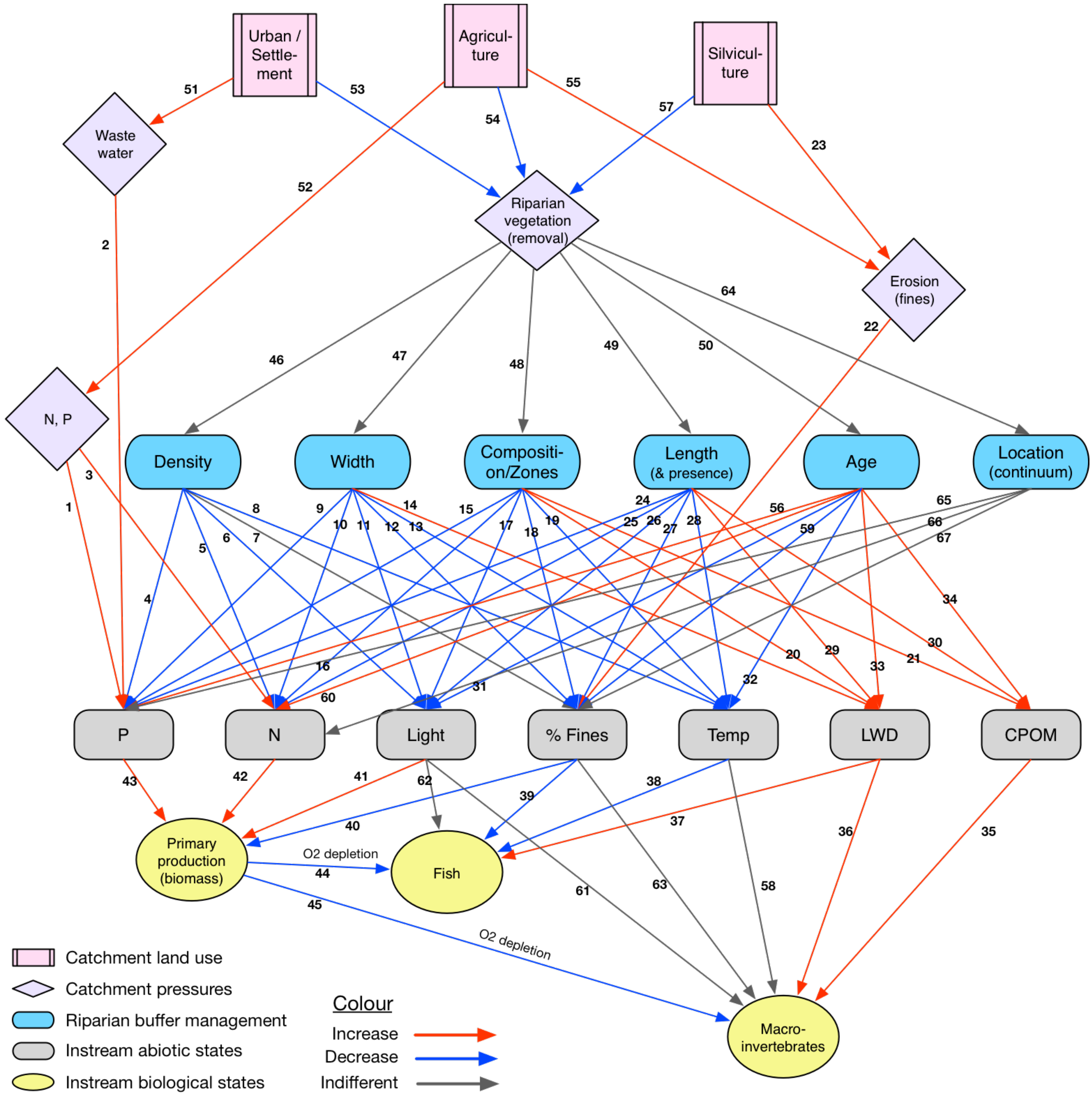

Figure 1: Conceptual model showing the hierarchical relationships between catchment land-use, catchment pressures, riparian buffer management, instream abiotic states and instream biological states. Blue arrows represent negative relationships, red arrows indicate positive relationships and grey arrows show unclear effects (both positive and negative). Arrow numbers are referenced to the core literature in Table A1 (Annex).

Results

Reviewed literature

Altogether, we selected 135 studies for our review, 53 of which were finally suitable to populate the links (arrows) of our conceptual model. These 53 references constitute the core evidence of this study and address 'real' management effects, either investigated through monitoring surveys after the implementation of actual measures, through experiments or through (sub-)catchment-scale modelling. The remaining references encompass previous review papers and empirical studies, the latter of which usually addressed statistical relationships of stressors and riparian gradients. The overlap of our core papers with the original literature referenced in the considered review papers was small, so that double-counting of evidence was avoidable.

The 53 core studies were published between 1990 and 2017 and originate mainly from the USA (36%), Europe (32%), New Zealand (25%) and Canada (6%). Experimental studies (53%) dominated over modelling studies (25%), analysis of statistical gradients (17%) and reviews (17%). Only about 15% of the studies addressed *in situ* monitoring of the effects of actual management measures, which highlights the poor representation of monitoring surveys following actual management (e.g., river restoration, catchment management).

The majority of evidence originates from wet and temperate climates (59%, Cfa: 6%, Cfb: 53%; classification according to Geiger (1961), followed by continental (26% Dfa: 11%, Dfb: 15%) and Mediterranean studies (8%, Csa: 6%, Csb: 2%). Most studies were deployed in small rivers (64%), followed by headwaters (40%) and medium sections (26%). Only 2% of the studies stem from large rivers. This focus on small streams matches the longitudinal location of studies: headwater and upstream studies dominated (64%), whilst those from middle (34%) and downstream sections (11%) were less frequent. Regarding elevation, 53% of the studies were conducted in lowland regions (<200 m a.s.l.), 42% in uplands (200–500 m a.s.l.), 8% in mountainous (500–800 m a.s.l.) regions and only 4% in alpine streams (>800 m a.s.l.). Agricultural impacts were in focus of the vast majority of the studies (98%), whilst silviculture (17%) and urbanization (6%) were much less often studied. Dominant pressures include vegetation removal (51%) and diffuse nutrient pollution (fertilizers) (43%), surface erosion (23%) and waste water (4%).

Riparian management studies

Riparian management studies primarily addressed the reach (60%) and segment scales (43%), compared to sub-catchment (17%) and site scales (8%). More specifically, the length of the management section was generally less than 1 km long (30% of the studies) or ranged 2–10km (28%); studies addressing longer segments (>10 km) were rare (8%). Most often, riparian buffer width was <10 m (34%), followed by buffer width of 10–20 m (21%) and >20 m (21%). Buffer height was variable, but no indication was provided for two thirds of the studies. Buffer vegetation age was usually <5 a (49%), although long-term management effects were also represented in the literature (5–10 a: 17%, 10–20 a: 11%, >20 a: 19%). The type of vegetation managed in the studies was mainly trees (74%), followed by grass/forbs (57%) and shrubs (34%). The plant combinations used in the buffers were mostly single trees (26%) or multi-zone configurations (25%), whilst multiple trees and grass (9%), single grass (9%), multiple shrubs and grass (6%) and multiple trees and shrubs (4%) were less common.

Common abiotic state variables

Studies almost equally addressed nitrogen pollution (total N, dissolved soluble N, nitrate-N, nitrate) (40%), diffuse sediment pollution (40%), phosphorous pollution (38%) and water temperature effects

(32%). Light conditions (shade) (19%) and the provision of large woody debris (LWD) were less frequently addressed (9%) (Fig. 2).

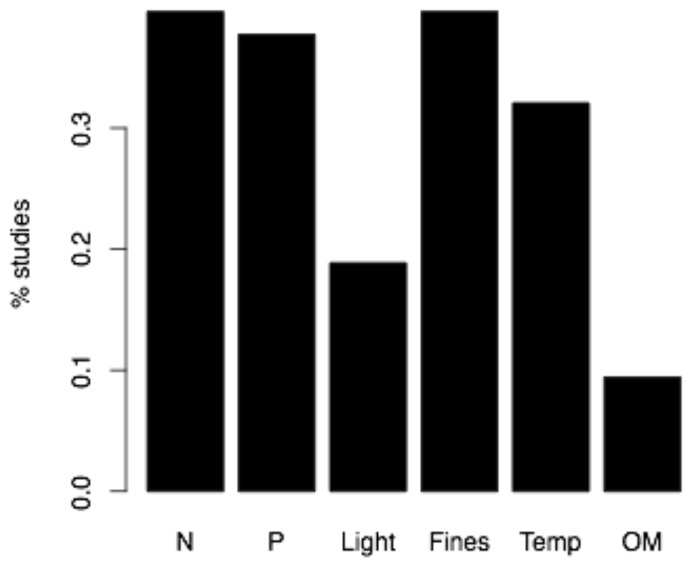

*Figure 2: Common abiotic state variables (stressors) addressed in the 53 core management papers (**N**itrogen, **P**hosphorous, **Temp**erature, **O**rganic**M**aterial).*

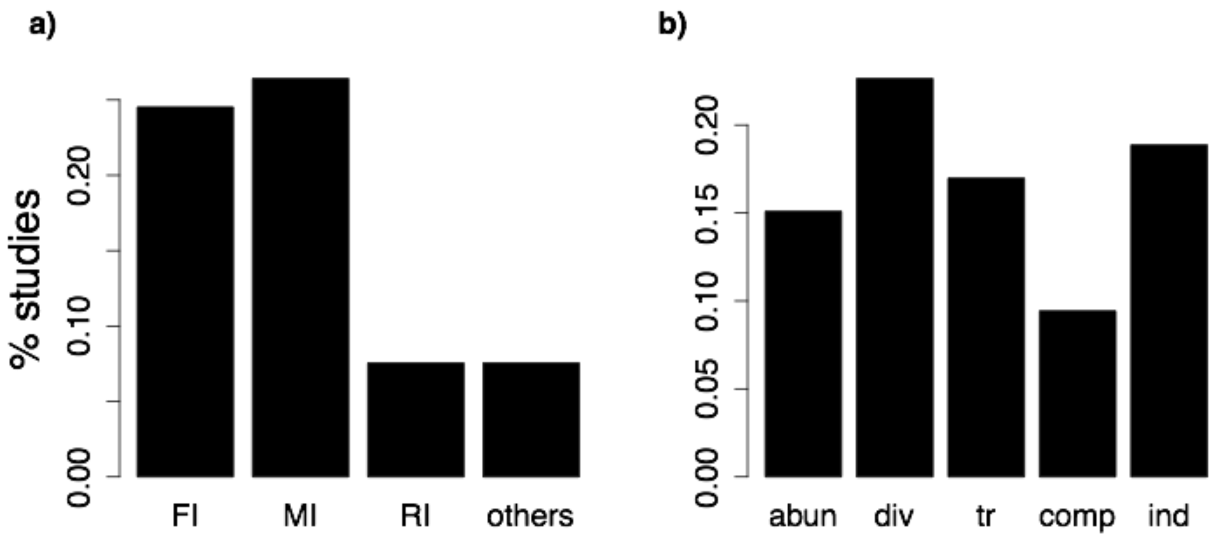

*Figure 3: a) Common biological response variables and b) community attributes addressed by the 53 core management papers (**FI**sh, **M**acro**I**nvertebrates, **R**iparian**I**nvertebrates, **abun**dance, **div**ersity, **tr**aits, **comp**osition, various assessment **ind**ices).*

Biotic states

Only 57% of the studies monitored affects on riverine or floodplain biota. Of these, macroinvertebrate (26%) and fish (25%) were most common, followed by instream primary producers (8%) and riparian vegetation (8%) (Fig. 3a). Most often, community diversity was used to quantify biological effects (23%), followed by various biotic indexes (e.g., national water quality indices) (19%), trait-based community metrics (e.g., feeding types, substrate preferences) (17%), measures of abundance (15%) and composition (e.g., No. of EPT taxa) (9%) (Fig 3b).

Conceptual Model

The most supported relationships between land-use and pressures were those linking agriculture with a reduction of riparian vegetation (30% of the studies), via removal, and an increase in soil erosion (11%), and silviculture with a reduction of riparian vegetation (30% and 8%, respectively) (Fig. 4). Although many studies support a significant influence of riparian vegetation removal on riparian buffer density (15%), width (15%), composition (30%), length (26%) and age (4%), the direct effects are variable and complex. However, the increase of fertilisers and surface erosion is generally linked to an increase in the percentage of instream fine sediments and nutrient concentrations (N, P compounds). Among the riparian buffer features, buffer width, composition/zones and length were most often addressed in the reviewed literature, as important attributes determining the abiotic states. Buffer density tends to decrease P concentration (4% of the studies) and light (6%), and has variable effects on the percentage of fines (15%). Buffer width is linked to a reduction of P (17% of the studies) and N concentration (15%), light (4%), the percentage of fine sediments (17%) and water temperature (9%), whereas it increased the availability of allochthonous organic matter on the stream bottom (8%). Similar positive and negative effects were found for the effects of buffer composition/zones (P: 9% of the studies, N: 13%, Light: 6%, Fines: 19%, Temperature: 11%, LWD: 8%) and length (P: 4%, N: 6%, Light: 8%, Fines: 6%, Temp: 13%, LWD: 6%).

The reviewed studies suggest that buffer vegetation age has minor influence on the reduction of water temperature (4% of the studies). Only light, temperature and allochthonous organic matter have any supporting effect on biological river attributes. Light tends to reduce aquatic primary producer biomass (9% of the studies), whereas the percentage of fine sediments (4%) and temperature (11%) revealed unclear effects on aquatic macroinvertebrates. Water temperature frequently reduced fish biotic indicators (13%), whilst studies supported a positive relationship between allochthonous organic matter and fish indicators (8%).

Riparian management options to address the main stressor variables

Nutrient pollution management

Most of the studies of riparian buffer effects on nutrient retention address headwaters and small to medium-sized sections of streams in the lowlands. Apparently, lowland streams are more prone to nutrient pollution than upland and mountainous streams, probably because agriculture, as the main source of nutrient pollution, is widespread and often more intense in the lowlands. Stream course length is addressed as either site or reach (to segment) scale, which translates to several tens up to several hundreds of metres in length. Management studies addressing stream lengths >2 km are rare.

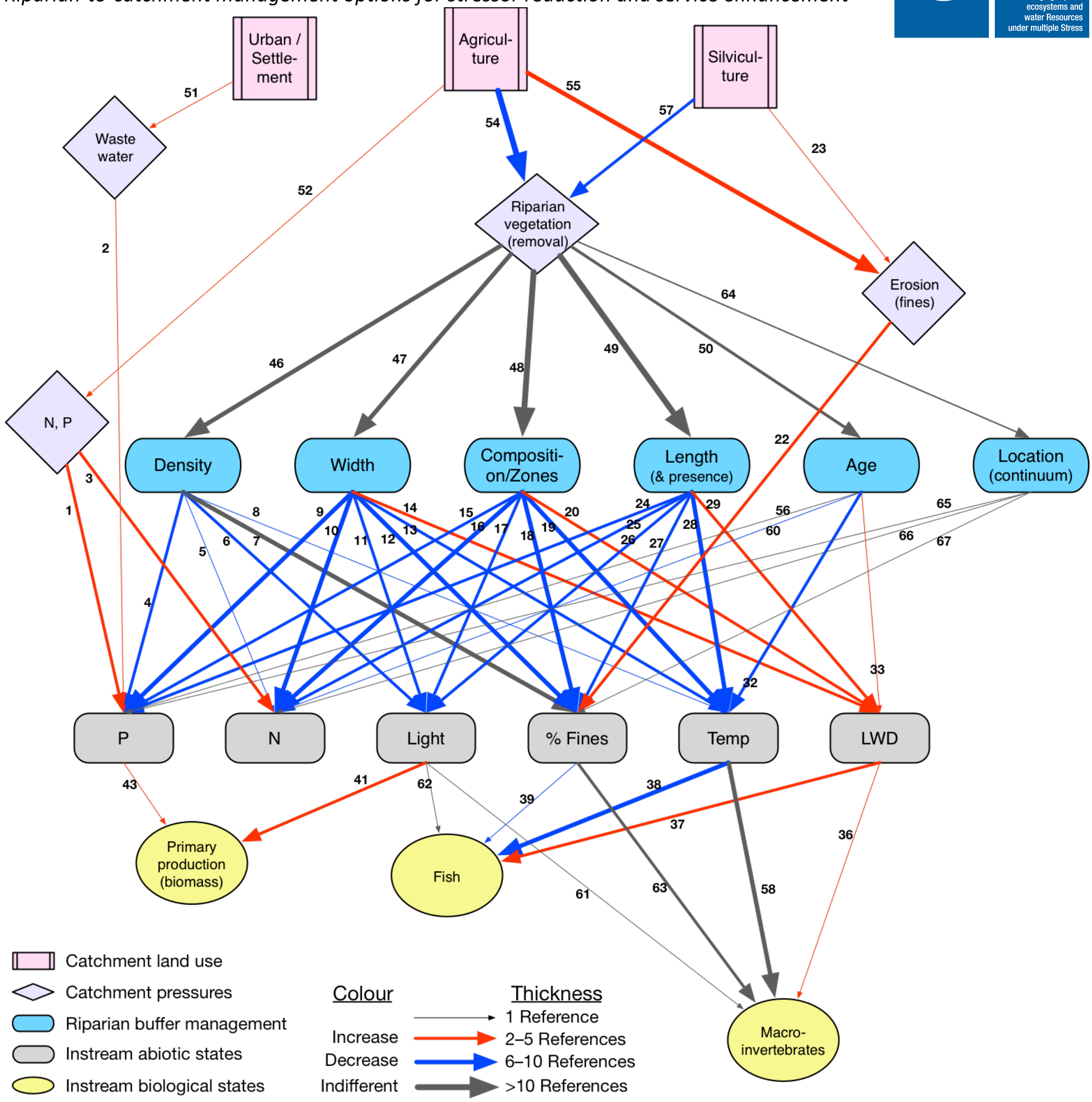

Figure 4: Conceptual model showing the hierarchical relationships between catchment land-use, catchment pressures, riparian buffer management, instream abiotic states and instream biological states. Blue arrows represent negative relationships, red arrows indicate positive relationships and grey arrows show unclear effects (both positive and negative). Arrow thickness is proportional to the number of studies supporting a significant relationships between two elements of the model (see legend). Arrow numbers are referenced to the core literature in Table A1 (Annex).

The retention of nitrogen and phosphorous was most frequently addressed in the reviewed literature (Fig 2). About 75% of the studies directly or indirectly report the effects of riparian buffers (mostly vegetated buffer strips) and riparian management (mostly fencing, to manage impacts by lifestock farming). Riparian buffers can retain up to 100% nitrogen (Feld *et al.* 2011), while most studies report retention capacities of 50–75% for nitrate or nitrate-N (Dosskey 2001, Broadmeadow & Nisbet 2004, Mankin *et al.* 2007, Krause *et al.* 2008, Dodd *et al.* 2010, Collins *et al.* 2012). Phosphorous retention is slightly less effective and mostly ranges 40–70% (Dosskey 2001, Dodd *et al.* 2010, Feld *et al.* 2011), while Kronvang *et al.* (2005) reported 90% retention of total phosphorous.

The role of riparian buffer configuration for nutrient removal is illustrated in Fig. 4 and suggests buffer length, width, zonation and density, but not age, to be the main descriptors of retention efficiency. Buffer width is directly related to N and P retention (Dosskey 2001, Feld *et al.* 2011, Sweeney & Newbold 2014) and, together with buffer zonation, determines the amount of nutrients retained in the surface runoff and upper groundwater layer (Dosskey 2001). A width of 30 m is reported to effectively retain N and P from surface and groundwater runoff, if buffers consist of mature wooded vegetation (trees, shrubs) and grass strips (Feld *et al.* 2011, Sweeney & Newbold 2014). Trees and shrubs are capable of removing 35% nitrogen from the upper groundwater layer (Clausen *et al.* 2000), which is more effective in wetlands and carbon-rich soils, both of which promote microbial denitrification (Mayer *et al.* 2005). TP is primarily retained through grass strips, which filter surface runoff and remove phosphorous adhered to fine sediment particles (Dosskey 2001, Yuan, Bingner & Locke 2009). This zonation, i.e. the simultaneous presence of a tree, shrub and grass zone, is key to the functionality of vegetated riparian buffer strips (Dosskey 2001).

Buffer length and density are also frequently addressed in the riparian management literature, although their relationship to nutrient retention is less often quantified. In general buffers >1,000 m long can effectively retain nutrients (Feld *et al.* 2011). However, although it seems trivial, it is not the mere length and density, but also the location of vegetated buffer strips that determine their effectiveness. In brief, nutrient pollution requires management at the source, i.e. along (all) the agricultural areas adjacent to the stream course. Notably, the role of buffer location is rarely addressed in the riparian management literature (but see Dosskey 2001 for a review).

Furthermore, buffer strips need to be dense enough to prevent from gully erosion (Dosskey 2001), which is usually occurring in the lowest parts of riparian zones and which is promoted by agricultural management (e.g. ploughing). Ploughing perpendicular to the stream course can initiate gully erosion and thus can easily counteract riparian buffer management irrespective of its width, length and density. In contrast, contour tilling (i.e. ploughing along the contour line) can avoid gully erosion (Dosskey 2001). Surprisingly, drainage, and its putatively counteracting effect on riparian buffer management, was not addressed in the reviewed body of literature.

The role of riparian buffer vegetation age for nutrient management remains unclear. Trees and shrubs, with their deeper and denser root system, can retain nitrogen effectively, in particular at intermediate ages (Mander *et al.* 1997); older stands of wooded vegetation then are less effective. However, due to the shade the trees and shrubs throw on the stream banks, they reduce the understory vegetation and hence the stability of the stream banks. As a consequence, riparian shade can induce phosphorous pollution through bank erosion (Hughes & Quinn 2014).

With regard to nutrient retention, the effects of riparian lifestock exclusion by fencing is less effective than vegetated riparian buffer strips. Collins *et al.* (2012) and Muller *et al.* (2015) reported insignificant effects of fencing on riverine nutrient concentrations, while Parkyn *et al.* (2003) found enhanced TP concentrations after fencing and thus highlight the unclear nutrient effects of livestock exclusion from the riparian zone. Mitigating effects of fencing on fine sediment retention and erosion prevention, however, are less ambiguous (see below).

Fine sediment pollution management

Most studies of riparian buffer effects on fine sediment retention address headwaters and small sections of streams in the (agricultural) lowlands. Sediment studies from upland areas address the effects of silviculture (e.g., Kreutzweiser *et al.* 2009). Stream course length is addressed as either site or reach (to segment) scale, which translates to several hundreds of metres up to several kilometres.

Riparian buffers can retain 60–100% fine sediment from surface runoff (Dosskey 2001, Hook 2003, Mankin *et al.* 2007, Yuan, Bingner & Locke 2009, Feld *et al.* 2011, Sweeney & Newbold 2014). The retention capacity is higher for sand-sized particles (up to 90%) than for silt and clay-sized particles (20%) (Dosskey 2001). Sediment retention is primarily linked to grass strips, which act as mechanical filters and which provide full functionality at widths between 3 and 8 m (Hook 2003, Mankin *et al.* 2007). However, Dosskey (2001) found riparian stiffgrass to almost completely retain sand-sized sediments at width <1 m. In contrast, wooded vegetation (trees, shrubs) is much less effective in the retention of fine sediments (Sovell *et al.* 2000, Yuan, Bingner & Locke 2009).

Besides buffer width and composition (i.e. the presence of a grass zone), buffer density can influence fine sediment retention, mostly through shading by trees and shrubs, which reduce understory (grass) vegetation and hence sediment filter functionality (Hughes & Quinn 2014). Hughes and co-authors also reported stream bank vegetation removal through shading, which decreases bank stability and promotes bank erosion. Thus, riparian wooded vegetation can inhibit the functionality of riparian grass strips. With regard to vegetation age, older stands with a dense canopy cover are likely to throw more shade than younger stands.

The role of riparian vegetation length and density is rarely targeted in the management literature, but it is addressed several times in the discussion of studies, when it come to the limitations of vegetated riparian buffers. Collins *et al.* (2012) assumed that gaps in the riparian buffer system, together with insufficient buffer width, were responsible for weak buffer effects. Parkyn *et al.* (2003) reported insufficient buffer lengths and location to be responsible for ambiguous buffer effects on fine sediment retention. This again points at the key role of the location of riparian buffers, which must match the scale of the source(s) of fine sediment pollution. Once entered the stream system, fine sediments (and nutrients) are no longer directly subjected to riparian buffer capabilities, but require instream retention structures and processes. Such structures (e.g., sediment accumulation behind LWD) and processes (e.g., nutrient uptake through primary production), however, although influenced by riparian vegetation, are not fully controlled by riparian buffer conditions.

The effects of riparian fencing are similar to those reported above, since fencing primarily induces the establishment of riparian vegetation, a prerequisite for the retention of fine sediments. The effects of fencing are detectable shortly after instalment of fences (Carline & Walsh 2007), since grass strips grow fast and may provide full functionality after one or several years. However, fine sediment retention in course of riparian fencing reveals less consistent results compared to buffer vegetation management. This may be due to the rather passive character of fencing that alone does not guarantee the establishment of a functional riparian buffer strip.

Light/shade and temperature management

Most studies of riparian buffer effects on instream light and temperature management address small sections of streams in lowland and upland regions. Stream course length is addressed as either site or reach scale, which translates to several tens up to several hundreds of metres. Few studies address stream course lengths up to several kilometres (review provided by Broadmeadow & Nisbet 2004).

The mitigating effect of riparian vegetation on instream water temperature was frequently subjected in the reviewed body of literature. Most studies report a cooling effect linked to the width of riparian wooded vegetation (Collier *et al.* 2001, Broadmeadow & Nisbet 2004, Whitledge *et al.* 2006, Broadmeadow *et al.* 2011, Sweeney & Newbold 2014). Accordingly, a buffer width of 20 m is sufficient to keep water temperature below critical values for many salmonid fish, while 30 m buffer width would be required to keep water temperatures at levels of natural forested stream sections (Beschta *et al.* 1987, Sweeney & Newbold 2014). Temperature reduction by riparian vegetation was most effective at streams below 5 m width (Whitledge *et al.* 2006) and at shading levels of 50–80% (Broadmeadow *et al.* 2011). Hence, shading effects are linked to buffer density, with denser buffers providing stronger shade.

Interestingly, the role of buffer length is not well highlighted in the literature, suggesting that buffer length is less important. A rare example is provided by Collier *et al.* (2001), who found a 150 m long riparian buffer to reduce water temperature by 3 °C. The authors, however, suggest that reheating is as quick, if riparian shade is lacking. This is confirmed by studies on the temperature effects of riparian clear-cutting. Harvesting of riparian trees along stretches of 185 m–810 m length lead to an increase of 4–6 °C (Macdonald, MacIsaac & Herunter 2003). Parkyn *et al.* (2003) concluded from modelling studies that at least 1–5 km was required for first-order streams versus 10–20 km for fifth-order streams to reduce water temperature to more typical reference conditions. A width-length function of shading effects is illustrated by Broadmeadow & Nisbet (2004). The function can help estimate required buffer width/length combinations to limit the maximum summer water temperature by riparian shading.

As to the question of buffer composition and age, it is evident from the reviewed literature that mature riparian trees are required to achieve full functionality (see also reviews by Broadmeadow *et al.* 2011, Feld *et al.* 2011 and Sweeney & Newbold 2014). Altogether 15 out of 17 reviewed studies of temperature effects addressed single or multi-zone riparian buffer strips comprising trees (the remaining two studies did not provide details on the riparian composition/zonation).

With regard to buffer location, it is trivial that riparian temperature management has to take place upstream of a targeted reach/site. The buffer length upstream that is required to keep water temperature within certain limits (e.g., suited for salmonid spawning) is conditional on buffer width, density, composition and age, and in addition controlled by natural co-variates (e.g., current velocity, latitude) (Collier *et al.* 2001, Hook 2003).

Unlike the effects of riparian shade on water temperature, the direct effects of shade as a control of instream processes (e.g., primary production) are less often addressed. Hutchins *et al.* (2010) found riparian shade to be a much more effective control of Chl *a* (phytoplankton) as compared to phosphorous reduction in sewage treatment plants and nitrogen reduction through agricultural management. Davies-Colley & Quinn (1998) found periphyton biomass to vary four orders of magnitude and to correlate with light conditions on the stream bottom. This highlights the most often unrealised potential to manage excess plant growth, and thereby adverse side affects such as oxygen depletion following biomass decay, by riparian shade.

Instream habitat management

Large Woody Debris (LWD)

Studies exploring the effect of riparian management on LWD were performed generally in headwaters and small rivers, i.e. in the upper parts of the catchment. The targeted pressures were agriculture (usually in the lowlands) and silviculture (usually in the uplands), with emphasis on the removal of buffer vegetation at the reach scale. The riparian management included fencing and tree planting.

In generally, riparian buffer width, composition/zones and length are linked to the presence of LWD. Opperman & Merenlender (2004) showed that fencing riparian vegetation over long periods (10–20 a) increased the amount of LWD and subsequently enhanced the conditions of river biota. The density of trees, their basal area and the number of LWD pieces was higher in restored reaches than in unrestored reference reaches. Debris jams were five times higher at restored reaches. McBride, Hession & Rizzo (2008) revealed that passive restoration of the riparian zone, over a course of >40 a increased the presence of LWD. In particular, forested reaches are reported to have 40% more pieces of LWD as compared to non-forested reaches. However, the total LWD volume, number of debris dams and volume of LWD in debris dams was similar between both groups of reaches. Other studies showed that forested reaches and reaches buffered by 15 m-wide tree zones have almost four times as much LWD volume per bottom surface as compared to pasture reaches, although there was a very strong seasonal variation (e.g., Lorion & Kennedy 2009). Davies-Colley *et al.* (2009) ran a modelling experiment to simulate the response of forest and river characteristics conditional on different types of restoration. Accordingly, active native planting of riparian trees provided the best results.

Coarse Particulate Organic Matter (CPOM)

Evidence of the effect of riparian management on instream CPOM was very limited and addressed only by one study (Thompson & Parkinson 2011). The authors investigated the effects of riparian management (planting a multi-zone riparian buffer) and compared the results with those from open-canopy reaches. Leaf litter input was about 40–50% higher in the restored reaches, while invertebrate biomass responded negatively and decreased. However, macroinvertebrate shredder density increased in the restored reaches. Algal biomass showed no significant differences between restored and unrestored reaches.

Catchment management options to address the main stressor variables

Studies on management options taken at the scale of entire catchments or sub-catchments are rare, probably because of two reasons. First, catchment-scale management requires concerted efforts of practitioners and stakeholders, including land owners, which probably renders this broad spatial scale impracticable in many cases. And second, catchment-scale management requires time to plan and implement measures and to monitor and evaluate the effects of the measures. This may explain, why only one (!) out of a total of 135 reviewed studies addressed the effects of agricultural land use change at the catchment-scale *in situ*: Hughes & Quinn (2014). The authors report the results of a 13-year study on the effect of cattle exclusion from the riparian zone (total area: 153 ha). Although it is arguable, whether the study addresses 'real' catchment management, it is the only study that subjected the implementation of an integrated catchment management plan, encompassing land use change and riparian management in concert.

All other studies of catchment-scale effects of land use change and riparian buffer management are modelling studies that applied some form of mechanistic or empirical model (or combinations thereof) to predict the effects of selected management scenarios. Hence, we found the tremendous evidence of riparian management effects contrasted by an almost complete lack of evidence of catchment management effects.

Nutrient pollution management

Hughes & Quinn (2014) provided evidence, that cattle exclusion and riparian planting within a headwater catchment in New Zealand had significant effects on water quality. However, while riparian fencing lead to an increased water quality, riparian planting had the opposite effect and lead to enhanced instream concentrations of nitrate and phosphorous, probably mediated by increased riparian shade and thus reduced nutrient uptake by instream macrophytes and algae. The authors point at the complex nature of the effects of catchment rehabilitation measures.

Based on water quality models applied to a 1,000 km^2 sub-catchment of river Havel (Germany), Krause *et al.* (2008) found riparian land use change (extensification of the current agricultural land use practices, with different protection/conservation targets) to reduce nitrate leaching from the root zone into the riparian groundwater zone by 43–85%, which translates to a 70% reduction of the net contribution of nitrate from the floodplain (due to denitrification). However, total floodplain nitrate contribution accounted for merely 1% of the total river's nitrate load and thus rendered the modelled effects negligible.

(Lam, Schmalz & Fohrer 2011) modelled the effects of several best management practices (BMP) (extensive land use management, grazing management practice, field buffer strips and nutrient management plan) on nutrient loads within a 50 km^2 sub-catchment of the Kielstau river (Germany). The authors found a combination of several BMPs to be most effective and to achieve reductions in nitrate loads by up to 54% (1.1–5.3% for total phosphorous). However, the results translate to a reduction of only 8.6% of the annual nitrate loads.

(Panagopoulos, Makropoulos & Mimikou 2011) evaluated the effects of BMPs (filter strips at the edge of fields, fertilization reduction in alfalfa, contour farming and zero-tillage in corn, reduction of animal numbers in pastureland) on nitrate-N and total phosphorous losses to surface waters within a 940 km^2 sub-catchment of the Arachtos river (Greece). Testing the effects of different combinations of BMPs, the models resulted in up to 50% less total phosphorous and 25% less nitrate-N lost to the surface waters.

The notably extensive modelling study by (Weller & Baker 2014) addressed the effects of riparian buffers on the cropland nitrate load to streams throughout the entire Chesapeake Bay watershed (USA). Across the watershed, croplands release 92,300 t of nitrate-N, of which 19,800 t (21.5%) were removed by riparian buffers. At most, 29,400 t more might have been removed if buffer gaps were restored so that all cropland was buffered. The other 43,100 t (46,7%) of cropland load cannot be addressed with riparian buffers.

In summary, the modelled effects of riparian and land use management at the (sub-)catchment scale are fairly variable. Various numbers and combinations of BMP resulted in reductions of 25–50% of nitrogen and 8–50% of total phosphorous, while a direct comparison of nitrogen effects is hampered by different units (nitrate, nitrate-N, total nitrogen) of the studies. Furthermore, the modelled catchments are subjected to different landscapes (lowland, uplands) and climatic regions (temperate, mediterranean) and thus different natural co-variates (temperature, precipitation, soil characteristics) that might have largely influenced the results. Nevertheless, there is evidence for considerable reductions in nitrogen loads achievable through catchment-scale management. At the same time, however, the model studies suggest that catchment-wide riparian buffer strips are unlikely to retain much more than about 50% of nitrogen and phosphorous (Weller & Baker 2014). Consequently, river basin managers should be concerned about the other 50% that continue to impose a serious threat to surface water quality and that require additional management.

Fine sediment pollution management

Only two catchment-scale studies addressed management effects in fine sediment pollution. For the 50 km^2 sub-catchment of the Kilstau river (Germany), Lam *et al.* (2011) evaluated a 0.8–4.9% reduction of fine sediment loads following the modelled implementation of several BMPs (land use, grazing and fertilizer management, buffer strips). As net sediment loads were much higher, the authors concluded that bank erosion dominated within the (lowland) model region and that therefore, the potential for surface erosion in general was very low.

For the Greek Arachtos catchment (see above), the estimated reduction in fine sediment loads from corn fields following the modelled implementation of riparian filter strips was around 5% (Panagopoulos *et al*. 2011).

In summary, although based on limited evidence, the results suggest that sediment management is complex and, besides the influence of natural factors (slope, climate, soil type), underlies various anthropogenic impacts operating at the whole watershed scale.

Biological effects of stressor management

Primary production

For plant biomass management, shading is reported to be more effective than nutrient reduction (e.g. through sewage treatment), which in combination lead to a reduction in 44% of peak phytoplankton as compared to 11% in unshaded reaches (Hutchins *et al.* 2010). Shading seems to be effective also to reduce periphyton and macrophytes, whose cover correlates positively with light (Davies-Colley *et al.* 1998, Parkyn *et al.* 2003).

Benthic macroinvertebrates

Macroinvertebrates were found to be mainly respond to changes in fine sediment coverage and water temperature. However, the reviewed studies found both positive and negative responses to both abiotic variables. Sediment reduction resulted in an increased of a macroinvertebrate index (Collier *et al.* 2001) and macroinvertebrate density, but not macroinvertebrate diversity (Carline & Walsh, 2007). Reduced water temperature was linked to an increase of several macroinvertebrate indices (Parkyn *et al.* 2003, Quinn *et al.* 2009, Dodd *et al.* 2010), reflecting generally the presence of taxa with affinity for cold, oxygenated waters. However, macroinvertebrate diversity or growth could not respond (Quinn *et al.* 2009) or even decrease (Weatherley & Ormerod 1990) after water temperature reductions.

Fish

The reduction of water temperature had variable effects on river fish. In two studies, fish showed reduced growth (Weatherley & Ormerod 1990) and density (Sovell *et al.* 2014) in forested reaches as opposed to open-canopy sites. However, other two studies revealed positive effects of reduced temperature on fish (Whitledge *et al.* 2006, Hickford & Schiel 2014), but in both cases, under particular circumstances. Whitledge and co-authors found that riparian shading and reduced temperatures increase growth potential of small-mouth bass (*Micropterus dolomieu*), which tend to inhabit warm waters (optimum temperature: 22 °C). However, the potential to achieve the optimum water temperature is limited in non-ground-fed or large rivers, compared to small (<5 m width) ground-fed rivers, where riparian shade can effectively mitigate maximum temperatures during summer. In another study, riparian shading had also a positive effect on egg production of *Galaxias maculatus*, a species that requires riparian habitats to spawn (Hickford & Schiel 2014).

LWD addition had positive effects only for some fish species (Jowett *et al.* 2009), (Sievers, Hale & Morrongiello 2017). A recent meta-analysis demonstrated that the abundance of trout species (brook trout *Salvelinus fontinalis*, brown trout *S. trutta*, cutthroat trout *Oncorhynchus clarkii*, and rainbow trout *O. mykiss*) increased, on average, by 87.7% after the addition of LWD. However, Jowett *et al*. (2009) found that reduced LWD inputs reduced pool and habitat heterogeneity and diminished the density of *G. maculatus*, and longfin eel (*Anguilla dieffenbachii*), whereas other species responded with an increased density. Lorion & Kennedy (2009) found that the density of herbivorous and detritivorous fish species was higher in open reaches (pastures) as compared to forested reaches, while the density of the other feeding types remained similar between reaches.

Synthesis: Riparian vs. catchment-scale management

Conflicting scales

After being populated with evidence from 53 published studies, the conceptual model (Fig. 4) assisted the evaluation of riparian *vs.* catchment-scale management options. In general, we are able to distinguish stressors (grey boxes) that are exclusively linked to riparian buffer characteristics (blue boxes) from those that are additionally linked to catchment-scale pressures (purple diamonds). While the former group (light, temperature, LWD) represents stressors that are entirely manageable at the riparian scale, the latter group (N, P, % fines) contains stressors that require catchment-scale measures in addition to riparian management, to effectively reduce nutrient and fine sediment loads. Hence, we can distinguish scale-tolerant (no conflict of scales) from scale-sensitive management (conflict of scales).

The evidence of scale-sensitive riparian management options is summarised in Tab. 1. Accordingly, numerous studies have shown or suggested that the riparian and floodplain (land use) conditions upstream can largely influence and even counteract site or reach-scale restorations (Mayer *et al.* 2005, Richardson *et al.* 2010, Feld *et al.* 2011, Lorenz & Feld 2013, Giling, Mac Nally & Thompson 2016). These watershed-scale adverse impacts may operate up to 5–10 km upstream (Lorenz & Feld 2013) or further (Feld *et al.* 2011).

Table 1: Evidence of riparian management effects and limiting factors at broad spatial scales.

Riparian management option	Abiotic effect	Biological effect	Limitation	Reference [type of study]
Wooded multi-zone riparian buffer strips, 5–30 m wide and >1,000 m long.	Retention of nutrients (up to 100% N/P) and fine sediments (up to 100%), reduction of stream temperature, habitat improvement (LWD, CPOM).	Increase of macroinvertebrate and fish diversity, improvements of functional traits, improved community composition, enhanced fish biomass, less studies effects of riverine plants.	Land use further upstream in the continuum continues to limit restoration success; poorly designed buffers (too narrow, too short) do not function.	Feld *et al.* (2011) [review of 46 riparian management papers, various regions and stream types worldwide]
Scenario 1 covers partial land use change on sensitive floodplain areas (e.g. hydromorphic soils, erodable soils) and 20 m-wide riparian forested buffers along the river course; scenario 2 covers full land use change on sensitive areas and 50 m-wide riparian forested buffers.	Reduced nitrate leaching from the root zone (43–85% for scenarios 1 and 2, respectively); reduced nitrate contribution from the floodplain (70–100%); floodplain can even constitute a sink for river-derived nitrate.	--	Floodplain nitrate contribution constitutes only about 1% of total river nitrate loads per year; thus modelled management effects are negligible.	Krause *et al.* (2008) [modelling of land use and management effects of two scenarios within a ca. 1,000 km^2 sub-catchment of River Havel, Germany]
Comparison of pasture sites with unlimited lifestock access and fenced sites without lifestock access and riparian trees/shrubs present.	Bank erosion processes vary throughout catchments (with particular reference to their scale dependence); only two studies specifically attributed reduced stream bank erosion to the presence of riparian vegetation.	--	The exclusion of livestock from riparian areas is generally reported as the principal factor in the measured improvements or differences; planting of riparian vegetation in headwater streams and the subsequent shading of stream banks can reduce bank stability and promote channel widening (and hence a release of sediment; see also Hughes & Quinn 2014).	Hughes (2016) [review of various studies with and without livestock access to river banks and riparian trees/shrubs]

Riparian management option	Abiotic effect	Biological effect	Limitation	Reference [type of study]
Riparian management targeting at the provision of riparian habitat that fulfils critical functions for fish (e.g., bank stability, shade/temperature, large wood, water clarity, sediment retention).	Riparian habitat is crucial for the provision of shade, control of channel complexity and sediment inputs through bank stabilization, input of large wood and allochthonous energy sources, and filtering of nutrients and toxins from adjacent land.	Riparian habitat should be considered biologically critical for most species of freshwater fish, unless the habitat requirements of individual species indicate insensitivity to the ecological functions as- sociated with riparian zones.	Protecting the riparian zone may not in itself be sufficient to maintain stream ecosystem integrity or species at risk if development throughout a watershed (e.g., agriculture or urbanization) significantly alters hydrology or water quality.	Richardson *et al.* (2010) [review of various riparian management studies in light of habitat demands of fish]
Riparian land use in buffers of 100–200 m width and 500–10,000 m length upstream, and riverine hydromorphology 500–10,000 m upstream of -- biological sampling sites.	--	Upstream land use and hydromorphology are stronger determinants of ecological success after restoration than local land use and hydromorphology at restored sites.	Land use and hydromorphological degradation in the sub-catchment upstream can limit the success of local restorations.	Lorenz & Feld (2013) [analysis of biological effects of riverine hydromorphology and riparian land use at several distances upstream of restored and unrestored lowland and mountaineous stream sites in Germany]
Comparison of modelled nitrogen loads from cropland conditional on the amount of buffered stream length and streamflow.	In the entire watershed, croplands release 92.3 t of nitrate nitrogen, 19.8 t of which is removed by riparian buffers; 29.4 t more might be removed with all buffer gaps closed; the remaining 43.1 t of cropland load cannot be removed by riparian buffers.	--	47% of cropland nitrogen load cannot be reduced by riparian buffers and must be addressed by other management options.	Weller & Baker (2014) [modelling of riparian buffer effects on cropland nitrate loads at 1,964 sub-basins of Chesapeake Bay, USA]

Riparian management option	Abiotic effect	Biological effect	Limitation	Reference [type of study]
Analysis of the response of aquatic macroinvertebrate assemblages to riparian replanting (8–22 a before monitoring) at agricultural streams.	--	Macroinvertebrates did not respond to replanting over the time gradient, probably because replanting had little benefit for local water quality or in-stream habitat. Invertebrate assemblages were influenced mainly by catchment-scale effects, but were closer to reference condition at sites with lower total catchment agricultural land cover.	Reach-scale replanting in heavily modified (agriculturally-used) landscapes may not effectively return biodiversity to pre-clearance condition over decadal time-scales.	Giling *et al.* (2016) [analysis of riparian vegetation replanting of different ages at streams in south-eastern Australia]
Meta-analysis of the effects of riparian buffer width and buffer vegetation type on the removal of nitrogen from surface and groundwater flow paths.	Riparian buffers effectively remove nitrate through uptake and denitrification (mean: 74%), but the relation to buffer width is not strong.	--	Riparian buffers are a best-practice management option, but only in concert with other management options at the watershed scale. Soil characteristics can promote denitrification (high organic content, water-saturated soils).	Mayer *et al.* (2005) [review of the effects of riparian buffers on nutrient and fine sediment retention]
Passive ecological restoration (excluding livestock through fences along an entire stream, 1 m from the stream bed) with the assumption that recovering riparian habitat will restore ecological processes (e.g., filtration, soil stabilization).	After eight years, the restored stream had complex riparian banks, similar to that of reference streams (more trees, less bare soil, increased habitat heterogeneity).	--	Water quality did not improve; the same low water quality in the reference stream demonstrated the need for a watershed-scale approach and for actions to improve agricultural practices before implementing restoration practices at a smaller scale.	Muller *et al.* (2015) [monitoring of water quality and riparian habitat heterogeneity of an entire stream in France, eight years after lifestock exclusion through fencing]

Riparian management option	Abiotic effect	Biological effect	Limitation	Reference [type of study]
Analysis of the capability of longitudinally restricted riparian forest buffers to enhance in-stream nutrient retention in nutrient-enriched headwater streams.	Riparian forested buffers can increase instream ammonia (but not phosphate) uptake through enhanced hydrologic retention (reduced flow) induced by LWD on the bottom.	--	Already highly eutrophied streams seem to have a limited retention capacity for N and P components; instream nutrient retention cannot compensate for deficits in riparian nutrient retention when the nutrient supply exceeds the demand significantly.	Weigelhofer *et al.* (2012) [experiment and modelling of the effects of riparian forested buffers on instream nutrient uptake]
Measurement of water quality along four Australian tropical streams in two catchments with similar agricultural development (mainly sugarcane growing) but contrasting riparian vegetation (intact native rainforest vs. exotic weeds).	Nitrate and nitrite (NOx) concentrations and loads were significantly lower in streams with greater riparian vegetation; yet, NOx concentration significantly increased with distance downstream (i.e. with the amount of fertilized agricultural land in the catchment).	--	An adequate reduction in NOx in streams can only be achieved by reduced fertilizer application rates in the catchments.	Connolly *et al.* (2015) [comparison of N reduction along buffered and unbuffered streams in four agricultural catchments in Australia]

Nutrient pollution management

Numerous riparian management studies and several reviews provide sufficient evidence of the nutrient retention capabilities of riparian buffers. Nitrogen and phosphorous losses from agricultural areas on the floodplain can be effectively retained, with retention levels frequently ranging 50–70% (up to 100%) (Dosskey 2001, Feld *et al.* 2011, Wahl, Neils & Hooper 2013). However, to develop full functionality, riparian buffers need to i) be 20–30 m wide, ii) consist of multiple zones (trees, shrubs, grass strips) and iii) accompany the entire stream length impacted by diffuse nutrient and sediment pollution. Gaps in the buffer can significantly reduce its functionality (Weller & Baker 2014). Furthermore, riparian buffers cannot address impacts from further upstream; once they entered the stream network, nutrients and fine sediments are controlled by different mechanisms, such as instream nutrient retention through primary production. Actually, primary production is controlled by riparian shade (Hutchins *et al.* 2010) and thus inhibited by riparian buffers.

Consequently, riparian management must address headwater and upstream sections of the stream network, if sources of diffuse pollution already occur thus far upstream (Parkyn *et al.* 2003). This raises buffer location, i.e. the location within the stream continuum, an important aspect. Notably, buffer location is rarely addressed in the relevant management literature (Fig 4).

In contrast to the riparian management literature, catchment-scale (modelling) studies result in much more pronounced limitations as to the retention capacity of riparian buffers. Accordingly, about half of the diffuse pollution cannot be managed at the riparian scale (Weller & Baker 2014) and thus requires further catchment management such as fertiliser management or land use change (Mayer *et al.* 2005, Panagopoulos *et al.* 2011, Connolly *et al.* 2015, Muller *et al.* 2015).

It follows that with regard to diffuse nutrient pollution, there is a conflict of management scales, and management at one scale alone is unlikely to effectively increase water quality.

Fine sediment pollution management

There is evidence that relatively small (3–8 m wide) grass strips can effectively buffer fine sediment pollution from floodplain agriculture. Retention capacity ranges 60–100% (Dosskey 2001, Carline & Walsh 2007, Feld *et al.* 2011), but retention of sand-sized particles is much more effective than retention of silt and clay-sized particles (Dosskey 2001). Trees and shrubs are less effective buffers of fine sediment pollution (Sovell *et al.* 2000) and may even counteract the functionality of grass buffer strips through riparian shade (Hughes & Quinn 2014). Regular harvesting of riparian wooded vegetation may reduce the adverse effects of riparian shade (Kronvang *et al.* 2005). However, similar to diffuse nutrient pollution, buffer location turns out to be of paramount importance, because buffer effectiveness is linked to the location of the sources of diffuse sediment pollution. It is likely that large gaps in the riparian buffer can lead to increased losses of fine sediments through (point-source) gully erosion (Dosskey 2001).

In contrast to the high riparian buffer capacity for fine sediments, the few catchment-scale modelling studies reviewed here imply that diffuse fine sediment pollution is fairly variable and that catchment-wide retention capacity, at most, is around 5%. Furthermore, instream fine sediment loads are not only controlled by surface erosion from the floodplain, but also by instream bank erosion, in particular in lowland regions, where surface erosion is fairly low (Lam *et al.* 2011).

Hence, it is difficult to conclude appropriate management guidance from this limited evidence. As a rule of thumb, riparian management may effectively reduce diffuse fine sediment pollution, if buffers are sufficiently wide and accompany the entire stream length prone to diffuse fine sediment pollution. Management may focus on headwater and upstream areas, to prevent from fine sediments already entering the stream system thus far upstream. Once, excessive fine sediment enters the stream system, riparian management options are very limited (see LWD below).

If compared to the conflicting scales of diffuse nutrient pollution, the case is much less clear with fine sediments, i.e. riparian management might be effective also without further catchment-scale management. Further evidence from catchment-wide modelling would be required, to be able to better estimate the potential limitations that catchment-scale impacts may impose on the functionality of riparian buffers.

Light and temperature management

In-stream light conditions and the resulting increase of water temperature is controlled solely by riparian wooded plants. Hence, light and temperature management does not underlie a conflict of management scales. Any riparian management that increases the amount of shade thrown on the stream channel will have mitigating effects on water warming (see riparian management results). To keep water temperature within ranges exposed by natural (forested) streams, forested buffer widths of 20–30 m are required. Evidence on the required lengths of buffers is less clear and spans 150 m up to several kilometres required to decrease water temperature by some 3–4 °C. With regard to composition and age, it is evident that mature trees with dense canopies (50–80% shaded stream channel surface) reduce light and water warming most effectively (Broadmeadow & Nisbet 2004). Although trivial, it might be worth stressing that with riparian shade effects, the location of riparian management is disconnected from the targeted instream management reach, i.e. where cooling effects are actually desired.

Water managers, however, need to be aware that riparian shade may have potential undesired side effects. First, riparian shade may increase bank erosion and thus promote instream fine sediment pollution (Hughes & Quinn 2014). And second, instream shade inhibits macrophyte and algal growth and thus can significantly reduce instream nutrient retention by aquatic plants (Davies-Colley & Quinn 1998, Huntchins *et al.* 2010). Yet, this loss of plant biomass may be balanced by a lower risk of oxygen depletion following the microbial biomass decay at the end of the vegetation period.

LWD and CPOM management

Organic matter management of both wood and coarse particulate organic matter requires the establishment of mature riparian wooded buffers, old enough to provide natural amounts of large wood to the stream system. This may require several decades (Feld *et al.* 2011) up to centuries. However, LWD and CPOM dynamics are not in conflict with catchment-scale management and thus constitute another 'no-regret' management option. LWD, if occurring in natural amounts, can form debris dams and thus can influence the sediment retention capacity of the stream system; fine sediments are typically accumulated behind larger debris dams and tree trunks. In addition, the surface of LWD constitutes a stable substratum for algae and fungi (biofilm) and thus promotes the instream retention of nitrogen and phosphorous.

The amount of LWD provided to the system is depending on the width, length, composition and age of the riparian vegetation, all of which require consideration for related management activities. CPOM provided by riparian plants is an important (allochthonous) carbon source and as such largely drives the instream food web (Vannote *et al.* 1980). This role of CPOM most pronounced in the upstream and middle sections of the network, where autochthonous carbon usually dominates over the autochthonous (instream) biomass production.

Biological effects

The response of biological groups to riparian management was highly variable, especially for macroinvertebrates and fishes. Only primary producers showed a clear consistent biomass decrease to reduced light, as a result of riparian restoration. Light is a primary driver of primary producer biomass across ecosystems. However, limiting primary producer growth in upstream reaches may result in higher nutrient concentrations downstream due to the reduced uptake. Therefore, increasing riparian shade should be recommended only if the downstream rivers are not nutrient-sensitive or if the focus of the management is in the upper part of the catchment (Hutchins *et al.* 2010).

Regarding macroinvertebrates and fishes, riparian management should be context-dependent and adapted to species ecological requirements and management goals. Also, we should consider intrinsic limitations, such as the trade-off between instream productivity and water temperature. Reduced productivity could result in reduced macroinvertebrate or fish biomasses (e.g., Lorion & Kennedy 2009). However, allochthonous inputs from the broadleaf forests or terrestrial fauna could enhance invertebrate biomass and compensate the reduced instream productivity in forested rivers (Thomas, Griffiths & Ormerod 2015, Thomas *et al.* 2016), but this would depend on climate and nutrient availability.

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MARS PROJECT

Managing Aquatic ecosystems and water Resources under multiple Stress

Deliverable 4.2-2: Stressor propagation through surface-groundwater linkages and its effect on aquatic systems

Lead beneficiary: **DELTARES**

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Non-technical summary

The good ecological status of Europe’s freshwaters is still lacking. This paper reviews the role of groundwater in these systems and demonstrates that it is an important factor to include in surface water management. Groundwater influences streamflow, water chemistry and water temperature and connects rivers and streams with their catchment and thus functions as a pathway for stressors to reach the surface water. A new ‘Groundwater DPS’ framework is proposed which shows how groundwater fits in the system of a stressed aquatic ecosystem. The functioning of this framework is demonstrated using examples from four different European lowland catchments: the Thames, Odense, Regge and Dinkel catchments. The importance of groundwater varies between scales, between catchments and within catchments. The Groundwater DPS will aid water managers in understanding the importance of groundwater in their management areas and promotes the consideration of groundwater in future water management practices.

Contents

Introduction / Background

The goals of good ecological status for Europe's freshwaters are still to be met (EEA, 2015). Although much effort from water managers and researchers has resulted in improvements, by 2015 one in every two water bodies failed to achieve good ecological status (EEA, 2015), and the extended deadline set in the Water Framework Directive (WFD) of 2021 to 2027 now approaches quickly. Surface waters are affected by multiple stressors due to anthropogenic alterations; the effect on aquatic ecosystems of this multi-stress is researched in the EU FP7 project MARS (Hering et al., 2014).

Much research has already been undertaken on the effect of stressors on surface waters (e.g. Feld and Hering, 2007; Stendera et al., 2012; Nõges et al., 2015; Piggott et al., 2015; Baattrup-Pedersen et al., 2016; Schülting et al., 2016). However, the inclusion of groundwater in this research is still limited, despite the fact that it has been known for several decades that groundwater and surface water are essentially one resource (Brunke and Gonser, 1997; Winter et al., 1998). Many aquatic ecosystems in lowland streams are dependent on a supply of groundwater (Brunke and Gonser, 1997; Hatton and Evans, 1998; Power et al., 1999; Wriedt et al., 2007) and together with specific terrestrial ecosystems referred to as Groundwater Dependent Ecosystems (GDEs) (Hancock et al., 2005; Kløve et al., 2011).

Groundwater influences the quantity and quality of surface waters in several ways: the amount of discharge because a significant part of surface water originates from groundwater, the timing of discharge because groundwater provides water with a delay as opposed to surface runoff, water chemistry because this groundwater provides water with a specific chemical composition, and temperature because the relative constant temperature of groundwater differs from that of surface water. Through these processes, groundwater provides water, nutrients and energy to aquatic ecosystems (Bertrand et al., 2011) and creates refugia in the surface water to for instance fish (Power et al., 1999).

Surface water managers are slowly giving increased attention to the importance of groundwater. For instance, in Australia water managers started integrating groundwater and surface water management in the last decade (Lamontagne et al., 2012). In the US groundwater needs of ecosystems are being taken into account in conservation plans (Brown et al., 2007) and in Europe groundwater is now included in the Water Framework Directive, Groundwater Directive, Habitats Directives and the CIS Working group on Groundwater (European Commission. 2000; 2006). Despite this, many water managers still consider groundwater and surface water separately. There is now a need to further integrate groundwater and surface water management.

The objectives of this paper are to (1) review the role that groundwater plays on freshwater ecosystems under multiple-stress, (2) propose a new framework that helps water managers understand the importance of groundwater in their management areas, (3) illustrate the framework by applying it to four European catchments, and to (4) discuss the differences in the importance of groundwater between these catchments.

Review of the effect of groundwater on surface waters and aquatic ecosystems

Discharge

Functioning of aquatic ecosystems is strongly dependent on flow (Arthington et al., 2006; Poff and Zimmerman, 2010). Aquatic species have adopted their life strategies to specific flow regimes and habitats (Bunn and Arthington, 2002; Lytle and Poff, 2004). In these flow regimes, discharge peaks are generally linked with surface runoff, while a streams baseflow is linked with groundwater. Discharge from groundwater is delayed compared to discharge from direct precipitation and overland flow and therefore leads to a more stable streamflow in time (Smakhtin, 2001). Many flow regimes are transformed by human interference in catchments which affects the aquatic ecosystems (Feld et al., 2011). Changes in flow regime of groundwater dominated rivers lead to more generalist and tolerant species (Blann et al., 2009) because species that evolved in a more variable environment are less vulnerable to environmental changes than habitat specialists (Schlosser, 1990).

A catchment contains a certain amount of surface and groundwater which continuously interact. Groundwater and surface water are basically the same resource and activities affecting one will affect the other (Winter et al., 1998). The abstraction of groundwater changes groundwater flow, recharge and discharge to the surface water (Zhou 2009); the water that is removed from a catchment's water balance is no longer available for surface waters. Research has shown how groundwater abstractions decrease stream baseflow (Henriksen et al., 2008; SKM, 2012; Hendriks et al., 2014) and because of this, groundwater pumping is an important stressor on a stream's ecosystem. While the abstraction of surface water has a clear immediate effect on stream discharge (Winter et al., 1998; SKM, 2012), the effect of the abstraction of groundwater is delayed in time (Custodio, 2000).

The importance and effect of groundwater on surface water is steered by the combination of different groundwater flow paths which each have a different travel time. These flow paths and their travel times are governed by catchment characteristics like geology and land use. Because these characteristics vary between catchments, the contribution of groundwater to streamflow does as well. This groundwater contribution is significant; research by Sinclair and Pitz (1999) for instance indicates that 68% of total annual streamflow in Washington State originates from groundwater discharge. In many catchments, part of the groundwater discharge comes from subsurface drainage pipes because agricultural and urban areas are often artificially drained. Studies in the USA showed examples where between 41% and 81% of annual discharge from such catchments came from drainage pipes (Xue et al., 1998; King et al., 2014). Subsurface drainage has an effect on peak flows and baseflow and therefore on a catchment's flow regime. Depending on the local settings, subsurface drainage decreases peak flows and increases baseflow (Irwin and Whiteley, 1983; Schilling and Libra, 2003; Blann et al., 2009; King et al., 2014) or increases peak flows by providing a fast flow path to the surface water (Irwin and Whiteley, 1983; Carluer and De Marsily, 2004).

Groundwater discharge is influenced by climate change because different temperatures and precipitation patterns lead to changes in evaporation, groundwater recharge and groundwater levels (GENESIS, 2011). Increasing evaporation and a decrease in precipitation reduce groundwater recharge and lower groundwater levels (Singh and Kumar, 2010). Therefore the amount of groundwater available to surface waters is lowered and consequently the amount of baseflow provided by groundwater seepage.

Water Quality

Surface water chemistry is a mixture of the chemistry of all the (groundwater) flow paths it sources from. As such, freshwaters are directly influenced by the quality of groundwater (Rozemeijer and Broers, 2007). Timescales of groundwater flow are important because groundwater with different ages is characterized by different chemical compositions. The water chemistry is dependent on the flow path and travel time through the subsoil which determine the loading during recharge / infiltration at source (land use, landscape), chemical interaction with sediments and time available for chemical reactions.

Water chemistry is an important habitat factor for aquatic species. For instance, nutrients control the distribution of species and dictate the occurrence of algae and diatoms, which form the basis of ecosystems. A change in nutrient levels can therefore lead to a change in food webs and ecosystem functioning (Blann et al., 2009). Nitrate can also be directly toxic to aquatic species when exposure to high concentrations continues for a longer time (Camargo et al, 2005). Low oxygen levels, leading to the death of fish, can be caused by eutrophication due to high nutrient levels and water temperatures but another cause of hypoxia can be the inflow of anoxic groundwater.

Anthropogenic nutrient inputs have increased levels of N and P by up to a 10-fold (Vitousek et al., 1997). The most important anthropogenic sources of nutrients are direct through waste water treatments plants and diffuse through agriculture. Nutrients from agriculture enter the surface water through overland flow, artificial drains and deeper groundwater. Of all groundwater bodies in Europe, about a third has been reported to exceed the guideline values for nitrate, which is acknowledge as a risk in causing nitrate pollution of surface waters (European Commission, 2008). Subsurface drainage has been found to be the most important route for nitrate loss from agricultural fields (Rozemeijer et al., 2010; Blann et al., 2009) as they provide a short-cut towards the surface water and thus provide groundwater with a short travel time in which little time was available for denitrification processes. Phosphorous is considered the most important factor in causing eutrophication because most surface waters are P-limited (Elser et al., 2007). P is easily bound to sediments and is therefore often retarded in the unsaturated zone (Hamilton, 2012). However, phosphorus is transported by the groundwater when it is released within the groundwater or when groundwater levels rise up to and dissolve P-containing sediments (Dupas et al., 2015).

Because of its longer residence times, groundwater creates a buffer for pollution in time which is governed by the specific flow paths and travel times of the groundwater. A catchment with a high percentage of old groundwater will be less susceptible to nitrate pollution thanks to the inflow of unpolluted old groundwater. On the contrary, long residence times also create a time lag in the contribution of historic pollution input which causes pollution even after management interference in a catchment (Hamilton, 2012). Groundwater fed surface waters often contain water with ages of several decades and older (Hamilton, 2012) which means that current pollution inputs will propagate through the groundwater system and form a future pressure on surface water ecosystems. The chemistry of infiltrating polluted water changes along its flow path and with travel time, depending on the composition of the subsoil. For instance, nitrate may be reduced and pollutants removed by biodegradation.

Water Temperature

The temperature of surface water is an important parameter for aquatic ecology as it influences the occurrence and speed of chemical and biological processes such as denitrification. In addition, species are adapted to a certain temperature range, and cannot tolerate extended exposure to temperatures above or below this range. Stream temperature is determined by a complex interplay of processes with strong spatial and temporal differences (Cassie, 2006).

When precipitation infiltrates and becomes groundwater, it quickly changes temperature to around the annual mean when it reaches a depth of generally up to several meters. This is for instance a temperature of around 10-11 °C for the Netherlands (Bense and Kooi, 2004) and around 9 °C for Denmark (Matheswaran et al., 2014). The contribution of groundwater to a stream is therefore characterized by a stable temperature, as opposed to the seasonally and diurnally fluctuating temperature of surface waters. Because of this, groundwater upwelling is a crucial component in the formation of river habitats by providing thermal refugia during warm or cold periods of the year, for instance for fish (Power et al., 1999). Streams with a high contribution of groundwater have a more stable water temperature, which is crucial for e.g. stenothermic aquatic species.

With increasing mean annual air temperatures as a result of climate change, the input of groundwater to streams will be even more crucial in mitigating high summer temperature peaks. However, the average groundwater temperatures will also slowly increase which means that summer thermal refugia will disappear or shrink (Meisner 1988; Isaak et al., 2012).

Groundwater Connectivity

The contribution of groundwater to streams and rivers is spatially and temporally heterogeneous, and changes from upstream to downstream (Modica et al., 1997; GENESIS, 2011). Streamflow is a mixture of water from different flow routes: overland flow, flow through shallow groundwater including subsurface drains, and deep groundwater flow. These flow routes act on different spatial and temporal scales and because of their longer flow paths and time, the groundwater routes facilitate as a buffer in time and space. Groundwater yields a 'mean' environmental flow and buffers chemistry and temperature in time and space, and as such also buffers the effect of stressors. This means that groundwater fed surface waters are more stressor resilient than surface water without a groundwater input.

Additional to buffering flow, chemistry and temperature, groundwater also functions as a connection between a catchment and its stream. Water from throughout a catchment is transported through the subsoil as groundwater before ending up in the surface water. This way, a stressor located somewhere in a catchment will have an impact on the surface water downstream, even when there is no connection over the surface. Because of its larger temporal scale, the effect of stressors via the groundwater system is indirect and has a longer response time than the direct effect on surface water. The connections between the groundwater and surface water are affected by anthropogenic influences (Brunke and Gonser, 1997; Kløve et al., 2011) and thus also under stress.

A DPS framework to illustrate the contribution of groundwater

The importance of groundwater for the abiotic and biotic status of surface waters in lowland catchments can be shaped to fit the DPS from the DPSIR (Driver-Pressure-State-Impact-Response) model used in European environmental assessments and various European projects (e.g. REFORM, MARS, GLOBAQUA) (European Environmental Agency, 1999; Kristensen, 2004). This Groundwater DPS(IR) framework is shown in Figure 1 and covers the most important processes in which groundwater plays a role in stressed ecosystems and additionally shows how surface waters are influenced by these processes. The abiotic states are proxies for the ecological status of surface waters (as described in Grizzetti, et al., 2015). The groundwater system is located between the source of various stressors and the aquatic ecosystem and therefore functions as a bridge between Drivers and Pressures, as will be demonstrated using examples later in this paper. The effect of groundwater on the Diver—Pressure relation is governed by the connectivity and residence time of the groundwater. The groundwater DPS framework can be applied to a wide range of scales varying from stream stretch to catchment scale. It can assist water managers to understand how groundwater is important in their management areas and promote the inclusion of groundwater in water management plans.

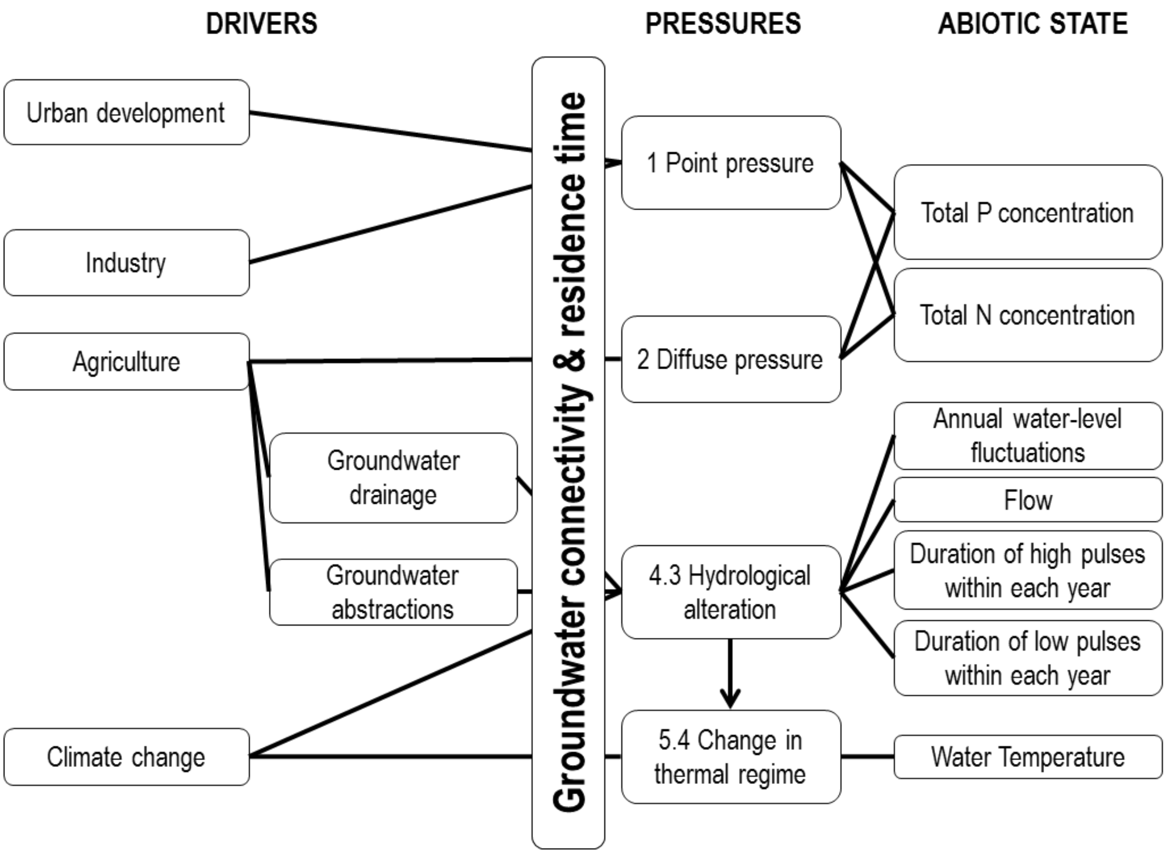

Figure 1. The Groundwater DPS(IR) shows how drivers are connected through groundwater with surface waters where they function as a pressure and affect abiotic state.

Four European catchments to illustrate the Groundwater DPS framework

The proposed DPS framework is used to describe the role of groundwater on the abiotic characteristics of four European catchments, and shows the functioning of the framework based on available data and literature. These catchments are the catchments of the Thames in the United Kingdom, the Odense in Denmark and the Regge & Dinkel in the Netherlands (Figure 2 and Table 1). All four are lowland agricultural catchments and as such they have strong similarities in the effects of groundwater on surface waters; even though setting, geology and scales are different. Main stressors in all catchments are diffuse pressures from agriculture, climate change and abstractions of both groundwater and surface water.

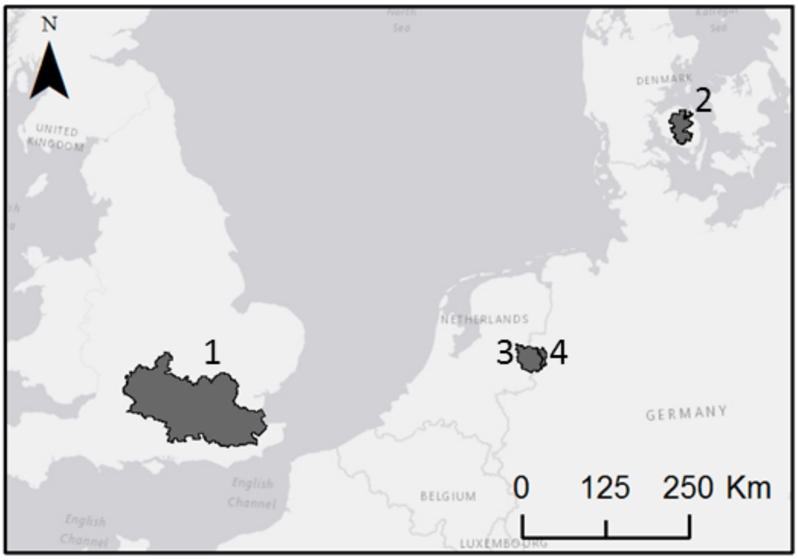

Figure 2. Location of the four study catchments.

Table 1. Selected characteristics of the four study catchments.

Catchment	Hydrogeology	Size	Primary land use	Mean annual flow
1. Thames	Limestones and chalk	9948 km^2	Agriculture (45%)	65.7 m^3s^{-1}
2. Odense	Clayey moraines	1061 km^2	Agriculture (68%)	10.4 m^3s^{-1}
3. Regge	Sand and gravel	340 km^2	Agriculture (60%)	6.95 m^3s^{-2}
4. Dinkel	Sand, gravel and clayey moraines	630 km^2	Agriculture (70%)	5.50 m^3s^{-3}

In the following paragraphs give a short description of these catchments will be given, additional information can be found in the case study report of the MARS project (Ferreira et al., 2016).

Thames catchment

The Thames River Basin (Figure 3a) is situated in the south east of the United Kingdom and covers an area of 9984 km2. It consists of a mixture of rural areas, primarily grassland, arable, and woodland in the east and south of the region, and urban areas, dominated by Greater London with a total population of about 15 million, but also including numerous other towns and cities. The Basin is underlain by two major bedrock aquifers, the Chalk of the Chilterns, Berkshire Downs and North Downs, and the Oolitic Limestones of the Cotswolds in the west of the Basin, separated by a series of aquitards (Bloomfield et al., 2009) (Figure 2b). Groundwater supplies from overlying Palaeogene to Holocene-aged Superficial Deposits are also locally important (Bricker and Bloomfield, 2014). The River Thames, the principal water course, has a length of about 235 km and a mean flow of 65.7 m^3s^{-1} down to the lowest gauge in the Basin at Teddington Lock, which also marks the non-tidal limit of the river. The mean annual rainfall varies across the Basin, but is typically in the range of 600 to 900 mm (Marsh and Hannaford, 2008), of which only ~250 mm pa is effective recharge (Environment Agency, 2014). The Baseflow Index (Gustard, 1992) at Teddington Lock is 0.64, but in sub-catchments that are dominated by the Chalk and Oolitic Limestone aquifers baseflow is typically in the range of 0.85 to 0.95, indicating a high dependence on groundwater.

As with other basins with both intensive agriculture and large urban populations, the Thames Basin is subject to a variety of stresses, these include: abstraction and artificial flow regulation; loading of organic pollutants (contributing to incidences of eutrophication) and faecal organisms from sources such as manure or sewage; loading from agricultural nutrients, primarily nitrate and phosphate, and loading from pesticides and other chemical pollutants, for example related to urban areas and the transport network, including a growing range of personal care products and hazardous substances (Environment Agency, 2015). There are 489 surface water bodies in the Basin. The Thames Basin River Management Plan (Environment Agency, 2015) states that pollution from waste water affects 45% of water bodies, from rural sources 27% of water bodies, and from towns, cities and transport 17% of water bodies; and changes in natural flows and levels affect 12% of water bodies. 47 of the water bodies are groundwater bodies, of which 22 have been assessed as having poor quantitative status and 18 have poor chemical status.

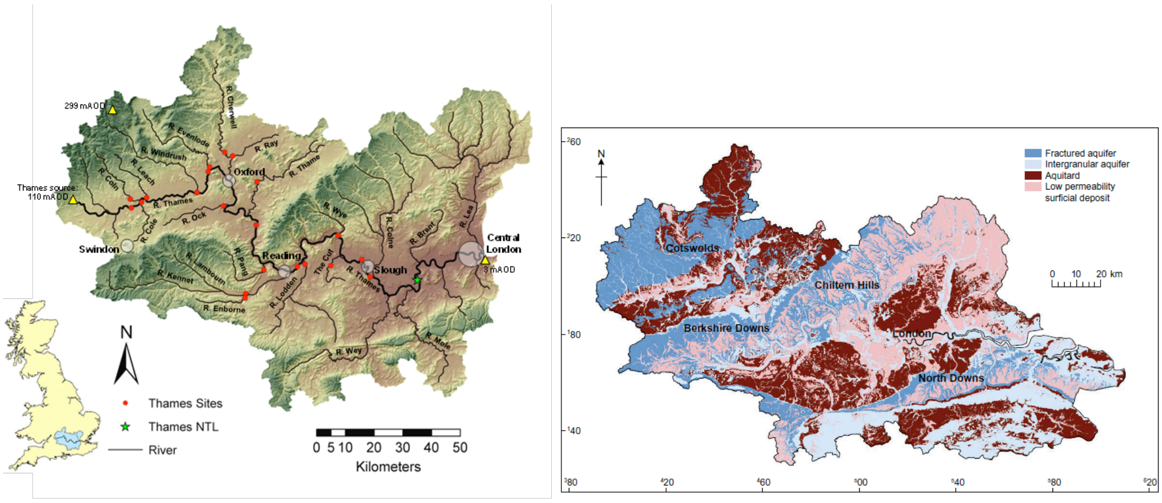

Figure 3. a. Thames Basin with main river channels and urban areas named, b. distribution of main hydrogeological units within the Basin.

Odense catchment

The Odense catchment, which drains into the Odense Fjord estuary, covers the central area of the island of Funen in Denmark (Figure 4). It has a catchment area of 1061 km^2 and is the largest among those draining into the fjord. Its climate is oceanic (warm temperate, fully humid, Kottek et al., 2006) with a mean annual temperature of around 9°C and mean annual precipitation of around 800 mm with no pronounced seasonality. The altitude in the catchment ranges from -9 to 125 m.a.s.l. and half of the landscape has surface slopes below 2%. The catchment geology consists of slightly undulating clayey moraine deposits with end moraines in the south (Smed, 1982). Aquifers constituted by sand and gravel deposits are also present (Troldborg et al., 2010) and sandy-loam soils are present throughout the catchment. The Odense River has a mean annual flow of 10.4 m^{3}s^{-1}, but experiences a marked seasonality, with higher streamflow in winter and spring and lower in summer and autumn. Land use in the catchment is dominated by agriculture (68%), followed by urban areas (16%) and forest (10%). Groundwater is a key resource in the basin, being the primary source of drinking water. The annual groundwater abstraction is ca. 20x10^6 m^3 (Naturstyrelsen, 2011).

The basin has undergone substantial hydrological and hydromorphological modifications, including channelization, damming and culverting of streams, draining of wetlands and conversion into agriculture, and subsurface drainage of agricultural soils (Larsen et al., 2008, Lu et al., 2015). The city Odense is located where the river flows into the estuary (Figure 4) and with 187,000 inhabitants is the main city in the catchment, which has a total population of 246,000. As a result, aquatic resources in the Odense Fjord catchment are under pressure, impaired due to excess nutrients, organic matter and toxic substances including pesticides derived from agriculture, urban and industrial sources, hydromorphological alterations of streams and groundwater abstraction (Naturstyrelsen, 2011). Among them, the main threat to the ecological status of the basin´s aquatic ecosystems is the nutrient loads from diffuse sources, i.e. agricultural areas. Many of the water bodies, including the estuary comprised by the Odense Fjord, do not meet the WFD criteria of good ecological status (Miljø- og Fødevareministeriet, 2016). Of the 600 km of streams only 36% have a good or high ecological status, and for the 17 lakes larger than 5 ha the corresponding number is 12% (Miljø- og Fødevareministeriet, 2016).

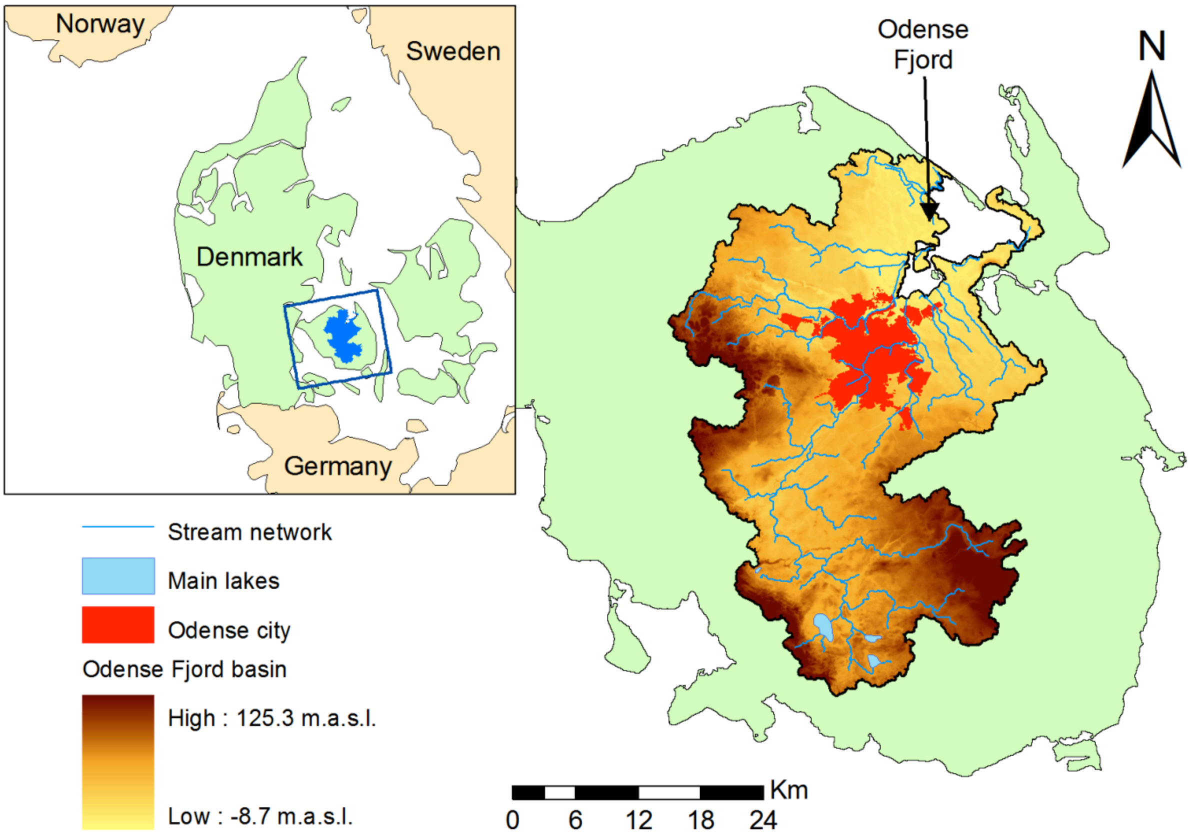

Figure 4. Location of the Odense Fjord catchment.

Regge & Dinkel catchments

The Regge and Dinkel catchments are two lowland catchments located in the east of the Netherlands (Figure 5). The rivers are managed by Waterboard Vechtstromen, classified as heavily modified and most surface waters in the catchments don't have good chemical or ecological states. Both rivers mouth in the river Vecht. The catchments have a temperate marine climate with a mean temperature of 9.9 °C, annual precipitation of about 800-850 mm and evaporation of 560-570 mm (KNMI, 2016).

The Regge has a catchment size of 340 km^2 and flows from about 70 m.a.s.l in the east towards 4 m.a.s.l in the northwest, over a length of 47 km. The western Regge catchment has sedimentary aquifers up to a depth of about 150 m with multiple clay layers in between. These aquifers wedge out towards the east, where they only reach a depth of 10 to 20 m below surface. The Regge is fed by multiple tributaries and has a mean discharge of 6.95 m^3s^{-1} at the catchment outlet.

The Dinkel springs in Germany, flows to the Netherlands and back to Germany over a total length of 92 km and a height difference of about 40 m. Its catchment area is 630 km^2 of which 230 km^2 is located in the Netherlands. In the Dutch part, the river flows from south to north from a height of about 35 to 20 m.a.s.l over a length of 46 km from the one to the other border. The Dutch part of the Dinkel catchment is characterised by a valley with sandy deposits located between clayey ice-pushed ridges which have shallow aquifers on top that feed several tributaries such as the Springendalse Beek and Elsbeek. Mean runoff of the Dinkel river is 5.5 m^3s^{-1} when it flow back into Germany.

Many land use changes and stream alterations took place in the Regge and Dinkel catchments in the 20th century (Hendriks et al., 2015b). An increase in agricultural land use led to higher manure use and hydrological alterations such as the installation of subsurface drainage systems. Population grew and led to urban development, increased groundwater abstractions and the installation of sewage systems. The catchments have several urban as well as forested areas but the main land use is agriculture (60 – 70%), for which the hydrology of the catchments is intensively managed using ditches, waterways, subsurface drains and weirs. Water is abstracted from both the surface and groundwater for irrigation and drinking water purpose. In addition, parts of the streams and rivers have been straitened, deepened and diverted for flood protection.

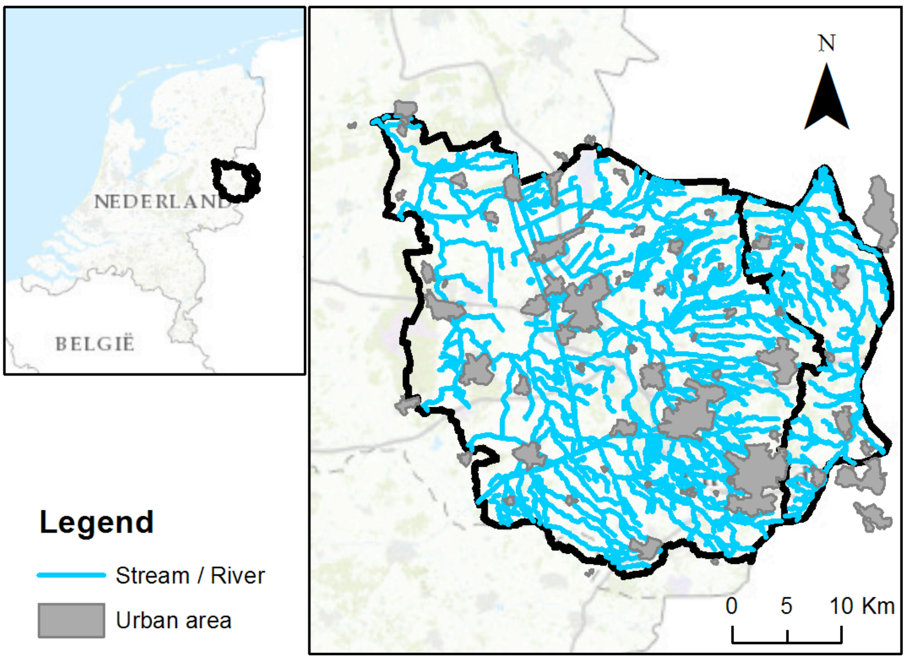

Figure 5. Location of the catchments on the Regge (left catchment) and the Dinkel (right catchment).

Review and results of groundwater using the Groundwater DPS framework

Thames catchment

There have been only been a few studies directly analysing the role of groundwater in the health of aquatic ecosystems in the Thames Basin. Consequently, below we review the nature of groundwater-surface water interactions in the Basin and evidence for stresses on aquatic ecosystem health through a series of proxies, namely groundwater influenced flows, quality and temperature.

Groundwater-surface water connectivity

The nature of groundwater surface water connectivity in the Thames Basin is highly scale dependent. Based on a classification system of groundwater–surface water interaction developed by Dahl et al. (2007), Bloomfield et al. (2009; 2011) and Darling et al. (2016) have shown that at the basin scale catchments in the Thames Basin broadly fall into the ‘landscape type’ category, a complexly interbedded sequence of aquifers and confining layers, where groundwater flow systems are principally influenced by factors related to regional geomorphology, hydrogeological setting and aquifer structure and heterogeneity rather than specific riparian zone processes. This is reflected in substantial variation in BFI of catchments within the Basin, typically in the range ~0.2 to ~0.95 (Bloomfield et al., 2009). At the catchment to sub-catchment scale, local hydrogeological processes dominate (Gooddy et al., 2006). Using chlorofluorocarbons (CFCs) and sulphur hexafluoride (SF6) to characterise groundwater movement and residence time in a lowland Chalk catchment within the Basin, Gooddy et al. (2006) showed that ‘piston’ flow dominates in the interfluves of Chalk catchments, with resulting average groundwater ages of several decades. In the valley bottoms, mixing between shallow groundwater and stream water occurs down to depths in excess of 10 m. At the river reach scale, Allen et al. (2010) further refined the conceptualisation of groundwater-surface water interactions to take into account the presence of river gravels and peats immediately beneath the River Lambourn, a Chalk stream in the west of the Basin. They documented a particularly complex system with the stream in hydraulic contact with streambed sediments and adjacent gravels and sands, and, depending on the local geometry of the Superficial Deposits, variably in hydraulic connection with the underlying Chalk contingent on the nature of the intervening Superficial Deposits.

Groundwater contamination – point pressure

Groundwater in the Thames Basin is susceptible to point source contamination from a variety of legacy activities. For example, ~40km^2 of the Chalk has been contaminated by bromate from a historic industrial site. The pollution has impacted a number of water supply sources in the region and represents the largest occurrence of point-source groundwater contamination in the UK (Cook et al., 2012). Dispersion of the bromate has been through 'double porosity' diffusive exchange and rapid advection through the solution-enhanced (sub-karstic) fracture network making remediation highly problematic.

Gooddy et al (2014) identified legacy landfills associated with peri-urban floodplains as a significant cause of pollution of groundwater and river bodies. Based on a case study of a historic landfill in Superficial Deposits (river gravels) above an aquitard, they investigated a peri-urban floodplain on the River Thames at Oxford. They found: high concentrations of ammonium emanating from the landfills; that there was no significant retardation of the ammonium by sorption to the aquifer sediments; and they estimated that over an 8 km reach of the river, which includes a number of other legacy landfills, 27.5 tonnes of ammonium may be supplied to the river annually (equivalent to up to 15% of the ammonium loading at the study site). They recommended that catchment management plans that cover peri-urban floodplains should take into account the likely risk to groundwater and surface water quality from such legacy landfills.

Stuart et al. (2014) characterised and contrasted sources of microorganic contaminants (also known as 'emerging contaminants') at two sites in the Thames Basin: one a Chalk sub-catchment on the River Lambourn previously used by Allen et al. (2010) and one at Oxford previously investigated by Gooddy et al. (2014). They found compounds at Oxford typical of a local waste tip plume (plasticisers and solvents but also barbiturates and N,N-diethyl-m-toluamide (DEET)) and of the urban area (plasticisers and mood-enhancing drugs such as carbamazepine). At the River Lambourn site the results were different. Agricultural pesticides, their metabolites and the solvent trichloroethene, as well as plasticisers, caffeine, butylated food additives, DEET, parabens and trace polyaromatic hydrocarbons (PAH) were all found. A significant finding was the relative absence or attenuation of many compounds in the Chalk hyporheic zone in the Lambourn catchment.

Groundwater contamination – diffuse pressure

Applying a Load Apportionment Modelling approach to sites across the Thames Basin, Bowes et al. (2014) identified phosphorous loading as being dominated by inputs from sewage treatment plants, particularly during the growing season, while all year round loading of nitrate was dominated by diffuse agricultural pollution and inputs from groundwater. In an analysis of nitrate in River Thames between 1868 and 2009, Howden et al. (2010) and Worrall et al. (2015) identified that the Basin had been saturated with respect to nitrate since the 1940s up until 1995, but that since then, due to memory effects within the catchment the relative contribution from groundwater to the river has been increasing in the Basin. The results of modelling of the fate of nitrate contributed by intensive farming within in the Basin (Howden et al., 2011) have been interpreted as showing that there is still a significant legacy of nitrate in the soil and groundwater compartments within the basin, and that restoration of nitrate concentrations in surface waters to levels similar to pre-intensification of farming 'would require massive basin-wide changes in land use and management that would compromise food security and take decades to be effective' (Howden et al., 2011). Wang et al. (2012; 2013) modelled the arrival of peak nitrate at the water table, and the quantity of nitrate remaining in storage in the unsaturated zone has also been modelled (Ascott et al., 2016) across the UK. Wang et al. (2012; 2013) and Ascott et al. (2016) identified large areas of the Thames Basin, primarily over the Chalk aquifer of the Chilterns and Berkshire Downs, where there remained both substantial quantities of nitrate in the unsaturated zone and that peak arrival of that nitrate is not yet due for at least another 50 years. In addition to the effect of existing nitrate loads in the Basin on groundwater and surface waters, Jackson et al. (2007) used a modified INCA-N model to account for Chalk unsaturated zone phenomena and modelled nitrate fate within the Lambourn catchment of the Basin. They demonstrated the high level of sensitivity of future nitrate concentrations in both groundwater and connected surface water in the catchment on current and future land use.

At the river reach scale there is evidence of hyporheic zone process that may locally affect nutrient fate. Pretty et al (2006) studied the nutrient dynamics of the hyporheic zone at two reaches on the River Lambourn. They found the river bed was predominantly aerobic, did not act as a major sink for nitrate, and that biogeochemical processing of nutrients is likely to be restricted to a thin, but biologically productive, layer in the shallow river bed sediments (Pretty et al., 2006). Recently, there has been interest in understanding the fate of Phosphorous (P) in groundwater within the Basin. Lapworth et al (2011) studied changes in the bioavailable phosphorus in the hyporheic zone on the River Lambourn. They found that the Chalk aquifer appears to provide some protection to surface water ecosystems by physiochemical removal of P where surface water flow is maintained by groundwater, although 'ecologically significant P concentrations (20–30 µg/L) are still present in the groundwater and are an important source of bioavailable P during baseflow conditions' (Lapworth et al., 2011).

Hydrological alteration

Water bodies within the Thames Basin have been assessed to be under 'severe water stress' (Environment Agency, 2014) and the balance between water supply and demand is in deficit (Wilby and Harris, 2006), making the Basin highly susceptible to natural climate variability and in particular to droughts (Folland et al., 2015). Major droughts have large spatial footprints, and most of the episodes of major droughts in England in the 20th century have affected the Thames Basin to some extent (Marsh et al., 2007; Bloomfield and Marchant, 2013). For example, as part of a study to develop an index of groundwater drought, Bloomfield and Marchant (2013) identified the groundwater droughts of 1976, 1990 to 1992, and 1995 to 1997 as being particularly notable within the Thames Basin, and Lange et al. (2017) describe how the impact of the 1976 drought was so severe that it led to restrictions in public water supply and to changes in regulation of national water resources. More broadly, the impacts within the Thames Basin of major droughts, such as the 1976 event, are: to reduced groundwater levels and streamflow – particularly in groundwater-fed headwaters; to lead to crop and grass stress and reduced agricultural production; and to contribute to fish kills associated with low oxygen levels in the streams (Lange et al., 2017). Westwood et al. (2006) investigated the effect of features of the hydrology and hydrogeology in groundwater-dominated, drought-affected Chalk streams in the Thames Basin on the health of the macrophyte communities they support. It was found that topography and channel morphology directly influenced the community structure at local level, and that abstraction and land use rates had an underlying effect at the catchment scale. They concluded that the optimal channel morphology to sustain species diversity and ensure resilience to drought was that of the 'the classic 'winterbourne' with its low-flow channel, extensive margins, gently sloping banks and high seasonal inundation' (Westwood et al., 2006).

In an attempt to better understand the natural climate variability in the region and of droughts and dry spells in particular, Folland et al. (2015) investigated the major drivers for dry winter half-years and hence of groundwater droughts, including forcing from the El Niño–Southern Oscillation (ENSO) and other teleconnections. No single driver was found to adequately explain major droughts in the historical record for the region. However, some evidence was found for a link between episodes of La Niña and reduced winter rainfall during some major episodes of drought.

There is some evidence that leakage from the aging water mains network may be significant and may be both modifying the surface water flow regime within the Basin and potentially effecting surface water quality. Bloomfield et al. (2009) modelled BFI in the Basin based on the underlying hydrogeology. They noted that while undertaking validation of the regression models some catchments had observed BFIs significantly higher than modelled BFIs and that the deviations between modelled and observed BFI scaled as a function of the urban area within the catchments. They inferred that this was due to additional baseflow from sewage discharge, mains leakage, and leakage from septic tanks. Subsequently, Gooddy et al. (2016) have shown that phosphate used to dose raw water supplies (to reduce lead and copper concentrations in drinking water) enters the environment via mains water leakage, bypasses wastewater treatments and enters the surface water systems. They estimate that phosphorous from this source could be equivalent to up to about 24% of the P load from sewage treatment works and up to about 16% of the load derived from agricultural non-point sources.

The River Basin Management Plan for the Thames Basin explicitly recognises that climate change needs to be taken into account in the Management Plan (Environment Agency, 2016). It notes that the latest projections for the UK show that temperatures will continue to rise, that there will be increased winter rainfall and that rainfall will be more intense, but it also notes that the "impact on river flows, water quality and ecosystems is less clear". For example, Wilby and Harris (2006) presented a framework for assessing uncertainties in climate change impacts on low flows for the River Thames and demonstrated that modelled low flows were most sensitive to uncertainty in the climate change scenarios and downscaling of different global climate models (GCMs) than to hydrological model structure. However, there have been a number of studies related to the Thames Basin that have attempted to reduce the uncertainty in predictions of climate change impacts on groundwater levels and river flows. Jackson et al. (2011) and Prudhomme et al. (2013) modelled changes in Chalk groundwater levels across the Thames to 2080s, based on medium–high emissions scenarios for thirteen GCMs. They found an average reduction in annual potential groundwater recharge of ~ 5%, although this was not statistically significant, but also noted a wide range in results from a 26% decrease to a 31% increase in recharge by the 2080s. Seasonal variation in the groundwater levels was predicted to significant, with increased rates of recharge during a reduced period of winter. It was also found that baseflow was likely to decrease by the 2080s, although there was considerable uncertainty in the model results.

House et al. (2016) modelled the impacts of climate change on groundwater dominated wetlands on the River Lambourn within the Basin. They modelled changes in precipitation, potential evapotranspiration, channel discharge and groundwater level based on UK Climate Projections 2009 (UKCP09) ensemble of climate models for the 2080s under a range of scenarios. A highly heterogeneous response over short distances across the wetland was seen depending on the nature of groundwater/surface-water interaction. Localised areas of groundwater upwelling were found to be most sensitive to climate change signals. The modelled groundwater levels were linked to the needs of the Cynosurus cristatus – Caltha palustris grassland (MG8) plant community and Desmoulin's whorl snail (Vertigo moulinsiana) for which the site is designated, highlighting the linked sensitivity of the hydrogeo-ecology of the wetland to climate change impacts.

Groundwater temperature

At the site scale, House et al. (2015) undertook a detailed investigation of the effect of groundwater on the species distribution of the wetland on the River Lambourn using ground thermometry. They found that anomalous warm zones (during winter monitoring) were associated with areas of groundwater upwelling, and a targeted vegetation survey identified the presence of *Carex paniculata* as an indicator of the areas of groundwater discharge. It was postulated that groundwater contains relatively high concentrations of nitrate as so was thought to support the growth of *Carex paniculata* against a background of poor fen and wetland communities located in reducing higher-phosphate waters. Garner et al. (2014) analysed the form and magnitude of stream temperature variations across England and Wales, including a site within the Thames Basin, and investigated the links between stream and air temperature. They found that the strongest links where groundwater contribution to runoff was smallest. The site on the Thames, at Caversham, was classified as being relatively warm with high seasonal variability, although given the variability in baseflow across the Basin (Bloomfield et al. (2009) it may be reasonable to expect significant variation in the influence of groundwater on stream temperatures. The biggest expected driver of change in groundwater temperature in the Thames Basin is that of anthropogenic climate change. However, as part of a national assessment of past changes and future prospects for climate change on water resources in the UK, Watts et al. (2015) note that there is very little reliable information on long-term changes in groundwater temperature and what longer-term data for groundwater that is available may be contaminated by ambient air temperatures.

Odense catchment

An adequate functioning of the aquatic ecosystems in the Odense Fjord catchment, which includes rivers, lakes and transitional waters, depends on keeping sufficient flow level and water quality, in which groundwater plays a vital role. In addition, groundwater might interact with pressures in the catchment. Despite this, studies analysing the role of groundwater in the preservation of the aquatic ecosystems in the Odense catchment are scarce.

Comprehensive hydrological and nutrient transport modelling has been carried out in the catchment within the framework of the MARS project (Molina-Navarro et al., 2017, in preparation). Results suggest that the average groundwater contribution to total flow was around 76% for the period 2001-2010. The Baseflow Index (BFI, baseflow volume divided by total volume) for the different sub-basins in the catchment varied between 0.63 and 0.92 (Figure 6), supporting the relevance of groundwater in the Odense Fjord catchment. Besides its absolute contribution, groundwater determines the streamflow regime. Figure 7 shows the monthly contribution of the groundwater flow component to total streamflow compared to the average monthly precipitation for the period 2001-2010 according to MARS modelling results. Results reveal that groundwater plays a key role especially in the drier seasons, providing water to ensure the sustainability of the aquatic ecosystems.

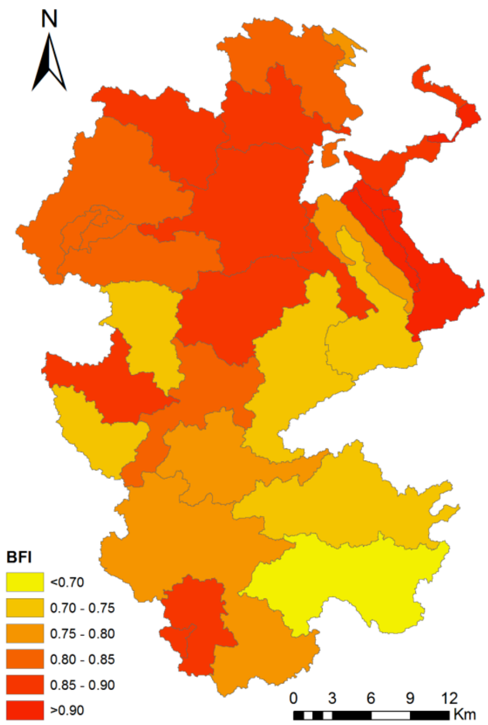

Figure 6. BFI values in the sub-basins modelled in the Odense Fjord Basin.

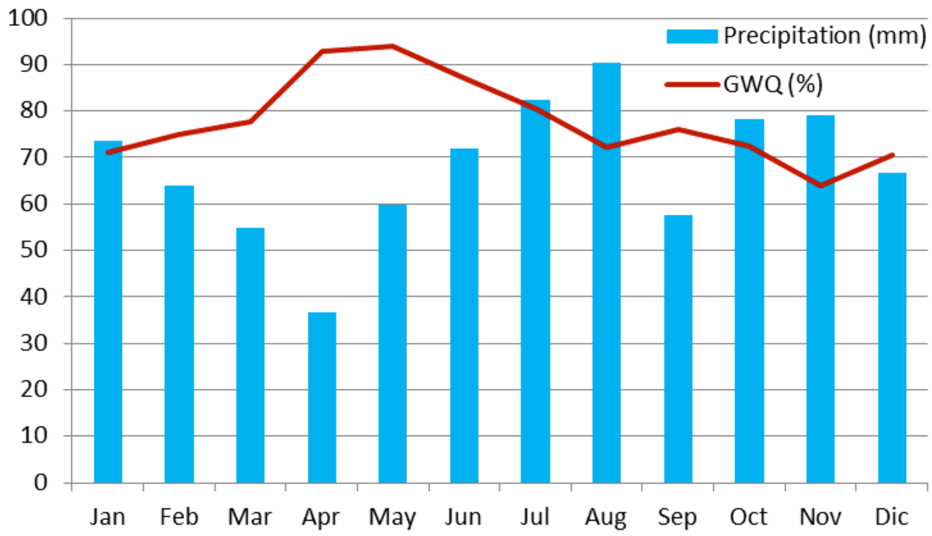

Figure 7. Monthly contribution of the groundwater flow component to total streamflow (GWQ, %) and monthly precipitation (mm) for the period 2001-2010.

Diffuse agricultural nutrient loss is one of the main threats to aquatic ecosystems in the Odense Fjord catchment (Miljø- og Fødevareministeriet, 2016; Molina-Navarro et al., 2017, in preparation), and agricultural activities are the main pollutant source of Danish groundwater (Blicher-Mathiesen et al., 2014). Several studies have addressed the nutrient problem in the catchment (e.g. Larsen et al. 2008, van der Keur et al. 2008), but they are mostly focused on nitrogen and the role of groundwater has been scarcely discussed.

Nitrate leached from the root zone can be reduced during its transport via groundwater both by microbial denitrification and pyrite oxidation denitrification which means that groundwater can attenuate N concentrations (Blicher-Mathiesen et al., 2014), which is strongly dependent on hydraulic residence time (Humborg et al., 2015). For 17 Danish catchments, Andersen et al. (2001) reported groundwater retention of N of 20-80% along a gradient with increasing retention time. Particularly, for the Odense Fjord catchment, Blicher-Mathiesen et al. (2014) found that nitrate reduction in aquifers and surface waters varied between less than 40% and up to 70-80% of the root zone N leaching. However, the specific role of groundwater was not analysed. The modelling work done in the catchment within the MARS project suggests, for the period 2001-2010, that 48.3% of the nitrate that percolates past bottom of the soil profile returns to the rivers via groundwater flow (9.38 vs 19.42 Kg N ha^{-1} yr^{-1}). In other words, simulated nitrate attenuation just by groundwater transport was around 51.7%. Nitrate is by far the largest N fraction being loaded into the Odense Fjord estuary (Molina-Navarro et al., 2017, in preparation), and it constitutes a big concern regarding the preservation of the marine ecosystem. The modelling work carried out confirms the relevance of nitrate reduction exclusively by groundwater, which had been previously pointed out, but scarcely supported by data.

Another major pressure for aquatic ecosystems in the Odense Fjord catchment is groundwater abstraction. Henriksen et al. (2008), by means of a comprehensive hydrological model, have evaluated several sustainability criteria in assessment of exploitable groundwater resource on regional and national scale for Denmark. These criteria were translated into four "sustainable groundwater abstraction" indicators, focused on avoiding significantly negative impacts on groundwater abstraction on both surface water ecology and groundwater quality, in line with the underlying WFD principles. For the Funen Island, of which the Odense basin is a major part, Henriksen et al. (2008) estimated a sustainable groundwater yield varying from 10 to 29 mm year^{-1}, although the lowest value was chosen for the assessment of sustainable abstraction. They also reported an actual abstraction of 12.8 mm year^{-1} (year 2000), which would mean slight over-exploitation. However, an additional evaluation at a sub-area level, including the Odense basin, showed that the over-exploitation was much higher, probably due to abstraction for the water supply of Odense city. Some of the plausible effects of abstracting groundwater in a non-sustainable manner include excessive streamflow depletion, consequently reducing the fraction available to supply aquatic ecosystems, or increased release of toxic solutes from aquifer sediments caused by lowered groundwater tables.

All these pressures ultimately affect the ability of the rivers in the catchment to achieve a good ecological status. A Danish national dataset comprising 263 variables and 131 observations has been used to evaluate the relationship between different river ecosystem stressors and ecological indicators, and to ultimately develop empirical methods to estimate ecological status indices based on those hydrological and water chemistry stressors (more information can be found in Ferreira et al., 2016). A first step was the correlation of the ecological status indices (Danish indices for fish fauna (DFFV), macrophytes (DVPI), macroinvertebrates (DVFI) and the Average Score per Taxon for macroinvertebrates (ASPT)) with the variables in the dataset, to pre-select those stressors that might be relevant for the development of the empirical models. Among those indicators found to be relevant, Q_{90} (the flow below the 90th percentile of the flow duration curve divided by the median flow) showed a positive correlation with all the indices (see Table 5.28, Ferreira et al., 2016). Q_{90} is strongly dependent on the groundwater contribution, and a value close to 0 means that the flows are much more extreme (lower) than if the Q_{90} is close to 1. Thus, groundwater contribution, which maintains the flow during dry seasons, is essential to keep high Q_{90} values. Modelling results yielded Q_{90} values between 0.03 and 0.39 in the Odense Fjord catchment (*Figure 8*), with an average of 0.17. This means that flows are extreme in this catchment, but they would be even more extreme without the existent groundwater contribution. According to correlation results, higher Q_{90} values might favour better ecological status, so groundwater might play a relevant role preventing even more extreme flows and providing better conditions that allow a higher ecological status. The Baseflow Index (BFI) also showed a positive correlation with three of the four indicators

(DFFV, DVFI and ASPT), revealing again the potential role of groundwater favouring a good ecological status.

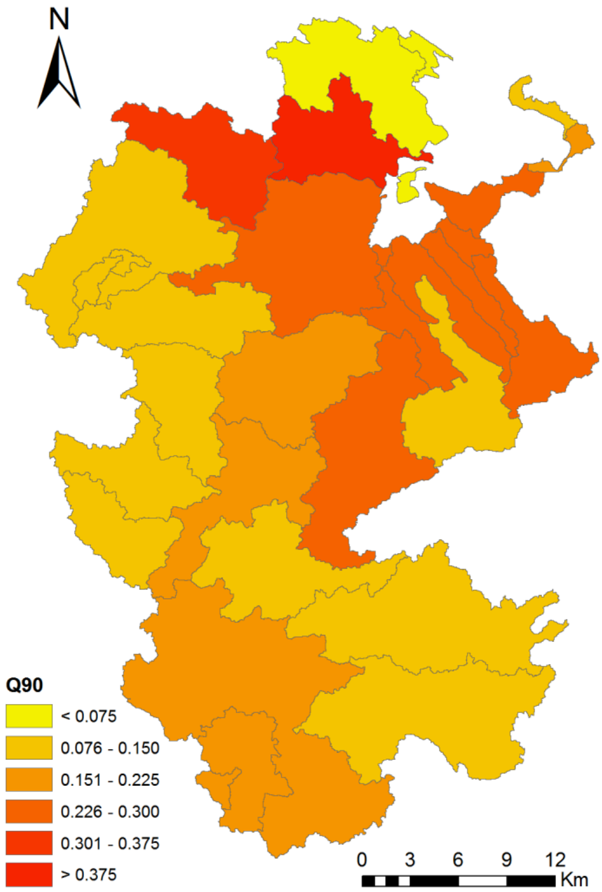

Figure 8. Q_{90} values in the sub-basins modelled in the Odense Fjord Basin.

However, the final development of the empirical models to estimate ecological status indices (see Ferreira et al., 2016) showed contradictory results for Q_{90}. Although it was included as a relevant stressor in all the equations, its influence was negative for fish and macrophytes, but positive for both macroinvertebrate indices:

- **DFFV_EQR** = $0.199 - 0.020 \cdot Q_{90} + 0.562 \cdot BFI - 0.041 \cdot BI5\_max + 0.018 \cdot Fre_{25}$
- **DVPI_EQR** = $0.887 - 0.004 \cdot drain\_tot - 0.025 \cdot Temp\_std - 0.009 \cdot dur_3 - 0.100 \cdot Q_{90}$
- **DVFI_EQR** = $0.40 + 0.085 \cdot Sin + 0.144 \cdot Q_{90} - 0.920 \cdot TP\_mean$
- **ASPT**(BInd12) = $4.54 + 0.261 \cdot Sin + 0.658 \cdot Q_{90} - 3.311 \cdot TP\_mean$

Nevertheless, for the fish indicator (DFFV), the Baseflow Index (BFI) exerted a positive influence with a much higher coefficient than Q_{90}, so it can be derived that groundwater plays a vital role and is related to a higher ecological status in terms of fish fauna. For the plant index (DVPI) the plausible effect of groundwater remains unclear from the equation. Q_{90} exerts a negative influence, but the standard deviation of the temperature (Temp_std) has a negative coefficient too, and a higher groundwater contribution would favour a lower Temp_std. Thus, regarding hydrological stressors affected by groundwater flow, the database analysis and empirical model development suggest that groundwater may play a significant role in favouring a good ecological status in at least three of the four indices studied. It must be acknowledged, though, that the study used a database from all around Denmark and not only data from the Odense Fjord catchment, although Denmark is a lowland country as a whole, and the correlations found are expected to be applicable to the study case.

The analysis also included water quality stressors. Total phosphorous (TP) was seen to be relevant in the estimation of ecological status through macroinvertebrate indices and, as expected, exerting a negative influence. Monitoring data revealed that mineral P represents slightly over half of the TP load in the Odense Fjord catchment, and the modelling suggested that the main source of mineral P in the catchment is groundwater (Molina-Navarro et al., in prep). Thus, for macroinvertebrates and based on the catchment model, groundwater has mixed effects on the ecological status of the rivers by providing a hydrological regime that favours a high status but on the contrary being an important source of TP.

It can be concluded that groundwater plays a vital role in the Odense Fjord catchment. Groundwater is by far the main component of streamflow and it ensures the sustainability of the aquatic ecosystems, especially in the drier seasons. However, this role is threatened by unsustainable groundwater abstraction. Groundwater flow also plays a key role in nitrate attenuation but, on the other hand, seems to be an important source of phosphorus in the catchment. Groundwater-related indicators have been seen relevant when predicting the ecological status in the catchment´s rivers through empirical modelling. It must be also noted that climate change impacts might modulate the role of groundwater in preserving the aquatic ecosystems in the catchment. Air temperature is expected to increase, up to 2.8°C for 2050, so the role of groundwater of mitigating summer temperature peaks will become crucial. Nevertheless, under high emissions, future scenarios also predict and increase of precipitation and consequently streamflow, which might occur mostly via groundwater flow (Molina-Navarro et al., 2017, in preparation).

Regge & Dinkel catchments

The Regge and Dinkel catchments have an extensive network of surface waters with stream systems that function in different ways. By comparing three tributaries of the Dinkel a good description can be given of these different systems; these tributaries are the Springendalse Beek, Roelinksbeek and Elsbeek. For these streams as well as for the Dinkel and Regge rivers, Table 2 shows the Baseflow Index (Gustard, 1992) which is often used as an indicator for the amount of groundwater in a stream. The discharges of both the Regge and Dinkel have a BFI of 0.6, which indicates a significant input of groundwater. Of the three tributaries, the Springendalse Beek has a high baseflow index of 0.8, while the other two have a value of 0.4. This indicates that groundwater is a more prominent factor in the discharge of the Springendalse Beek, which is also shown by the occurrence of many springs in the upstream area of the Springendalse Beek. Because the flow regime of a stream is an important habitat characteristic (Lytle and Poff, 2004; Arthington et al., 2006; Poff and Zimmerman, 2010), these differences in BFI indicate different habitats.

Table 2. Calculated Baseflow Indices and flow statistics (m3/s) for the Regge, Dinkel and three Dinkel tributaries.

River / Stream	BFI	Q95	Mean	Q5	Model Groundwater outflow route		
					Streams	Drainage	Overland flow
Regge	0.6	1.910	9.073	24.04	-	-	-
Dinkel	0.6	0.410	5.069	16.80	-	-	-
Springendalse Beek	0.8	0.016	0.043	0.090	78%	2%	20%
Elsbeek	0.4	0.000	0.104	0.363	85%	7%	9%
Roelinksbeek	0.4	0.000	0.091	0.335	84%	4%	12%

The groundwater discharge to the three Dinkel tributaries can be broken down into different flow routes using a groundwater model created by Kuijper et al. (2012). A distinction can be made between the amount of groundwater discharging through overland flow, subsurface drainage pipes and groundwater. Overland flow from the model is discharge that occurs when the groundwater table rises above the land surface. Table 2 shows the contribution of these routes for the three tributaries. The catchments with the highest agricultural activity, the Elsbeek and Roelinksbeek, have the highest amount of discharge from subsurface drains. Overland flow is highest in the Springendalse Beek due to the fact that many of the springs in the upstream part of this stream are created by local depressions where the groundwater table rises above the surface.

In the last century, land use and the hydrological system have changed significantly in the Regge and Dinkel catchments. Hendriks et al. (2015b) showed that as a result of these changes, groundwater levels in the Regge catchment went down until the 1980s. Figure 9 presents three time-series of groundwater heads of relatively deep wells in the Regge and Dinkel catchments (45 – 80 MBGS) and shows the decrease in groundwater heads between 1940 and 1980. At measuring point B280028 (Figure 9B), which is located in the ice pushed ridge of Ootmarsum, groundwater heads have gone down by approximately 3 meters during 1965 and 1980, which is caused by a drinking water abstraction well. Data at measuring location B29C0039 (Figure 9C) shows that groundwater levels went down by more than a meter between the 1950s and 1970s. At B28B0021 (Figure 9A) groundwater levels have slowly decreased by about 1 to 1.5 m. After the 1980s, the abstraction and drainage of groundwater remained at a stable level which led to a new equilibrium of the water balance of system. In this equilibrium, groundwater heads did not return to their 1940-1950 values but have remained stable.

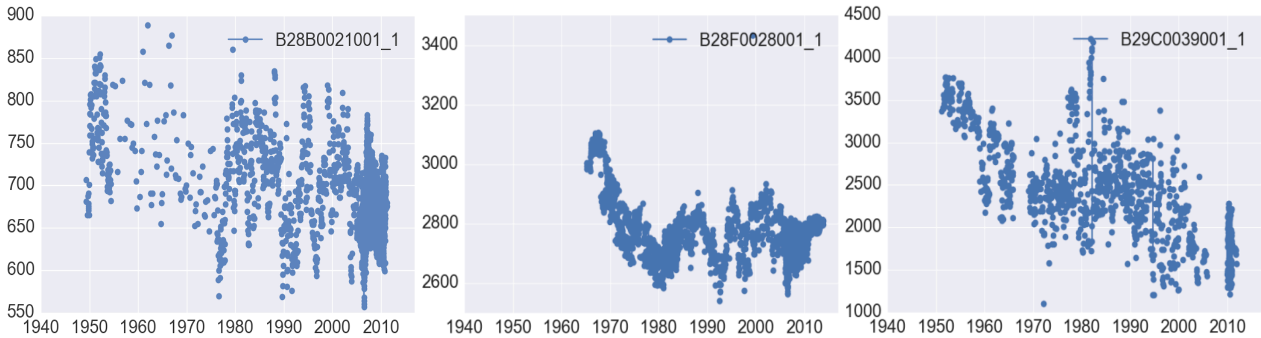

Figure 9. Time-series of groundwater heads [cm+NAP] in the Regge and Dinkel catchments: measuring points B28B0021 (filter 45 MBGS), B28F0028 (filter 60 MBGS) and B29C0039 (filter 80 MBGS).

The Regge and Dinkel catchments are sensitive to droughts. Based on the River Basin Management Plan certain streams are required to have year-round flow. However, during the 2003 drought many streams in the Regge and Dinkel catchment fell dry (Kuijper et al., 2012). One of the few exceptions was the Springendalse Beek, which kept flowing as a result of the high groundwater component of flow. Because groundwater pressures have been reduced since the 1940s, stream base flow has also decreased because groundwater input will have remained below the historical amount. A modelling study done by Hendriks et al. (2014) in the Hollandse Graven catchment, a tributary of the Dinkel, showed that the Q95 of the discharge has decreased by 16-30% due to subsurface drainage and 5-55% due to the abstraction of groundwater for irrigation. Kuijper et al. (2012) did a study on low river flows in the Regge and Dinkel catchments and found that groundwater input to many surface waters has been reduced by 20 to 50% as a result of groundwater abstractions and subsurface drainage. Additionally, they applied a climate scenario and calculated that summer discharge of many streams will further decrease by 25 – 30%.

The chemistry of groundwater influences surface water chemistry because groundwater and surface water are essentially one resource (Winter et al., 1998). A good example of this can be seen in the Springendalse Beek where downstream nitrate concentrations have gone up until the 1990s as result of agriculture (Figure 10A). After the Nitrate Directive was initiated in 1991, concentrations have slowly decreased again, although they are still higher than natural background levels. The concentration upstream in the Springendalse Beek shows a different trend than downstream: nitrate concentrations have not decreased (Figure 10B). This is caused by the nitrate concentration of the groundwater, which is shown in Figure 10C. Due to the long travel time of the groundwater, these concentrations have not gone down, even though the local nature organisation has even bought the agricultural fields in the surrounding area and converted them into natural fields (Nijboer et al., 2003). This case displays how historic pollution can cause long term pollution of the surface water due to groundwater residence times. Ironically, due to the high influence of groundwater and its positive effect on discharge and temperature, the upstream stream stretch actually has a high natural value and is a habitat of for instance the brook lamprey (*Lampetra planeri*) (Verdonschot et al., 2002). The groundwater downstream contains less nitrate causing the nutrient concentration to decrease in the downstream direction.

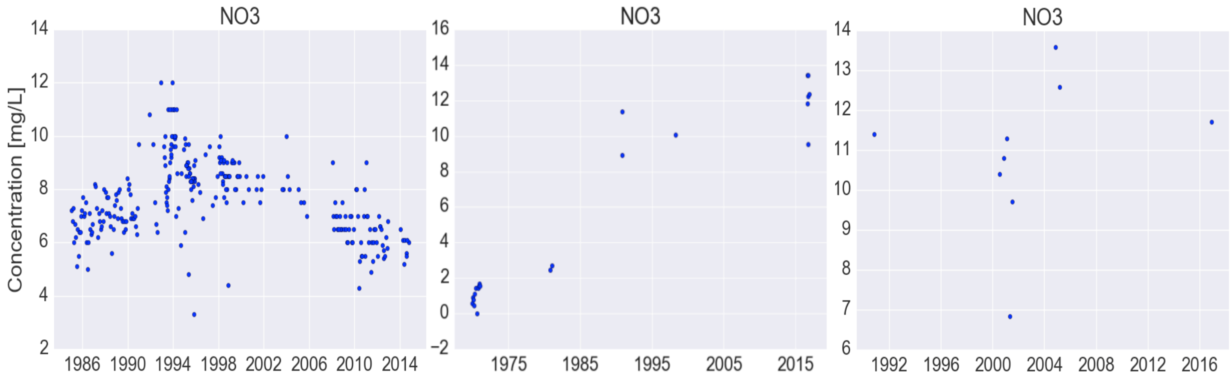

Figure 10. NO3-N concentrations downstream (A) and upstream (B) in the Springendalse Beek and in the groundwater upstream (C).

Figure 11 shows the daily average temperature of the Springendalse Beek and the Elsbeek during the summer of 2016. The measurements show that the Springendalse Beek has a more stable temperature, less temperature variation during the summer and lower temperature peaks, which are all caused by its higher input of recent groundwater. Table 3 shows the average monthly temperature for August and November both upstream and downstream of a ~500 m river stretch of the Springendalse Beek and Elsbeek. During summer, the difference in temperature between the up- and downstream of the Springendalse Beek is almost 2 °C while for the Elsbeek this difference is only $2/10^{th}$ of a degree. On the contrary the difference between up- and downstream is larger in the Springendalse Beek during a cold month. This is the result of the fact that the upstream part of the Springendalse Beek is highly influenced by groundwater and because the groundwater temperature has little variation throughout the year. Due to its stable temperature regime, the Springendalse Beek contains cold-stenothermic species (Verdonschot et al., 2002). The stream stretch of the Springendalse Beek is located closer to its source than the Elsbeek stretch, which means that the groundwater component is larger. The influence of groundwater generally decreases in the downstream direction because of influences at the surface such as the air temperature and because the mass of water in a stream increases in the downstream direction, so that the input of groundwater is relatively smaller. This does not mean that groundwater doesn't have an influence on downstream and larger water bodies, but this only becomes clear on spots with high local input of groundwater; diffuse over larger areas or within the hyporheic zone. The difference in stream temperature between a warm and cold month is also a characteristic of the temperature regime and influenced by groundwater. The average downstream temperatures between August and November differ about 5 degrees for the Springendalse Beek and 10 degrees for the Elsbeek, again showing the buffering temperature effect of groundwater in the Springendalse Beek.

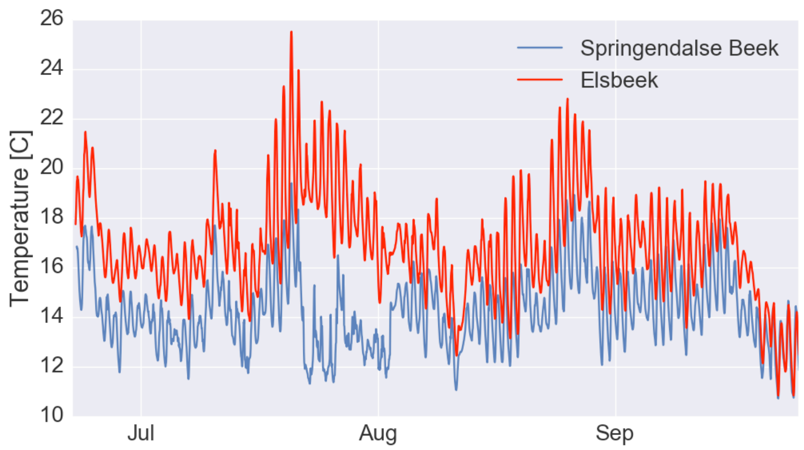

Figure 11. Average daily temperatures of the downstream parts of the Springendalse Beek and Elsbeek during the summer of 2016.

Table 3. Average temperatures [°C] of two Dinkel tributaries in August and November 2016

Stream	Average August Temperature			Average November Temperature			Difference Aug-Nov downstream
	Upstream	Downstream	Difference up-down	Upstream	Downstream	Difference up-down	
Springendal	12.45	14.41	1.96	9.86	9.69	-0.17	4.72
Elsbeek	16.59	16.81	0.22	6.90	6.86	-0.04	9.95

Climate change is causing increased air temperatures which will lead to increased stream and groundwater temperatures. Currently summer temperature peaks are mitigated by groundwater influence and stenothermic are able to reside in specific streams such as the Springendalse Beek. With rising temperatures, the capacity of the groundwater to decrease summer temperatures will no longer be sufficient to sustain these habitats, especially if flows simultaneously decrease due to climate change, as is expected in the Regge and Dinkel catchments (Kuijper et al., 2012).

The changes in land use and water management in the last century in the Regge and Dinkel catchments (Hendriks et al, 2015b) led to decreased groundwater levels, changed groundwater flow paths and altered groundwater inputs to the surface waters (Kuijper et al., 2012; Hendriks et al., 2014). This affected flow, chemistry and temperature regimes of surface waters and altered habitats.

Pollutants are slowly transported by the groundwater and historical input of fertilizers is now a source of nutrients to surface waters via the groundwater because of its long travel time. Decreased groundwater seepage directly affects flow but also changes the thermal regime of streams. Changes in flow regimes might not always be apparent from discharge measurements but this does not mean that groundwater flow paths have not been changed. Changed flow paths could lead to seepage of water of different quality and temperature. For instance, groundwater seepage from subsurface drains is different from deep groundwater seepage (Rozemeijer et al., 2010), so alterations in groundwater flow paths also change in-stream habitat conditions.

Discussion and Conclusions

Groundwater is an important component of flow in Europe's lowland catchments, and varies between scales: from upstream to downstream, and from basin to river stretch scale. Especially during summer, the input of groundwater prevents streams from falling dry. Often used metrics to indicate the relative contribution of groundwater are the BFI and low flow parameters such as Q_{90}. Groundwater feeds streams via different flow paths, which each have their own travel time, meaning that time scales are also important in catchments influenced by groundwater. The baseflow from groundwater is often crucial for the survival of specific ecology, but has been changed by anthropogenic influences such as the installation of subsurface drainage and initiation of groundwater abstractions, which changed groundwater levels and flow paths in all four studied catchments.

The catchments are under additional stress from high nitrate concentrations, caused by intense agricultural land use. Even though denitrification in groundwater attenuates nitrate concentrations, groundwater has been identified as the main transport path for nitrate. Additionally, a large part of the current nitrate output by groundwater is a remainder of historical land use, transported by long groundwater flow paths. This has important implications for water managers as the effect of catchment measures to reduce nitrate input can take multiple decades to show due to the continuous input of historic N to the surface water. This is for instance the case in the Springendalse Beek in the Dinkel catchment. In the Thames catchment too, the amount of nitrate in the unsaturated zone and groundwater is at such a level that the nitrate concentration in certain streams is expected to further increase during the coming decades, despite any future measures. This proposes a problem to water managers who have to deal with restoring such catchments as a good chemical and ecological status cannot be achieved with excessive nutrient levels. As these levels cannot be decreased on the short term, taking measures in such catchments is discouraged. However, evidence from the upstream part of the Springendalse Beek suggests that even with high nutrient levels, the ecological value of such stream can be high. This is also the case in steams in the south of the Netherlands where data seems to indicate that nitrate levels actually show some correlation with a high ecological status (Waterboard Limburg, unpublished), possibly because nitrate in these catchments is an indicator for groundwater influence.

Although in general the main source of phosphorous in the four catchments is effluence from waste water treatment plants, significant amounts of P can also be transported by groundwater. In fact, in the Odense catchment modelling suggested that the main source of mineral P was transport by groundwater, and in urban areas in the Thames catchment evidence indicates that phosphorous enters the groundwater from leaking water mains network and is subsequently transported to the surface water. Phosphorous is often related to surface runoff processes, but these examples show that groundwater should also be taken into account in managing catchments with phosphorous stress. Additionally, groundwater is important in transporting other (historic) pollution from amongst others industry and landfills, as was shown for multiple locations in the Thames catchment.

Groundwater is important in governing stream and river temperature by providing an input with a relative constant seasonal temperature. This way, it dampens summer temperature peaks of the surface water, like in for instance the Springendalse Beek. In addition, on locations with significant groundwater seepage, specific temperature habitats are formed as was shown for e.g. the wetlands in the river Lambourn catchment. Climate change is an important potential stressor on surface water temperatures. Although forecasts are uncertain and differ locally, temperatures are generally expected to increase leading to the disappearance of temperature habitats. Although the water temperature at locations with significant groundwater input will have a limited temperature increase, these habitats will also tip over a threshold if temperatures keep increasing. In addition to temperature increases, baseflow is expected to locally decrease, although much uncertainty exists.

This paper started with the premise that groundwater is not yet taken into account enough by Europe’s water managers. Freshwater ecosystems are under multiple-stress and groundwater has a crucial effect on many of these ecosystems. Using examples from literature and from four different European catchments, it was shown which effect groundwater has on surface waters. Groundwater provides a certain amount of water at a certain time and is crucial during low summer flows. It provides water with a specific quality, sometimes providing clean water and sometimes water containing historic pollutants. Groundwater provides water with a specific temperature, which is much more stable than the seasonal temperature of surface waters. Because of this, groundwater is important in the formation of thermal habitats for e.g. stenothermic species, and in providing thermal refugia during warm periods. In addition to influencing flow, quality and temperature, groundwater functions as an important connection between a catchment and its surface waters. Groundwater has essential implications for river basin management and this study thus supports the call to water managers made in the FP7 REFORM project to take groundwater into account in river basin management (Hendriks et al., 2015a). A framework was proposed which shows how groundwater in lowland catchments acts as a bridge between stressors and their effects within surface waters. This framework shows water managers how their management areas might be influenced by groundwater, and helps them include this important, but often overlooked part of the water cycle in their basin management plans.

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CO	Confidential, only for members of the consortium (including the Commission Services)	

Content

Introduction

The MARS project (Managing Aquatic ecosystems and water Resources under multiple Stress) supports water managers and policy makers at the water body, river basin and European scales in the implementation of the Water Framework Directive (WFD) (Hering *et al.,* 2015). It aims to address how a complex mix of stressors, for example resulting from urban and agricultural land use, water power generation and climate change, impacts European rivers, lakes, groundwater and estuaries, and what implications these stressor combinations have for ecological services, such as water provision (Ferreira *et al.,* 2016).

One of the three scales of investigation within the MARS project, includes the study of 16 river basins throughout Europe, which have been investigated to characterise relationships between multiple stressors and ecological responses, functions and ecosystem services (Ferreira *et al.,* 2016). Results of these studies are being synthesized and jointly analysed in the context of stressor combinations, climate scenarios and water and catchment management responses (including restoration scenarios), and how these can affect the ecological status, ecological functions and ecosystem services.

To assess the complex multi-stressor scenarios, it is necessary to test and improve existing modelling techniques including process-based models and empirical/statistical models; and to up-scale and generalize the results of the case studies, and contribute with these improved models to guide River Basin Management Plans (RBMP) and the best Programs of Measures (PoM) to achieve a good ecological status (Ferreira *et al.,* 2016).

The modelling process follows the MARS conceptual modelling framework that can provide a holistic approach to modelling multi-stressors across different scales. This approach manages to combine the use of a risk assessment framework, a DPSIR scheme and the ecosystem service cascade in a joint modelling framework that enables to investigate the impacts of multiple stressors on biotic/abiotic state and on ecosystem services (Ferreira *et al.,* 2016; Feld *et al.,* 2016).

Within MARS the climate change scenarios were based upon GFDL-ESM2M and IPSL-CMA-LR climate models, which were used for generating precipitation and temperature scenarios for two climate scenarios (RCP 4.5, RCP 8.5), available for period of 2006-2099. The period of 2006-2015 was used in general as the reference period and monthly linear correction was applied to observed climate data and scenarios outputs covering this period (for details, see Ferreira *et al.,* 2016).

In addition, three different storylines were developed for future land use scenarios. In each basin, these storylines were downscaled according to specific characteristics of land use and programs of measures to be undertaken for the purpose of achieving a better ecological status. The storylines were:

- Storyline 1 – 'Techno world' (economic focus): In this scenario, economic growth is the main focus. Higher economic growth accompanied increase in energy demands and resources which brings agricultural expansion. However, environmental measures will be applied to mitigate human disturbance.

- Storyline 2 – 'Consensus' (green focus): In this scenario, economic growth is as it is today. More efforts put in to promote sustainable use of sources. A lot of effort applied to promote conservation and to restore degraded ecosystems.
- Storyline 3 – 'Fragmented world' (Survival of the fittest): In this scenario, there is an increase in economic development, in some cases also considering a big economic crisis (recession). There is no room almost for environmental issues.

In order to study this, we used one of the case studies, which is the Nervión-Ibaizabal catchment (hereafter Nervión), especially focusing in the estuary, as a transitional system. Here, we have investigated: (i) the links between pressures, recovery of the system and provision of cultural ecosystem services (i.e. recreational fishing and bathing waters); (ii) the future climate change scenarios for these services; and (iii) the management implications.

Methods

Site description

The Nervión system is located in the Basque region on the north of the Spanish coast (Figure 1). This mountainous catchment, with an area of 1,755 km^2, drains into a 'Heavily Modified Water Body' (a morphologically modified Atlantic estuary, with an important commercial harbour) affected by urban, organic and metal discharges, which have decreased dramatically in recent years (Borja *et al.,* 2016; Cajaraville *et al.,* 2016). The water sources are located around 850 m height and the length of the river is 72 km. The geology of the catchment is mainly limestone.

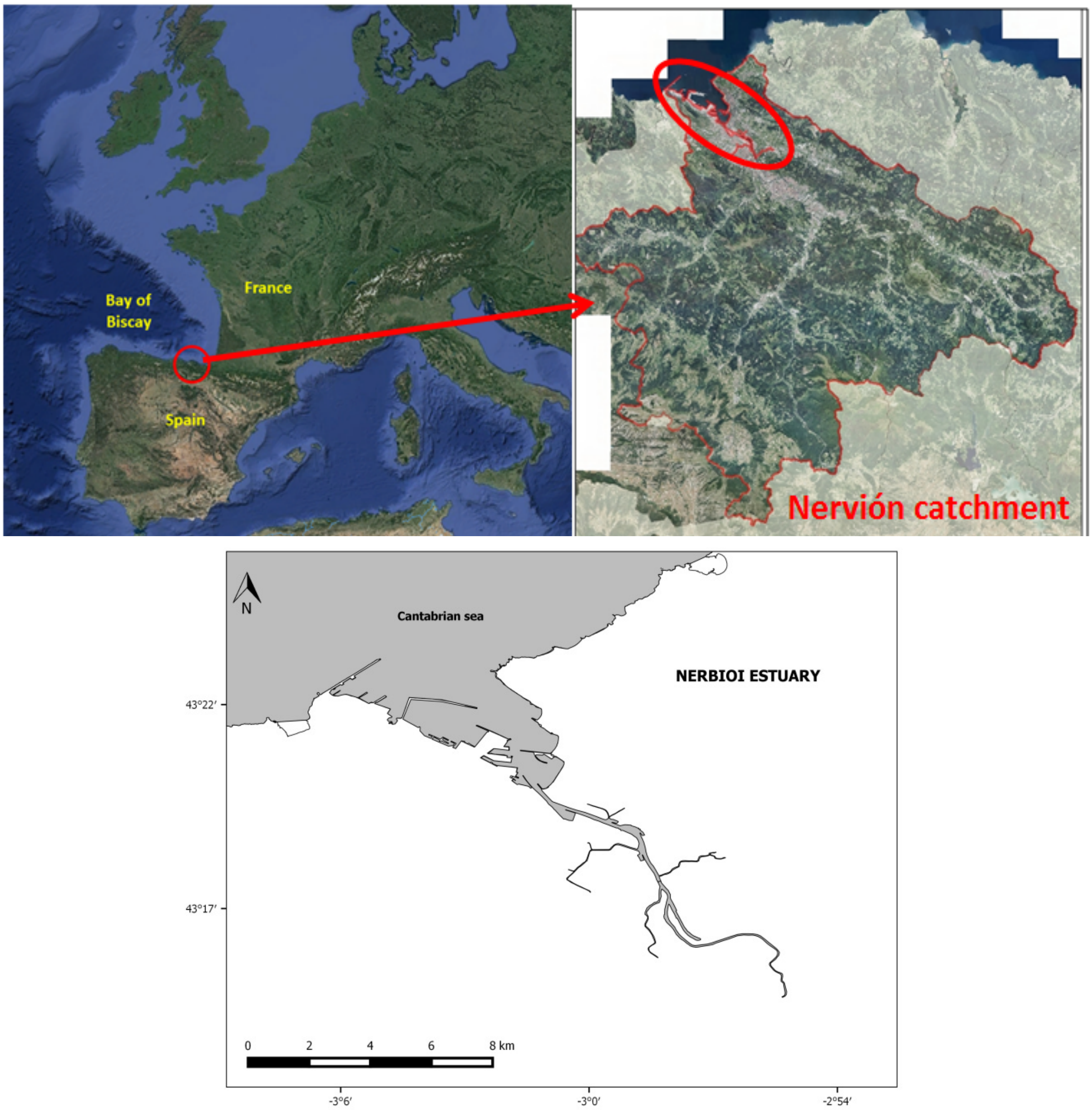

Figure 1. Study site: Nervión catchment in the Basque Country (North of Spain), including the Nervión estuary.

The natural features of the Nervión river and estuary have been dramatically modified by urban, industrial and port settlements (Cearreta *et al.,* 2000, 2004). The Nervión estuary is a mesotidal estuary, 15 km in length, placed on the north Spanish coast, close to the city of Bilbao. It receives fresh water from the Nervión River with a mean flow of 25-30 m^3s^{-1} (Borja *et al.,* 2006a). Over the last 150 years the Nervión has received wastes from different sources (i.e. urban effluents, industrial wastes, mineral sluicing), and suffered many morphological pressures (Cearreta *et al.,* 2004). In fact, the original estuary was rapidly reduced in size through land reclamation, to form a tidal channel (average dimensions: 100 m wide; <10 m deep), completed by 1885. The estuary was isolated by dyking from its original intertidal areas to allow a navigable watercourse from the city of Bilbao to the open sea, having lost around 1,000 ha of intertidal surface.

The system was severely degraded until the 1990's, showing low oxygen concentrations (even anoxia), high concentrations of bacteria and contaminants (metals and organic compounds), and disappearance of fauna (e.g., most of the estuary was azoic until mid-1990's) (Borja *et al.,* 2006b). It is classified as Heavily Modified Water Body (Borja *et al.,* 2006a). Then, a Water Treatment Plan was approved in 1979. The water treatment began in 1991 with physico-chemical treatment; the biological treatment was later introduced, coming into operation in 2001. It serves a total population of ~ 1 million people (García-Barcina *et al.,* 2006).

Available data

The estuary has been monitored since 1989 by the "Consorcio de Aguas Bilbao-Bizkaia" (García-Barcina *et al.,* 2006; Borja *et al.,* 2006b), with many physico-chemical and biological variables available (Table 1). Since 1995, the Basque Water Agency has also monitored the river and estuary, to assess the ecological status within the Water Framework Directive (Borja *et al.,* 2016). As such, additional physico-chemical variables and data on phytoplankton, macroalgae, macroinvertebrates and fishes are available (Table 1) in different sampling locations (Figure 2).

The Nervión river runoff has been monitored since 1995 by "Diputación de Bizkaia" through a hydrological station. These runoff data were used in the present study (Table 1). Other seven minor rivers that drain into the estuary were also considered, namely: Kadagua, Asua, Castaños, Gobela, Granada, Ballonti and Udondo. The runoff data for these small rivers were obtained from a simple lineal regression of mean daily runoff data, obtained from the implementation of the TETIS hydrological model in the Nervión catchment applied by the "Basque Water Agency".

Pollutant loads from the eight above mentioned rivers and the Galindo urban waste water treatment plant (UWWTP) were also used in the present study (Table 1). Sea surface temperature (SST) data series from period 1946-2015 recorded at a point close to the Nervión estuary (Aquarium of Donostia-San Sebastian) was used in the present study. Furthermore, solar radiation data from the Climate Forecast System Reanalysis (CFSR) grid was used. The local solar radiation data series for period 2013-2015 was extracted from the CFSR grid.

Table 1. Variables, periods and sources used in this study

Parameter / Variable	Format	Period Time Step	Unit	Sources
Climate				
Sea Surface Temperature	Time-series	1946-2015 - hourly	°C	Aquarium Donostia - San Sebastián
Precipitation	Grid	1992 –2015 hourly	mm	CFSR
Wind	Grid	1992 – 2015 hourly	$m\ s^{-1}$	CFSR
Solar radiation	Grid	1992 - 2015 hourly	$W\ m^{-2}$	CFSR
Air temperature	Grid	1992 – 2015 hourly	ºC	CFSR
Relative humidity	Grid	1992 – 2015 hourly	fraction	CFSR
Long wave radiation	Grid	1992 – 2015 hourly	$W\ m^{-2}$	CFSR
Estuary				
Temperature	Time series	1990-2015 monthly	º+C	Water Consortium (2) Monitoring Network (3)
Salinity	Time series	1990-2015 monthly		Water Consortium (2) Monitoring Network (3)
Dissolved oxygen	Time series	1990-2015 monthly	$mg\ l^{-1}$	Water Consortium (2)
Oxygen saturation	Time series	1990-2015 monthly	% saturation	Water Consortium (2)
pH	Time series	1990-2014 monthly		Water Consortium (2)
Organic carbon	Time series	1990-2014 monthly	$mg\ l^{-1}$	Water Consortium (2)
Nutrients – NH_4	Time series	1990-2015 monthly	$mmol\ m^{-3}$	Water Consortium (2)
Nutrients – NO_3+NO_2	Time series	1995-2015 trimestral	$mmol\ m^{-3}$	Monitoring Network (3)
Nutrients – Silicate	Time series	1995-2015 trimestral	$mmol\ m^{-3}$	Monitoring Network (3)
Nutrients – Phosphate	Time series	1995-2015 trimestral	$mmol\ m^{-3}$	Monitoring Network (3)
Total P	Time series	1995-2015 trimestral	$mg\ l^{-1}$	Monitoring Network (3)
Total N	Time series	1995-2015 trimestral	$mg\ l^{-1}$	Monitoring Network (3)
N/P Ratio	Time series	1995-2015 trimestral		Monitoring Network (3)
Organic matter (sediment)	Time series		%	Monitoring Network (3)
Bacteria (coliforms)	Time series	1993-2014 monthly	$UFC\ 100ml^{-1}$	Water Consortium (2)
Chlorophyll a	Time series	1999-2015 monthly	$\mu g\ l^{-1}$	Water Consortium (2) Monitoring Network (3)
Phytoplankton diversity	Time series	2002-2015 monthly (March-September)	$Bit\ cel^{-1}$	Water Consortium (2)
Secchi depth	Time series	1999-2014 monthly	m	Water Consortium (2)
Suspended solids	Time series	1995-2015 trimestral	$mg\ l^{-1}$	Monitoring Network (3)
Turbidity	Time series	1995-2015 trimestral	NTU	Monitoring Network (3)
Benthic invertebrates - abundance	Time series	1990-2015 yearly	$ind\ m^{-2}$	Water Consortium (2)
Benthic invertebrates - richness	Time series	1990-2015 yearly	N spp	Water Consortium (2)
Demersal fishes – abundance	Time series	1989-2015 yearly	$ind\ Ha^{-1}$	Water Consortium (2)
Demersal fishes - richness	Time series	1989-2015 yearly	N spp	Water Consortium (2)
Demersal fishes – AFI value	Time series	1989-2015 yearly		Water Consortium (2)
River				
Nervión-Ibaizabal runoff	Time series	1995 - 2015 – daily	$m^3\ s^{-1}$	Dip. Foral Bizkaia (1)
Faecal Coliforms loads	Time-series	1990-2015 yearly	$MPN\ m^{-3}$	Water Consortium (2)
Nutrients – NH_4 loads	Time-series	1990-2015 yearly	$t\ yr^{-1}$	Water Consortium (2)
Galindo UWWTP				
Faecal Coliforms loads	Time-series	1990-2015 yearly	$MPN\ m^{-3}$	Water Consortium (2)
Nutrients – NH_4 loads	Time-series	1990-2015 yearly	$t\ yr^{-1}$	Water Consortium (2)

(1) Red Meteorológica de Bizkaia, Departamento de Medio Ambiente de la Diputación Foral de Bizkaia; (2) Consorcio de Aguas Bilbao Bizkaia, EDAR de Galindo; (3) Basque Littoral Monitoring Network.

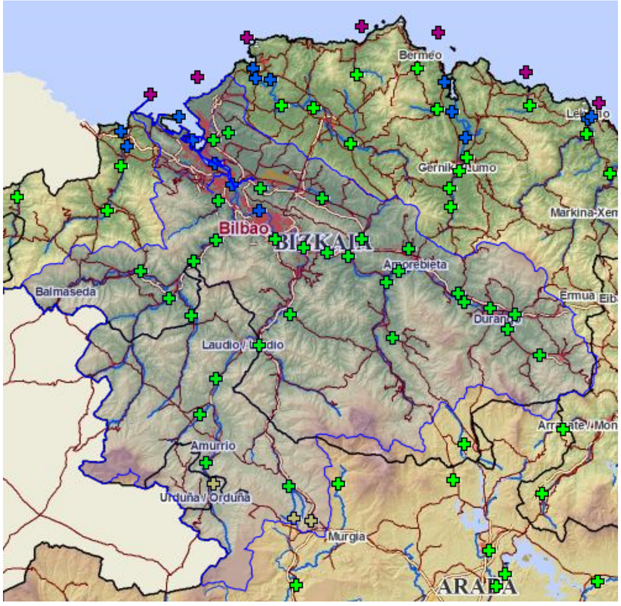

Figure 2. Sampling locations within the Nervión catchment (shaded area), as obtained from the Basque Water Agency. Green: river stations, blue: estuarine stations, red: coastal stations.

Conceptual MARS model for the Nervión estuary

The DPSIR (Driver-Pressure-State-Impact-Response) model for the Nervión estuary has been harmonized within the context of MARS Project. This model includes drivers, pressures and state variables both abiotic and biotic, together with the relationship of these variables with ecosystem services and Management measures (Figure 3).

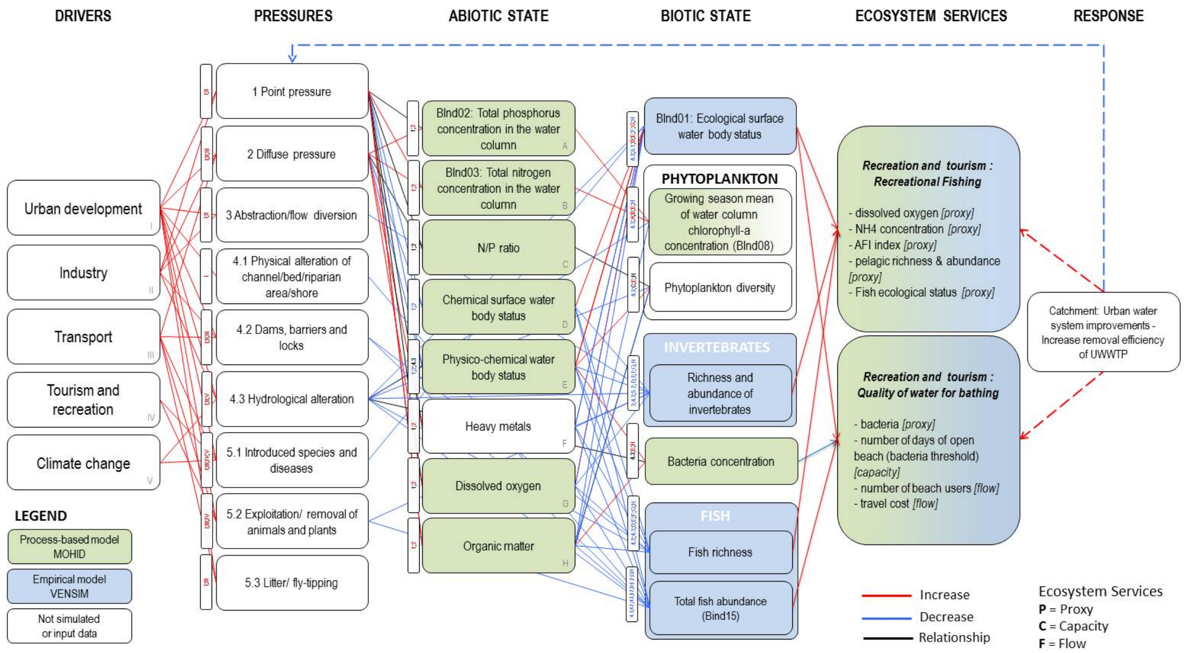

Figure 3. DPSIR model for the Nervión case study, including the two ecosystem services selected: bathing water quality and recreational fishing.

This model considers that impacts in biotic and abiotic variables can lead to changes in the provision of ecosystem services. For this purpose, it is important to well understand the changes produced in the Nervión estuary which may have affected the ecosystem services in the area.

The estuarine conditions began to improve in 1991, with the progressive reduction of the pressures (i.e., closure of polluting industries and the beginning of the WWTP) that allowed the progressive recovery, both spatial and temporally, as reflected on the biotic and abiotic data monitored for the last 25 years. In short, the improvement of abiotic conditions (e.g., increase in oxygen saturation, reduction in ammonia concentration, etc.) allowed the posterior recovery of biological parameters (benthic invertebrates, biodiversity, etc.). However, little is known about the influence that the ecological amelioration had in the provisioning of cultural ecosystem services.

This study focuses on the effect that the pressure reduction had in the ecological recovery and the provision of cultural ecosystem services at Nervión estuary. Specifically, this study will investigate the impact of the ecological recovery in two recreational activities, i.e. recreational fishing and bathing waters.

Fishing is considered recreational when "fishers do not sell the fish they catch", when it "is not undertaken for predominantly subsistence purposes" and when it "is not undertaken for primarily cultural or heritage purposes"; usually, it is performed by catching fish on hooks, but "may include the use of small boats, the capture of fish by divers with spear guns and hand-gathering of shellfish from the beach or shore" (Pawson et al., 2008). The activity in Nervión is regulated by the act 198/2000 for the *Maritime recreational fishing regulation of the Basque Country* (Basque Government, 2000).

In turn, bathing waters in Europe are regulated by the Bathing Waters Directive (2006/7/EC). This directive is focused in maintaining and improving microbial water quality of EU´s bathing waters (Quilliam *et al.,* 2015). Within Nervión estuary, there are three beaches (Las Arenas, Ereaga and Arrigunaga) and all of them located in the right bank at the outer estuary. The three beaches are recognized bathing waters, although Las Arenas is considered as at high risk of suffering from microbial pollution (Solaun *et al.,* 2016).

Modelling strategy

To create a numerical tool capable of simulate the DPSIR model, two modelling systems are being implemented: MOHID-Water and VENSIM® software. Figures 4 and 5 show the conceptual model of the modelling systems for the ecosystem services bathing waters and recreational fishing respectively.

For the link between the pressures and the ecosystem service bathing waters, MOHID-Water model has been implemented to simulate the hydrodynamics and dispersion of the indicator bacteria *Escherichia coli* in Nervión estuary. The concentrations of *Escherichia coli* in the three bathing waters of the estuary (namely Las Arenas beach, Arrigunaga beach and Ereaga beach, Figure 6) were extracted from the implemented model.

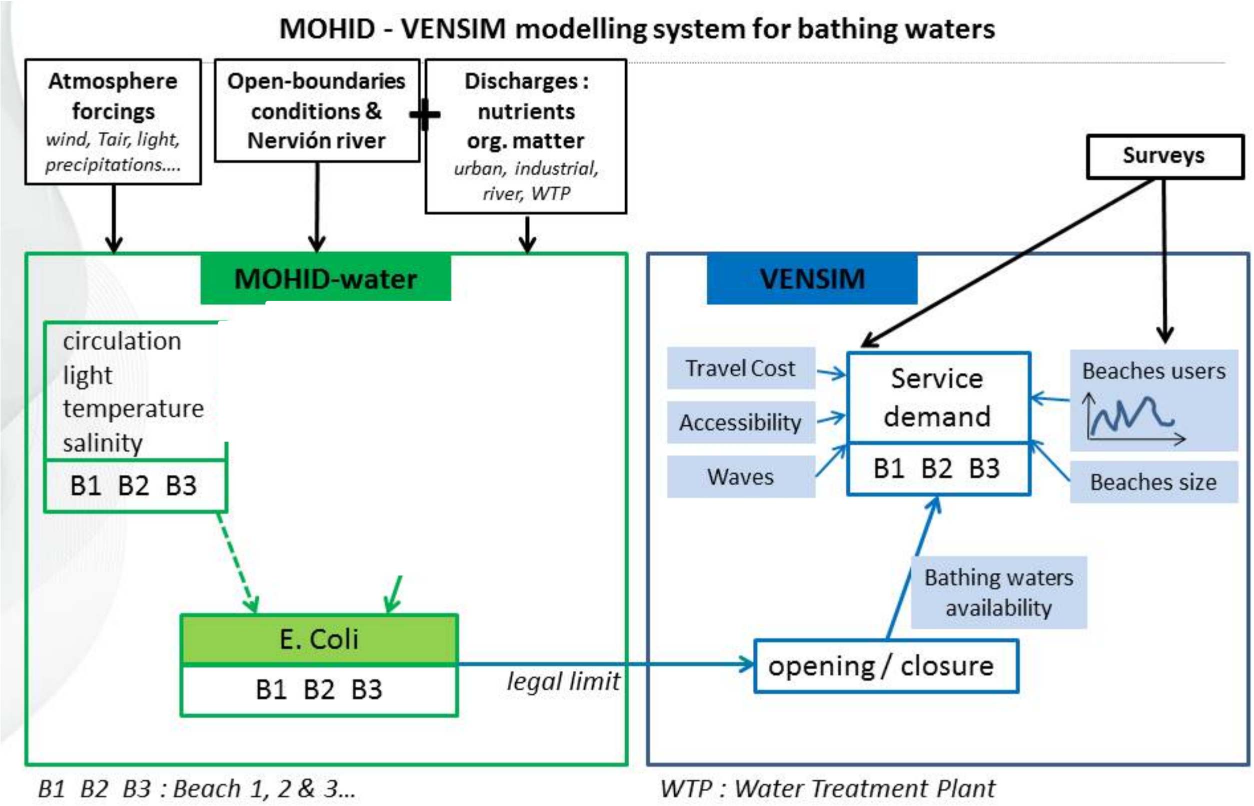

Figure 4. Conceptual model for the ecosystem service bathing waters in Las Arenas (B1), Arrigunaga (B2) and Ereaga (B3) beaches in the Nervión estuary.

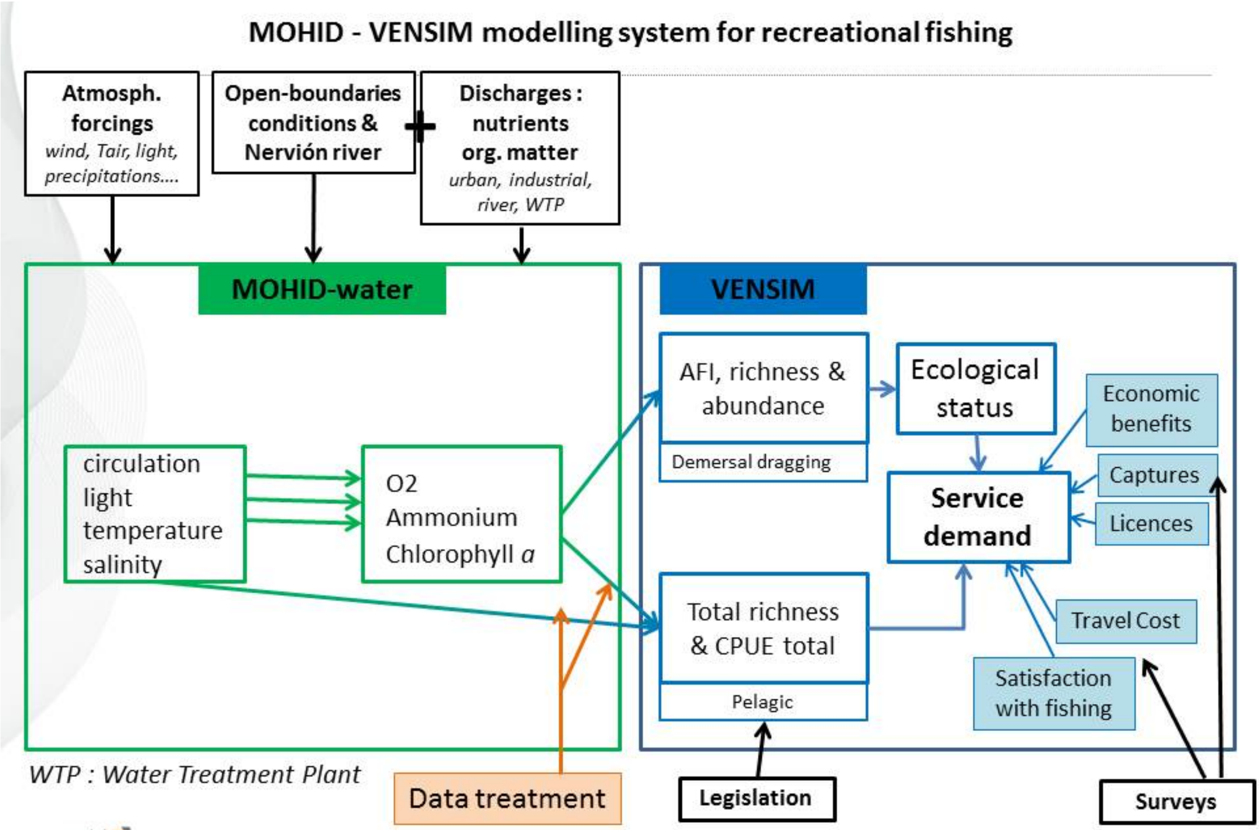

Figure 5. Conceptual model for the ecosystem service recreational fishing in the Nervión estuary.

Before the implementation of MOHID-Water, loads of faecal coliforms concentrations (FC) into the estuary were converted into *Escherichia coli* concentrations (*E. coli*) according to the following regression relationship:

Log_{10} FC = 0.968 Log_{10} E. coli + 0.376 R^2=0.81 (Marta Revilla, personal communication)

Furthermore, the algorithm used to simulate *E. coli* decay in MOHID-Water was the one proposed by Canteras *et al.* (1995). Mean *E. coli* concentrations during the bathing season, at the three bathing waters (Figure 6), were extracted from the implemented MOHID-Water model. The bathing season period considered was from the 1st of June to the 30th of September. The number of days during the bathing water season where the value of *E. coli* concentration was higher than 500 MPN 100 ml^{-1} was computed; this was the criterion used to define the quality of the bathing waters as an ecosystem service. Other criteria will be added after the analysis of the interviews made to the local beach users. This analysis is under development now.

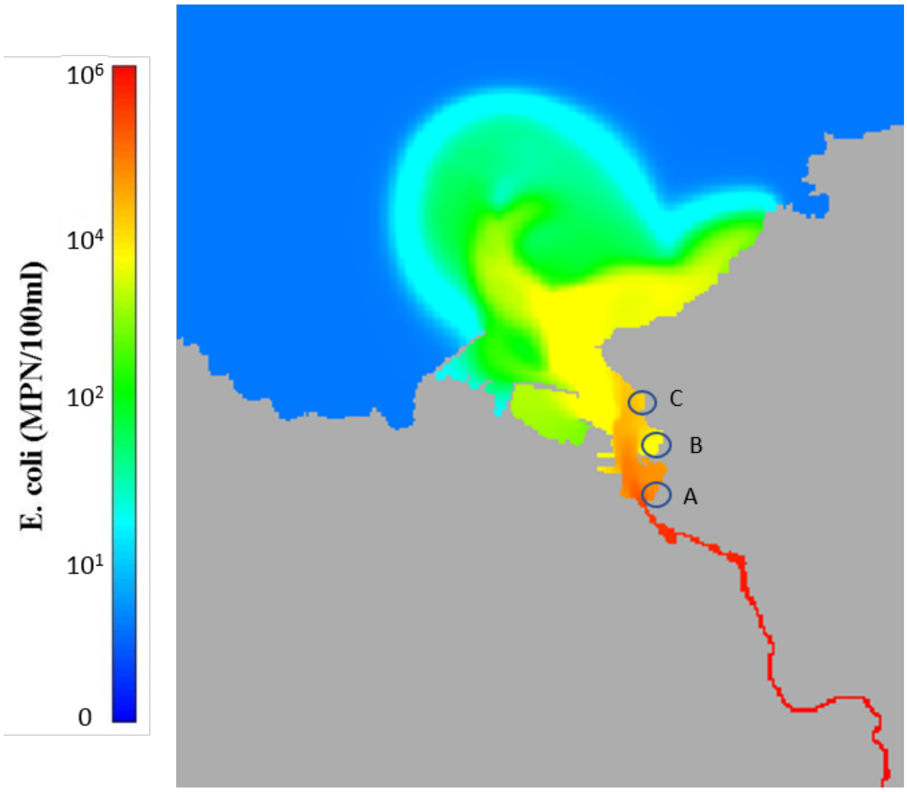

Figure 6. Location of the recreational beaches at Nervión estuary: A. Las Arenas; B. Ereaga; C. Arrigunaga. The colour scale refers to *E. coli* concentrations obtained in a simulation test performed with the implemented MOHID-Water model configuration.

In order to test the reliability of the implemented MOHID-Water model configuration, observed and simulated values of salinity were compared. The salt content is an indicator of the mixing between fresh and marine waters, and its flux and transport can be used to infer the transport of other conservative scalar variables (D'Aquino *et al.,* 2010). The bias and the vertical root mean square error (RMSE) computed between simulated salinities and the field measurements (Table 1) were –0.25 and 1.7 respectively, indicating good reliability of simulated values.

For the link between the pressures and the recreational fishing ecosystem service, the biogeochemical module of MOHID-Water will be implemented to obtain values of dissolved oxygen, ammonium and chlorophyll a in the estuary (or other important variables identified after the data analysis described below).

The output of the MOHID-Water model will be used to construct a system dynamic model using VENSIM® software. System dynamics can be used for non-linear and complex problems, involving delays, stock and flow relationships and realistic decision-making, among others (Sterman, 1987). In this study, VENSIM model construction will allow to introduce socio-economic variables that affect the provisioning of the two cultural ecosystem services (i.e. bathing waters and recreational fishing).

Linking pressures and ecosystem services

In order to explain the effect of environmental variables on the possibility of practicing recreational fishing, a Generalized Linear Model (GLM) was built up in R (R Core Team, 2015). For undertaking this, the MARS empirical modelling framework following the "Cookbook on data analysis" (Task 4.1) was used.

The abundance of demersal fishes was selected as the best available estimate for recreational fishing, and therefore used as the dependent variable in the GLM. The explanatory variables considered were: water temperature (at the bottom), salinity (at the bottom), turbidity, suspended particulate matter, total P, total N, N/P ratio, ammonia concentration (at the bottom), oxygen saturation (at the bottom), chlorophyll-*a* concentration, organic matter in the sediment, richness and abundance of benthic invertebrates and phytoplankton diversity.

Demersal fishes are sampled once a year at the end of summer-beginning of autumn in five different areas of the estuary (see Uriarte and Borja (2009) for more details on the sampling procedure). Thus, the estuary was divided in five different stretches in accordance with the sampling stations of demersal fish surveys (Figure 7). The explanatory variables were aggregated accordingly, to have a single mean value per stretch and per year.

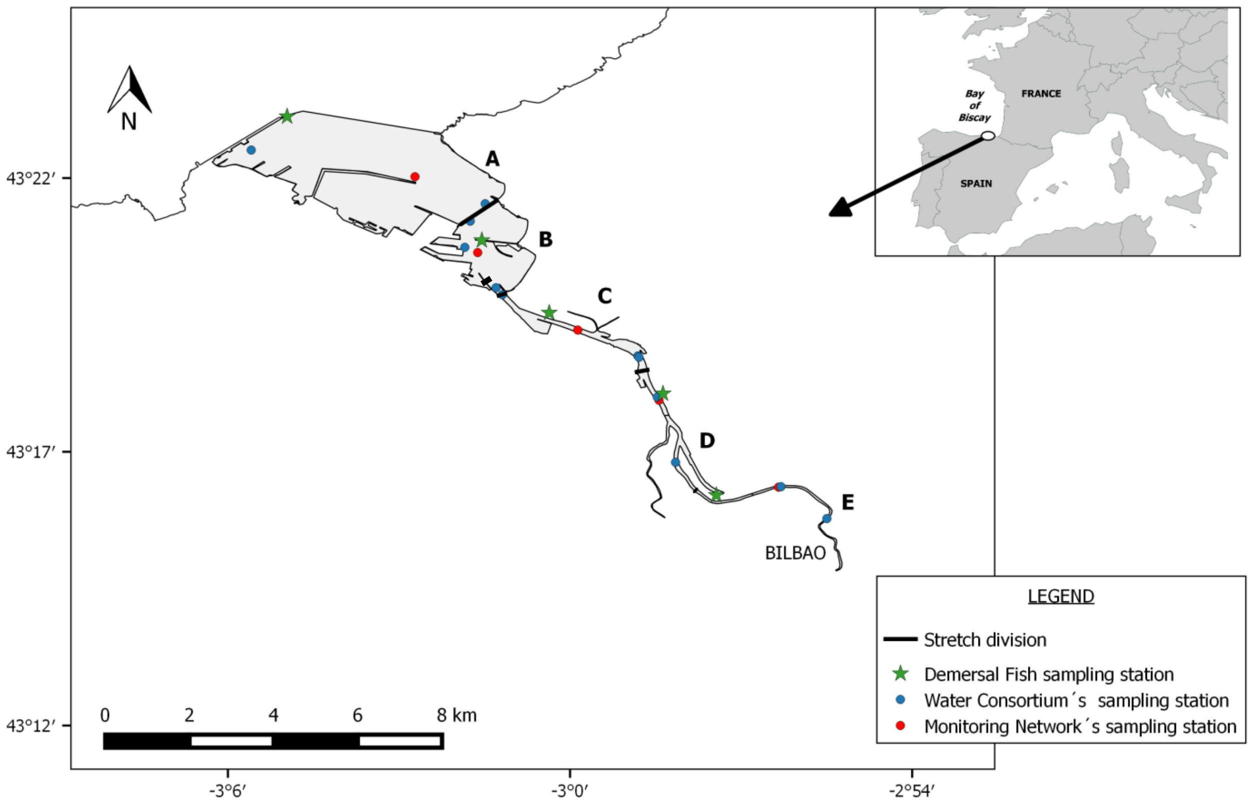

Figure 7. Location of Nervión estuary within the Bay of Biscay, stretch division and sampling stations used in this research.

To select the best model with the lowest number of independent variables, we followed the next approach:

1. Considering demersal fish abundance as the dependent variable, for each of the 14 environmental variables, simple linear regression models were built.
2. Environmental variables with statistically significant relationship (p-value<0.05) in the simple linear regression models were selected for performing (i) a correlation matrix and (ii) variance inflation factor (VIF). These two techniques are useful to ensure against multicollinearity among the independent variables (Zuur *et al.,* 2010). In the correlation matrix, if any environmental variables were highly correlated between them, the variable with the lowest number of data available or the one with the lowest r^2 value in the simple linear regression model was discarded. In the case of the VIF, when any independent variable´s VIF value exceeded 5, it was removed and the analysis was run again; this process was repeated until all the independent variables had a VIF<5.
3. Two GLMs were constructed using as independent variables the selected environmental variables from (i) the correlation matrix and (ii) the VIF analysis. Demersal fish abundance was the dependent variable in the two GLMs. *Dredge* function of 'MuMIn' R-package was used to generate a set of models with different combinations of the environmental variables (Barton, 2016). The models with the lowest AICc value (Akaike Information Criterion) corrected for small-sample sizes) were selected, as the lower the AICc the better the model fit (Burnham and Anderson, 2002).

On the other hand, to capture ecosystem service users´ behaviour and perceptions on recreational fishing, a questionnaire was designed and distributed to current recreational fishers who practice fishing inside the estuary. The questionnaire comprised 29 questions aiming at answering the following questions:

- *Which are the main fishing habits in the estuary? Are there any differences between the different areas of the estuary?* To capture the possible differences among estuarine areas, the estuary was divided in five stretches (see Figure 7), in accordance with the sampling points of the demersal fish monitoring programmes. For each of the five stretches, respondents indicated whether they have ever fished there and if so, when was the first time they did. For the stretches where fishers fish nowadays, they indicated the fishing methods they practiced there, which were the most frequently caught species when they began fishing in that stretch and which are now.
- *Which is the opinion of the recreational fishers about the current situation of the fishing activity?* Respondents had to give their opinion about the current conditions of 20 social and environmental characteristics in the estuary that potentially could be influencing fisher´s enjoyment and satisfaction with the fishing activity.

- *Have recreational fishers perceived changes in estuarine conditions?* Respondents had to indicate if they perceived changes in three characteristics (i.e. abundance of catches, species variety of catches, and water quality); the results were compared with changes in recorded related data (i.e. changes in demersal fish abundance, demersal fish richness and oxygen saturation).
- *Did recreational fisher´s satisfaction with the fishing activity change?* Respondents indicated if their personal satisfaction with recreational fishing activity in the estuary increased, decreased or if remained equal. Also, they answered if they would continue to fish in the estuary.

All the questionnaires were introduced in a database and statistically analysed using R (R Core Team, 2015).

Climate change scenarios and MARS storyline selected

Firstly, time projections of sea surface temperature from Combal (2014), for climate scenarios RCP 4.5 and RCP 8.5, were used. SST scenarios were available for decades 2010, 2020, 2030, 2040 and 2050. Secondly, runoff projections for year 2025 and 2050 obtained from WP2 were also used. The period of 2004-2014 was used as the reference period and monthly linear correction was applied to the observed local SST and runoff data. Factors derived from linear correction were applied to projected SST and runoff data series. Two distinct years were chosen for future scenario runs: 2025 and 2050.

Three different storylines were developed for future scenarios where the removal efficiency of the Galindo UWWTP was chosen as an element of change:

- In this Baseline scenario, the removal efficiency of the UWWTP is 70%. We assume the values for ammonia and faecal coliforms discharges of year 2014 (see Figures 8 and 9).
- In the Storyline 1, there is still a discharge from the UWWTP to the estuary. Nonetheless, there is an investment to improve the efficiency of the UWWTP, although the increase in population (Figure 10) limits this improvement. Therefore, we assume that the efficiency of the UWWTP increase 10% by 2050 in this storyline.
- In the Storyline 2, there is a big concern about the environmental consequences of the UWWTP in the estuary. Therefore, the efficiency increase 30% by 2050 (i.e. 100% removal efficiency).
- In the Storyline 3, there is no change in efficiency of the UWWTP, although the increase in population (Figure 10).

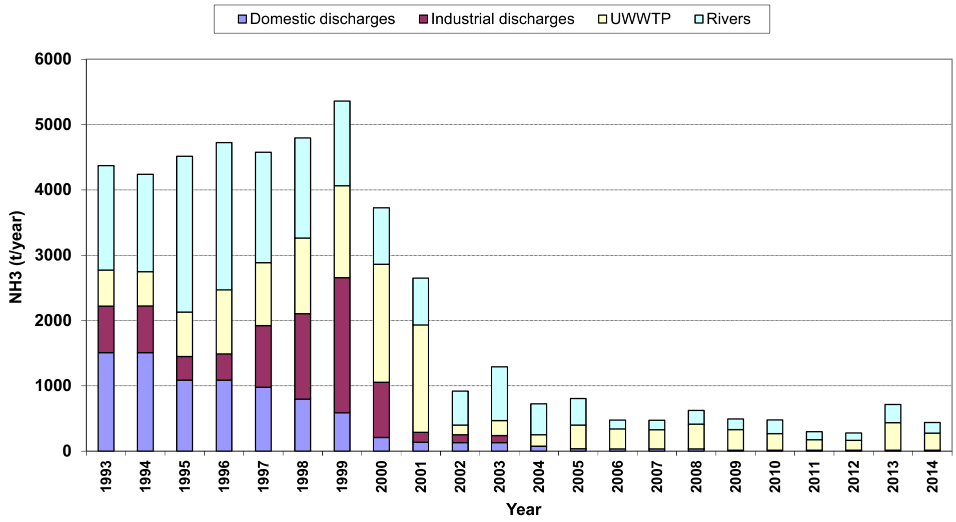

Figure 8. Evolution of annual NH_4 discharge in the Nervión estuary (source: Consorcio de Aguas Bilbao-Bizkaia).

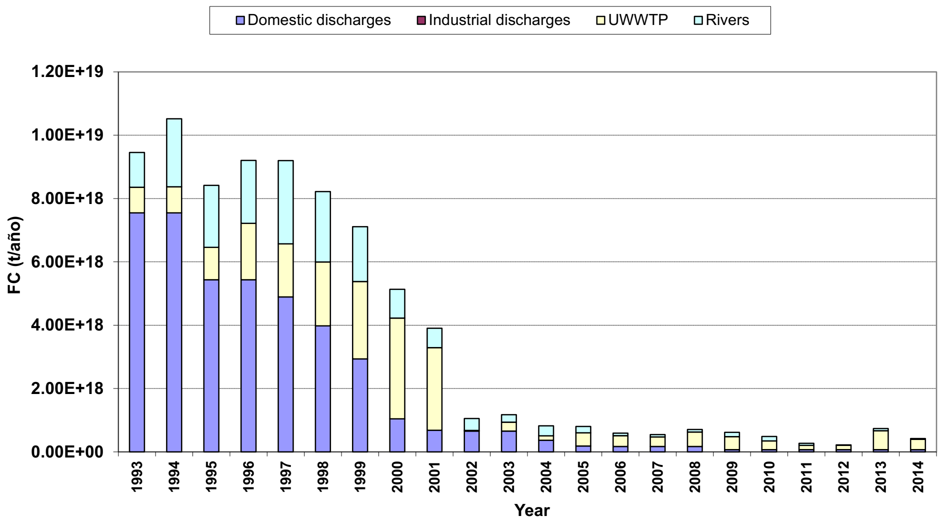

Figure 9. Evolution of annual faecal coliforms discharge in the Nervión estuary (source: Consorcio de Aguas Bilbao-Bizkaia).

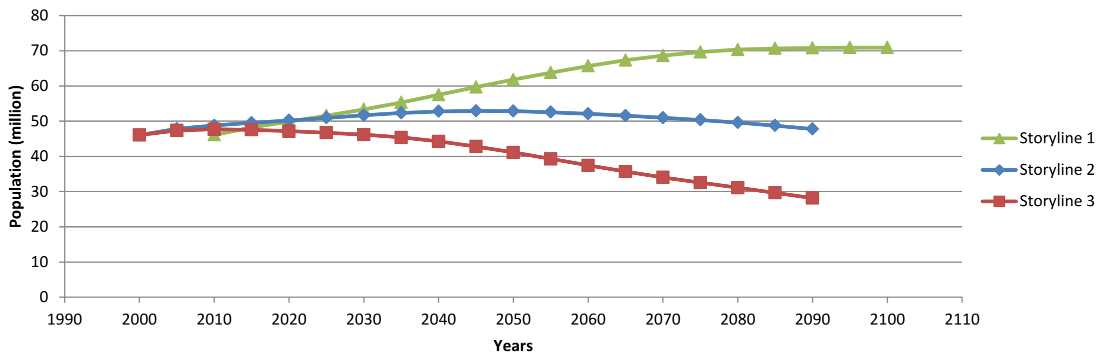

Figure 10. Annual evolution of the population in Spain (source: MARS WP2)

In Table 2 the variables that define each scenario are summarised.

Table 2. Scenarios for the Nervión estuary.

Scenarios		**Annual mean SST***	**Total annual runoff**	**Removal efficiency of UWWTP**
Baseline		16.1°C	1137.52 million m^3	70%
Storyline 1	**2025**	0.3°C increase (RCP 8.5.)	9.06% decrease	70%
	2050	0.9°C increase (RCP 8.5.)	29.40% decrease	80%
Storyline 2	**2025**	0.2°C increase (RCP 4.5.)	8.12% decrease	70%
	2050	0.4°C increase (RCP 4.5.)	27.76% decrease	100%
Storyline 3	**2025**	0.3°C increase (RCP 8.5.)	8.34% decrease	70%
	2050	0.9°C increase (RCP 8.5.)	28.18% decrease	70%

*SST: Sea Surface Temperature

Results

Linking pressures and ecosystem services

Modelling environmental potential of the estuary for recreational fishing

The simple linear regression models built with each of the 14 environmental variables as the independent variable, and the demersal fish abundance (natural logarithm) as the dependent variable, resulted on eight statistically significant relations (p-value <0.05) (see Table 3).

Table 3: Linear regressions explaining demersal fish abundance (dependent variable) as function of 16 environmental variables. Statistically significant results: *p*-value<0.05.

Independent variable	n	Term	Estimate	Std. Error	t value	Pr(>|t|)	Residual Std. Error	Multiple R^2	Adjusted R^2	F-statistic	p-value
Stretch (factor: A-E)	103	TransectB	1.234	0.499	2.474	**0.015**	1.669	0.0694	0.0315	1.828	0.129
		TransectC	0.309	0.490	0.632	0.529					
		TransectD	0.342	0.521	0.657	0.513					
		TransectE	0.756	0.604	1.252	0.213					
Salinity	103		0.001	0.092	0.008	0.993	1.704	<0.001	-0.009	<0.001	0.994
Water temperature (°C)	103		-0.252	0.213	-1.188	0.238	1.692	0.014	0.004	1.411	0.238
Oxygen saturation (%)	103		0.037	0.011	3.321	0.001	1.618	0.098	0.090	11.030	**0.001**
Ammonia concentration (µmol l^{-1})	65		-0.028	0.010	-2.710	0.009	1.546	0.104	0.090	7.344	**0.009**
Total Nitrogen (mg l^{-1})	76		0.330	0.185	1.784	0.078	1.494	0.041	0.028	3.184	0.078
Total Phosphorus (mg l^{-1})	76		2.795	1.320	2.118	0.038	1.481	0.057	0.044	4.486	**0.038**
N/P ratio	76		0.003	0.008	0.373	0.710	1.524	0.002	-0.012	0.1393	0.710
Turbidity (NTU)	76		0.087	0.035	2.489	0.015	1.465	0.077	0.065	6.195	**0.015**
Suspended particulated matter (mg l^{-1})	76		0.139	0.031	4.429	0.000	1.356	0.210	0.199	19.62	**<0.001**
Organic matter in the sediment (%)	68		-0.015	0.036	-0.409	0.684	1.465	0.003	-0.013	0.1674	0.684
Phytoplankton diversity (bit/cel)	63		1.021	0.304	3.359	0.001	1.417	0.156	0.142	11.28	**0.001**
Chlorophyll-*a* (µg l^{-1})	75		-0.178	0.239	-0.745	0.459	1.492	0.008	-0.006	0.5547	0.459
Abundance of benthic invertebrates (Ind m^{-2})	100		<0.001	<0.001	0.615	0.540	1.667	0.004	-0.006	0.3785	0.540
log(Abundance of benthic invertebrates)	100		0.314	0.126	2.504	0.014	1.619	0.060	0.051	6.27	**0.014**
Richness of benthic invertebrates	100		0.027	0.008	3.422	0.001	1.579	0.107	0.098	11.71	**<0.001**

Subsequently, the eight variables with significant correlation were crossed between them to check for collinearity.

GLM 1: variable selection based on correlation matrix

Spearman rank correlation test resulted in a total of 9 out of 28 significant correlations (Table 4).

Table 4: Spearman Rank correlation Test results. *rho* values are presented above the diagonal arrow and *p*-values (with Holm-Bonferroni correction) below.

	O_2 Saturation	NH_4	Turbidity	Suspended particulated matter	Total P	Phytoplankton diversity	Benthic invertebrates (richness)	Benthic invertebrates (log abundance)
O_2 Saturation		-0.763	-0.158	0.234	0.145	0.587	0.619	0.121
NH_4	<0.001		0.363	0.077	0.213	-0.729	-0.522	0.169
Turbidity	1.000	1.000		0.469	0.371	-0.310	-0.079	0.268
Suspended particulated matter	1.000	1.000	0.004		0.361	0.189	0.165	0.341
Total P	1.000	1.000	0.161	0.225		0.065	0.205	0.482
Phytoplankton diversity	<0.001	0.007	1.000	1.000	1.000		0.551	0.290
Benthic invertebrates (richness)	<0.001	0.002	1.000	1.000	1.000	<0.001		0.520
Benthic invertebrates (log abundance)	1.000	1.000	1.000	0.513	0.003	1.000	<0.001	

In order to avoid the effect of collinearity between the independent variables, only three out of the 14 variables were selected for GLM construction. The selection was based on the Spearman correlation test (*p*-values at Table 4) and on the number of available data (e.g. ammonia and oxygen were correlated (*p*-value<0.001) and oxygen was selected for inclusion in the GLM based on the higher number of available data (O_2 *vs.* NH_4 for ammonia, n=100 and 65, respectively)).

Finally, the selected variables for GLM construction were: oxygen saturation, turbidity and total phosphorus. Overall, a total of 76 complete observations were available.

Based on the AICc principle, the chosen model was based on oxygen saturation and turbidity which explain 15.88% of deviation (see Table 5):

$$\textbf{log (fish abundance)} \sim \mathbf{O_2}\ \textbf{saturation} + \textbf{Turbidity}$$

Table 5: Coefficients for the best Generalized Linear Model based on Akaike Information Criterion.

	Estimate	Std. Error	t value	Pr (>|t|)
Oxygen saturation	0.03099	0.01166	2.659	0.00963 **
Turbidity	0.10424	0.03420	3.048	0.00321 **

GLM 2: variable selection based on VIF

From the eight selected independent variables, only ammonia concentration was removed from the analysis, based on its high VIF value (<5). Therefore, a total of seven variables were selected for construction of the second GLM (Table 6).

Table 6: Variance Inflation Factor (VIF) values for the full models (8 variables) and for the reduced model (after removing NH_4).

Variables	VIF (full model)	VIF (reduced model)
Phytoplankton diversity	2.92	1.83
Benthic invertebrates (richness)	3.11	1.98
Benthic invertebrates (abundance)	2.18	2.32
Total P	1.82	1.66
Turbidity	2.58	1.99
Suspended particulate matter	1.86	1.79
Oxygen Saturation	13.47	1.93
Ammonia	14.33	-

Finally, a total of 60 complete observations were available for GLM construction. Based on the AICc principle, the chosen model was based on phytoplankton diversity, turbidity and suspended particulate matter, which explain 33.08% of deviance (Table 7):

log (fish abundance) ~ Phytoplankton diversity + Susp. particulate matter + Turbidity

Table 7: Coefficients for the best Generalized Linear Model based on Akaike Information Criterion.

	Estimate	Std. error	t value	Pr (>|t|)
Phytoplankton diversity	0.99005	0.32509	3.045	0.00354 **
Suspended particulate matter	0.09257	0.04001	2.314	0.02437 *
Turbidity	0.09311	0.04916	1.894	0.06342

GLM: Final selection

According to the percentage of deviance explained, the best model would be the GLM 2 (33.08% of deviance explained vs. 15.88% of deviance explained). However, based on the higher number of available data (for GLM1 and GLM2, n=76 and 60, respectively) and previous studies (Uriarte and Borja, 2009), we consider that GLM 1 (log (fish abundance) ~ O_2 saturation + Turbidity) reflects better the underlying process affecting fish abundance. Thus, we used GLM 1 to estimate fish abundance and projected changes under different scenarios.

Recreational fisher's behaviour and perceptions at Nervión estuary

From January to September 2016, we obtained a total of 146 questionnaires. The profile of a fisher in Nervión is a local (89% living in the villages along the estuary), middle-aged (51±14 years) man (93.2%) with 29±16 years of recreational fishing experience. Regarding employment, most fishers were inactive (17.2% unemployed and 34.2% retired). Only six out of 146 respondents (4%) did not hold an active fishing licence of any type.

The main motivations for practicing recreational fishing at Nervión are relaxation, to be with friends or relatives and to practice outdoor activities, as the three of them were mentioned by >30% respondents.

The most popular area for practicing fishing is the outer Nervión, and more specifically the area B, which comprised also the highest percentage of constant fishers (74% of respondents fish nowadays here) (Table 8). Angling from shore is the most popular fishing method in Nervión estuary, which is practiced in all the areas. Spear fishing and fishing from boat are mainly practiced in the outer Nervión.

Table 8: Fishing in Nervión stretch by stretch. Key: Ever fished and fished now: number of fishers who have ever fished or fish nowadays and the percentage from the total number of questionnaires (n=146); Year of the first fishing event: mean year of the first fishing event for respondents who have ever fished in the corresponding stretch.

Stretch	Ever fished		Year of the first fishing event		Fishes now	
	n	%	Mean	SD	n	%
A	103	70.55	1994	13	74	50.7
B	112	76.71	1996	14	108	74
C	75	51.37	1997	14	62	42.5
D	35	23.97	2003	12	25	17.1
E	12	8.22	2007	6	9	6.2

Regarding fisher behaviour, the most remarkable result was the year when fishers began to fish in each stretch (Table 8), as fishers progressively entered into the inner stretches over more recent years.

Fishers reported a total of 32 different species being caught in the estuary. From the 32 species, only two were mentioned in all the stretches: sea bass (*Dicentrarchus labrax*) and common sea bream (*Diplodus vulgaris*). Outer stretches and C and D stretches showed a similar species composition, with a decreasing importance of cephalopods as we got into the inner stretches. The innermost stretch shows a completely different species composition, with most frequent species being representative of an oligohaline environment, such as thicklip grey mullet (*Chelon labrosus*) and carp (*Cyprinus carpio*).

Fifty out of the 146 respondents indicated that the most frequently captured species changed in all or some of the stretches where they fish nowadays.

The respondents who reported a change in the most frequently caught species were later asked to name the most frequent species in the past, which were *Diplodus vulgaris* and *Dicentrarchus labrax*. These species are, according to fishers, the most frequently caught species nowadays too. In contrast, Blackspot seabream (*Pagellus bogaraveo*) and pouting (*Trisopterus luscus*) were mentioned as frequent species in the past, but hardly anyone (one and zero respondents, respectively) mentioned them as present nowadays.

All 20 characteristics considered as potentially influencing the enjoyment of the fishing activity were perceived to be generally in good condition in the estuary ($\bar{x}$= 2.39-3.79 in a scale from 4=excellent to 1=bad) (Table 9). The characteristics perceived in better conditions were "accessibility to fishing areas" and "proximity to home", with mean values > 3.5. On the contrary and with values < 2.5, the characteristics in worst conditions were "number of catches" and "controls of fisher´s catches".

Table 9. Perceived condition in Nervión of 20 characteristics potentially affecting recreational fishing. Each characteristic was valued independently by each respondent in a scale from 1 (excellent) to 4 (bad).

	n	**Mean**	**Stand Dev**
GENERAL CONDITIONS			
Proximity to home	141	3.77	0.56
Accessibility to fishing areas	140	3.79	0.57
Peace of the area / Tranquility	142	3.40	0.92
General cleanliness of the area	142	3.22	1.00
WATER QUALITY			
Water transparency	137	3.36	0.85
Absence of marine debris	141	2.78	1.10
Absence of foam in the water	142	3.23	0.97
Absence of oils in the water	140	3.13	1.02
Absence of water odour	141	3.38	0.97
Absence of residual discharges nearby	137	3.19	1.05
CONTROLS			

Fishing area delimited	111	3.02	1.14
Controls to fishers	125	2.50	1.21
Controls of fishers´ catches	122	2.39	1.21
INTERACTIONS			
Absence of numerous fishers	137	3.01	1.01
Absence of boats	136	3.18	1.09
Absence of people practicing aquatic sports	139	3.14	1.12
CATCHES			
Number of catches	139	2.44	1.10
Great size of catches	140	2.89	0.96
Great diversity of catches	138	2.99	0.96
Catches with food interest	137	3.58	0.73

Fishers´ perceptions towards changes in catches are generally more negative than reality. Indeed, although the data collected for the last 25 years on demersal fish abundance and richness pointed a clear improvement (from eight species in 1989 to 26 in 2015 and increase in abundance), fishers mainly perceived no changes in catches diversity (36.3% of respondents) and a decrease in number of catches (69% of respondents). On the contrary, perceived a positive change in water quality, as 69% of respondents indicated that water conditions have improved.

In relation to whether the personal satisfaction with fishing has changed over time, no clear pattern was found; as 33% perceived no changes, 24% a deterioration and 29% an improvement. In contrast, from the 146 respondents, 91% stated that they would like to continue fishing in Nervión.

Climate change scenarios

The mean *E. coli* concentrations during the bathing season at the three bathing waters for each scenario are shown in Table 10. In all scenarios, the highest *E. coli* concentrations were obtained at Las Arenas beach, being this the closest to the pollution source (i.e. the rivers and the UWWTP). There is a decrease in *E. coli* concentration by year 2050 in all the storylines. The best scenario is Storyline 2 by year 2050 with 17 MPN 100 ml^{-1} of *E. coli,* at Las Arenas beach. *E. coli* concentrations at Ereaga and Arrigunaga beaches are all lower than 10 MPN 100 ml^{-1}. Table 11 shows the number of days during the bathing water season where the value of *E. coli* concentration is higher than the local water quality standard of 500 MPN 100 ml^{-1} for the considered scenarios. In the best scenario (Storyline 2 in year 2050), the bathing water quality is not sufficient during 16 days at Las Arenas beach, while for the same beach in the baseline scenario the bathing water quality is not sufficient during 22 days (i.e. in the best scenario there is a 27% improvement of the water quality for bathing).

Table 10. Geometric mean *E. coli* concentrations during the bathing season for the scenarios. Results extracted from the MOHID implementation of Nervión estuary.

Scenarios		Geometric mean *E. coli* concentration (MPN $100ml^{-1}$)		
		Las Arenas beach	Ereaga beach	Arrigunaga beach
Baseline		146	<10	<10
Storyline 1	2025	111	<10	<10
	2050	68	<10	<10
Storyline 2	2025	113	<10	<10
	2050	17	<10	<10
Storyline 3	2025	112	<10	<10
	2050	86	<10	<10

Table 11. Number of days during the bathing season where the *E. coli* concentration value is higher than the local water quality standard for the scenarios. Results extracted from the MOHID implementation of Nervión estuary.

Scenarios		Number of days where *E. coli* concentration > 500 MPN $100ml^{-1}$		
		Las Arenas beach	Ereaga beach	Arrigunaga beach
Baseline		22	5	2
Storyline 1	2025	22	4	2
	2050	20	4	1
Storyline 2	2025	22	4	2
	2050	16	3	1
Storyline 3	2025	22	4	2
	2050	21	4	1

Although the biogeochemical module of MOHID has not been implemented yet to provide values of dissolved oxygen concentration, values of oxygen saturation were determined considering only the changes in the mean sea surface temperatures. For this purpose, we have considered the following:

- The estuary is well mixed and the mean temperature is the same at the surface and at the bottom.

- The mean dissolved oxygen concentration at the bottom of the estuary for the baseline scenario is 6.9 mg l^{-1} and it will not change in the future.
- The mean value of salinity for the estuary in the baseline scenario is 33.7 and it will not change in the future.
- The maximum dissolved oxygen concentration saturation at 33.7 of salinity:
    - and 16°C of water temperature is 8.0 mg l^{-1}
    - and 17°C of water temperature is 7.9 mg l^{-1}

    (Source: http://www.buzzardsbay.org/bbpreports/oxygen-saturation-table.pdf)

Table 12 shows the values of the bottom oxygen saturation for the scenarios considering the abovementioned assumptions. There would be an increase in the mean oxygen saturation with the increase of water temperature. The difference between the climate scenarios with the highest SST (i.e. Storyline 1 and 3 by year 2050) and the baseline scenario is a mean oxygen saturation of 1.5%.

Table 12. Scenarios of mean bottom oxygen saturation values at Nervión estuary, assuming changes in mean bottom water temperature only.

Scenarios	Mean bottom water salinity (ppt)	Mean bottom water temperature (°C)	Mean bottom dissolved oxygen concentration (mg/l)	Mean bottom oxygen saturation from formula(%)
Baseline	33.7	16.1	6.9	86.0
Storyline 1 (year 2025)	33.7	16.4	6.9	86.4
Storyline 1 (year 2050)	33.7	17.0	6.9	87.5
Storyline 2 (year 2025)	33.7	16.3	6.9	86.3
Storyline 2 (year 2050)	33.7	16.5	6.9	86.6
Storyline 3 (year 2025)	33.7	16.4	6.9	86.4
Storyline 3 (year 2050)	33.7	17.0	6.9	87.5

Table 13 shows the predictions of the mean total fish abundance obtained for each future scenario, applying the GLM equation that relates bottom oxygen saturation with fish abundance (Table 5) and considering no change in the water turbidity. The differences obtained between the mean total fish abundance in the baseline scenario and the storylines are negligible.

Table 13. Future scenarios of mean total fish abundance at Nervión estuary, assuming changes in mean bottom water temperature only.

Future scenarios	Differences in mean bottom oxygen saturation from formula (%)	Differences in mean total fish abundance from GLM (Ind/Ha)
Storyline 1 (year 2025)	0.4	2
Storyline 1 (year 2050)	1.5	6
Storyline 2 (year 2025)	0.3	1
Storyline 2 (year 2050)	0.6	2
Storyline 3 (year 2025)	0.4	2
Storyline 3 (year 2050)	1.5	6

Discussion

Linking pressures and ecosystem services

It is well-known the recovery of the Nervión estuary in recent decades (Borja *et al.,* 2010, 2016; Cajaraville *et al.,* 2016; Pascual *et al.,* 2012), but nothing is known on the recovery of cultural estuarine ecosystem services (i.e. recreational fishing) and benefits, derived from the environmental restoration in the area.

In the last 25 years, the improvement in environmental conditions led to radical structural changes in the estuary and influenced also the practice of recreational fishing, especially in the inner estuary. The abiotic data gathered prove a great improvement on water conditions and consequently a dramatic increase in the abundance and richness of demersal fishes (Uriarte and Borja, 2009), which has been demonstrated to be linked with the increase of oxygen saturation and decrease in turbidity, resulting from the water treatment (it has been shown here the dramatic decrease in nutrient load and the increase of oxygen). Also, the increase in the number of recreational fishing licences issued in the villages along the estuary suggests a growing interest in this recreational activity. All these data are indicators of better fishing conditions, and could be indicating an increase and an extension of the fishing activity throughout the Nervión estuary.

The results of the questionnaire survey support the hypothesis of a correspondence between improvement in environmental parameters and an increase in recreational fisheries, as a cultural ecosystem services. Specifically, the spatial and temporal differences found on the year of the first fishing event at Nervión, show a clear pattern on the development of the activity from the outer to the inner part of the estuary, which match pretty well with the milestones of the recovery pattern pointed out by Borja *et al.* (2010), who showed a progressive recovery in estuarine conditions from the outer to the inner part of the Nervión and related with three events. This pattern supports the conclusion of Fulford *et al.* (2016), who argued that angler's behaviour varies in response to changes in the habitat. Indeed, as a result of the different restoration events, fish have been able to recolonize the inner part of the estuary (Uriarte and Borja, 2009), leading to a subsequent increase of fishers in this area.

Recreational fishing has been previously considered as highly dependent on water ecological conditions (Ribaudo and Piper, 1991; Vesterinen et al., 2010), although this has been questioned recently (Ziv *et al.,* 2016). In a more general context, the Water Framework Directive (WFD) aims at achieving a good ecological status for all the European water bodies (Hering *et al.,* 2010), and it is essentially framed in the ecosystem approach (de Jonge *et al.,* 2012). Furthermore, the connection between improvements in water quality and in ecosystem service provision has been defended broadly not only by the scientists (Haines-Young and Potschin, 2010), but also by the European Commission (Ziv *et al.,* 2016). Nevertheless, the findings of the current study prove that better water quality conditions can support an improvement on the delivery of recreational services in an estuary.

Fishers´ perceptions on changes in catches abundance, variety and size, drawn a less clear picture of the situation; perceptions are more negative than the changes depicted by the environmental data. Taking into account that the presence of fish and the possibility of catching fish is considered an essential motivational factor for recreational fishers (Arlinghaus, 2006; Fedler and Ditton, 1986), and despite the clear improvements recorded on fish and other biological elements (Borja et al., 2016), the findings of this study show that fisher´s perceive just the opposite situation, with no-improvements or deterioration in parameters related with catches. Previous studies already highlighted the mismatch that often exists between science data and public perceptions (Danylchuk et al., 2016).

The concept of recreational fishing depending on a mixture of factors is of special relevance when service beneficiaries´ satisfactions are used to measure cultural ecosystem services, as environmental quality and catches characteristics are only part of the multiple factors that have an effect on fisher´s satisfaction (Griffiths et al., 2017; Sutton, 2007). Therefore, a restoration project that results in an improvement in the ecological conditions could not be enough to achieve an improvement in the delivery of cultural ecosystem services and both ecological and social factors involved in the service production must be considered (Reyers et al., 2013).

This study shows that the improvement in the physicochemical conditions in the Nervión estuary, and the subsequent progressive improvement in biological parameters, influenced positively the provisioning of cultural ecosystem services, attending to changes in the number of recreational fishing licenses and the behaviour of recreational fishers in the estuary. Indeed, data gathered in this study indicates that recreational fishing activity has moved to the inner parts of the estuary, following their recovery.

On the other hand, we have started also the study of data from a survey in three beaches of the estuary, trying to determine the perception of users on the quality improvement and the increase of uses linked to water (i.e. bathing, surfing, diving, etc.). However, as the study has not concluded yet, the results have not been included here, but the first results indicated a similar pattern as in the fishing recovery.

Climate change scenarios

The combination of SST increase, runoff decrease and improvement of the removal efficiency at Galindo UWWTP in the future scenarios has shown a general improvement of the bathing water quality in the beaches of the estuary, especially in Storyline 2 (Consensus World) in year 2050, where the removal efficiency at the UWWTP is of 100%. Nonetheless, even in the best scenario, Las Arenas beach (i.e. the closest beach to the sources of pollution) has shown not sufficient bathing water quality problems during the bathing season. Therefore, management measured should be taken to reduce the pollution loads from the rivers.

The maximum change in SST would be of 1°C by year 2050 in storylines 1 and 2. Considering only the effect of SST change, the oxygen saturation percentage would increase by 1.5% (in Storylines 1 and 2 by 2050). This would infer in a negligible change in fish abundance.

The oxygen saturation percentage is a water quality indicator proposed by the Water Framework Directive. This parameter is a ratio of the concentration of dissolved oxygen in the water to the maximum amount of oxygen that is dissolved in the water at that temperature and pressure under stable equilibrium. A change in water temperature only has effects on this maximum amount of oxygen (an increase in water temperature will decrease this maximum amount of oxygen). Therefore, assuming no change in dissolved oxygen concentration in the scenarios, an increase of SST would increase the oxygen saturation percentage and therefore, the water quality. Nonetheless the available oxygen for the fishes would not change. Therefore, the implementation of the GLM equation (Table 5), that relate fish abundance with oxygen saturation percentage, should be considered carefully.

The changes in dissolved oxygen concentration in estuaries are complex and involve numerous processes, such as diffusion from the atmosphere, photosynthesis by algae and aquatic plants and decomposition of organic matter. The biogeochemical module of MOHID will be applied to simulate the dissolved oxygen concentrations in the estuary to provide more reliable values of oxygen saturation.

It should be mentioned, that the studies of climate change point out that an increase in water temperature, would reduce the oxygen solubility and therefore, the dissolved oxygen concentration will decrease (Stortini *et al.,* 2017). This is true when the oxygen saturation percentage of a water mass is close to 100%. The water body of the Nervión estuary is normally under this 100% and, therefore, a change in water temperature will not modify its content in dissolved oxygen.

Management consequences

In this study, we have combined long-term monitoring data from different ecosystem components (i.e. physico-chemical, fishes, bacteria, etc.), hydrodynamic modelling and socio-ecological surveys. This approach has led us to demonstrate that the management decisions taken in the Nervión basin and estuary in late 1980's (i.e. removal of direct urban discharges, changes in industrial production, biological water treatment, etc.) have resulted in a clear recovery of the ecological quality of this system.

During this long process, some of the ecosystem services provided by the system, such as biodiversity supporting, have increased dramatically (Pascual *et al.,* 2012). However, until our study in MARS it was not clear to which extent other ecosystem services, such as those cultural (i.e. recreational fishing, bathing), have been recovered and are perceived by the users and citizens. Despite the demonstrated fact that these services have increased, there is some contradictory perceptions from the users (e.g. fishers say that they fish less, but the number of fishers has increased and the abundance of fish also). Probably this requires some ocean literacy actions (Uyarra and Borja, 2016) to make aware the citizens on the real recovery of the ecosystem services.

The link between the reduction of organic matter and nutrient loads to the estuary (because of the different phases of water treatment, between 1980 and 2015), the increase of dissolved oxygen and reduction of nutrient concentration and turbidity during that period, the subsequent increase of plankton, richness and diversity of macroinvertebrates and, finally, in the richness, abundance and quality of fish, have been demonstrated (Borja *et al.,* 2010) and have extended in this study with new data. This has resulted in management consequences, since the perception of an increase of the system quality and fish availability, led to an increase in the recreational fishing licenses and new regulations of fishing practice.

Regarding bathing, the water treatment reduced also dramatically the load of coliforms into the estuary, resulting in an improvement in the water quality in the estuarine beaches and a reduction in the number of days in which bathing activity was forbidden (Bald *et al.,* 2004). Although the study of the survey undertaken in beaches has not still finished, the first results indicate that the treatment management has been perceived by the beach users as the cause of quality improvement.

However, in both cases (bathing waters and fishing), as the discharge of the WWTP is still taking place in the estuary, there are some punctual problems in the area, which have been reduced after the biological treatment (Borja *et al.,* 2016). The water management authorities are considering new management measures to remove these punctual problems, by deriving the current discharge to the coastal area, by means of a submarine outfall, which would dilute and disperse the final discharge in a more efficient way.

On the other hand, some studies in areas where climate change will result in an increase of river runoff, have predicted an increase in bad quality of bathing waters, in the future (Schernewski *et al.,* 2014). However, in the Nervión estuary, under the different future scenarios, the predictions suggest small changes in the current situation, because an increase in the precipitation is not expected (Moncho *et al.,* 2009). Hence, only under Storyline 2 (Green Focus), by 2050, there is an improvement of the water quality for bathing in the closest beach to the WTP. This improvement was determined by the number of days during the bathing water season where the value of *E. coli* concentration was lower than 500 MPN $100ml^{-1}$. The improvement in quality would be reinforced by additional management measures, such as the derivation of the discharge to the submarine outfall. The results of the present study have shown that this could be a valid criterion to evaluate the provision of ecosystem services, such as bathing waters.

Regarding the fish abundance, it seems that the changes expected under each scenario are negligible, probably because the treatment has been completed and the future oxygen saturation does not represent a harm for the fish reproduction and life (Branco et al., 2016; Hrycik et al., 2017). However, the management consequences can be different, related to the recreational fishing itself, e.g. because the increase of the activity, needing responses in terms of regulation of fishing, control of fishers and captures, changes in the species permitted for capture, size of the captures and number of individuals (or weight) that can be captured daily (Cardona and Morales-Nin, 2013; Zarauz *et al.,* 2015). This management measures can include an engagement of recreational fishers in the management and conservation of the resources within the estuary (Granek *et al.,* 2008). However, to involve the recreational fishers in this management, their motivations to participate should be determined carefully (Copeland *et al.,* 2017). In this way, to promote sustainable recreational fisheries in the estuary, it is recommended the detailed suggestions for governance and management outlined in the United Nations Food and Agricultural Organization Technical Guidelines for Responsible Fisheries: Recreational Fisheries (Arlinghaus *et al.,* 2016).

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Content

Non-technical summary

This deliverable consists of the manuscript ‘Fisheries impacts on lake ecosystem structure in the context of changing climate and trophic state’ by Tiina Nõges (EMU), Orlane Anneville (INRA), Jean Guillard (INRA), Juta Haberman (EMU), Ain Järvalt (EMU), Marina Manca (CNR), Giuseppe Morabito (CNR), Michela Rogora (CNR), Stephen J. Thackeray (NERC), Pietro Volta (CNR), Ian J. Winfield (NERC), Peeter Nõges (EMU) that was submitted to Journal of Limnology (http://www.jlimnol.it/index.php/jlimnol) on March 8, 2017.

We analysed case studies from five European lake basins of differing trophic states (Lake Võrtsjärv, two basins of Windermere, Lake Geneva and Lake Maggiore) with long-term limnological and fisheries data. Decreasing phosphorus concentrations (re-oligotrophication) and increasing water temperatures have been reported in all five lake basins, while phytoplankton concentration has decreased only slightly or even increased in some cases. To examine possible ecosystem-scale effects of fisheries we analysed correlations between fish and fisheries data, and other food web components and environmental factors. Re-oligotrophication over different ranges of the trophic scale induced different fish responses. Although a general model predicted increasing fish production and biomass with increasing trophic state, parameters other than phosphorus including fisheries pressure and the balance between predatory and non-predatory fish species explained a significant part of the observed variability in fish abundance. In the deeper lakes Geneva and Maggiore, we found a stronger link between phytoplankton and planktivorous fish and thus a more important cascading top-down effect than in other lakes. This connection makes careful ecosystem-based fisheries management extremely important for maintaining high water quality in such systems. We also demonstrated that increasing water temperature may favour piscivores at low phosphorus loading, but suppresses them at high phosphorus loading and may thus either enhance or diminish the cascading top-down control over phytoplankton with strong implications for water quality.

INTRODUCTION

Changes in fish assemblages are commonly used to evaluate aquatic ecosystem stress because, with their relatively long lifespan, fishes integrate the effects of short- and longer-term stressors (Dobiesz *et al*., 2010). Sustainable fisheries are critical for human welfare and biodiversity conservation, with overfishing posing numerous threats to the functioning of the whole ecosystem by triggering trophic cascades and altering food web dynamics (McIntyre *et al*., 2007; Salomon *et al*., 2008).

The socio-ecological significance of fisheries, and major stressors that impact them, differ between marine and freshwater ecosystems. Fishing is recognized as one of the most important ecosystem services provided by the world's oceans but, in addition to this, lakes provide a diversity of other ecosystem services as well, for example the provision of drinking water. Thus, lake management is primarily focused on maintaining high water quality which in turn facilitates the multiple services that lakes are expected to provide. In oceans, fish stocks suffer mainly from overfishing, while in lakes a reduction in fish abundance is more often caused by human activities in the lake or its catchment other than intense fishing. Although many studies have addressed the impact of lake fisheries management on fish stocks (Cows, 2002; Pine *et al*., 2009; Cowx and Portocarrero, 2011; Everson *et al*., 2013; Kolding and van Zwieten, 2014; Persson et al., 2014; Suuronen and Bartley, 2014; Fraker *et al*. 2015; Anneville *et al*., 2015; DuFour *et al*., 2015), the variability in lake fish communities is still mostly related to changes in environmental pressures such as eutrophication (Vonlanthen *et al*., 2012) or species introductions (Gozlan, 2008). As a consequence, lake management practices focus on pollution control, habitat conservation and/or manipulation in order to enhance ecosystem health and assure the availability of all ecosystems services including professional and recreational fishing.

Both marine and freshwater ecosystems are driven by subtle and complex combinations of bottom-up and top-down controls which in turn are influenced by food web structure and composition. In addition, because food web structure and composition are sensitive to climate change and other environmental disturbances, there is also a need to consider and understand not only the interactions among and between species but also those with their environment. From the early 2000s, Ecosystem-Based Fisheries Management (EBFM), which originated in the marine realm and which incorporates a holistic approach as its basic tenet, has gained increasingly popularity around the world. As noted by Pickitch *et al*. (2004), EBFM represents a new direction for fishery management and essentially reverses the order of management priorities to start with the ecosystem rather than with the target species, with the overall objective to sustain healthy ecosystems and the fisheries they support.

Since the 1980s (e.g. Shapiro and Wright, 1984; Carpenter *et al*., 1985), detailed food web studies have recognized that cascading effects in lakes are particularly visible because aquatic organisms are characterized by strong trophic links which can be profoundly disturbed by changes in biodiversity. As an example, Gallardo *et al*. (2016) underlined the impact of invasive fish predators on different trophic levels of aquatic ecosystems. Because of strong cascading trophic interactions in lakes, Shapiro and Wright (1984) proposed using fish for lake restoration by either removing planktivorous fish directly or by introducing or favouring the growth of piscivorous fish. Both measures should favour zooplankton development and enable them to control efficiently phytoplankton biomass. This method, "biomanipulation", (Shapiro and Wright, 1984) has been implemented in many lakes to improve the water quality.

While top–down cascades from fish to phytoplankton have been a core topic in recent limnology, they have attracted far less interest in marine ecology because lake studies have been largely aimed at regulating eutrophication-induced algal blooms while marine studies have been more oriented towards fish yield (Hessen and Kaartvedt, 2014). However, there are still fewer directly EBFM-aligned studies in lakes compared to in marine systems, and in both environments the cascading effects of fisheries on the whole ecosystem are only rarely addressed due to their data-demanding nature. Nevertheless, because fishing can drive large-scale ecosystem changes, fisheries management should target the recovery of entire ecosystems to more desirable and resilient states. The partial recovery of fish stocks only is not a stable objective because a further change in another component of the ecosystem (e.g. in climate or alien species) may drive the system into another catastrophic loop (Daskalov *et al*., 2007). Understanding of the relative importance of top-down and bottom-up mechanisms in regulating ecosystem structure is a fundamental ecological question with implications for both fisheries and water-quality management. Accordingly, a recent study in the Laurentian Great Lakes justifiably underlined the importance of continued monitoring to extend time series, and of mechanistic research to test correlative findings, with the overall goal of enhancing the ability of managers to implement ecosystem-based management approaches (Bunnell *et al*., 2014).

The present study compiles and analyses long-term data from four European lakes, i.e. Lake Võrtsjärv, Windermere (with two basins), Lake Geneva and Lake Maggiore, which differ in trophic state and fishing pressure, to explore variation in the extent and strength of top-down cascading effects of fish and fisheries at the ecosystem level. In particular, we attempt to identify the potential driving factors that shape the community structure of these ecosystems by evaluating the effects of multiple stressors (e.g., nutrient loading, fishing pressure, and temperature) on the fisheries and food webs of these five lake basins.

METHODS

Study sites

We focus our study on four medium-to-large lakes for which long records of fisheries activity, and associated ecosystem variables, exist. The lakes were selected to represent a wide gradient in depth, trophic state and fisheries intensity (Fig. 1). The deepest and most oligotrophic lake, Maggiore, has experienced the strongest fishery pressure, followed by the shallowest and most eutrophic lake, Võrtsjärv. In both basins of Windermere the fishing intensity was low.

Lake Võrtsjärv

Lake Võrtsjärv is a large and very shallow lowland lake in Estonia (Table 1) which has suffered from increasing nutrient loads from agriculture since the 1950s (Nõges and Nõges, 2012). Among our case study lakes, Võrtsjärv is the second largest by surface area and the most eutrophic (Table 1). Since 1961, surface water temperature has significantly increased in spring, summer and autumn, at rates of up to 0.39 ^{o}C decade^{-1} (for August, Nõges and Nõges, 2014). Thirty-one fish species and one lamprey species inhabit Võrtsjärv and its tributaries permanently. Eels (*Anguilla anguilla* (L.)) have been introduced to and stocked into Võrtsjärv since 1956 and have become the most important commercial fish followed by pikeperch (*Sander lucioperca* (L.)), pike (*Esox lucius* L.) and bream (*Abramis brama* (L.)). Catches of roach (*Rutilus rutilus* (L.)), burbot (*Lota lota* (L.)) and perch (*Perca fluviatilis* L.) are also considerable. Ruffe (*Gymnocephalus cernuus* (L.)), bleak (*Alburnus alburnus* (L.)) and lake smelt (*Osmerus eperlanus* m. *spirinchus* Pallas) have lost their commercial importance following prohibition of the use of fine meshed trawls since the 1970s (Järvalt et al., 2004). The lake has an intensive commercial fishery with well documented yearly catches for all commercial fish species since 1971, and at a lower resolution since 1935 (Nõges et al., 2016). In years when a severe winter coincides with a low water level, serious fish kills may occur (Nõges and Nõges, 2012).

Windermere

Windermere is a large and deep meso-eutrophic lake comprising elongated northern and southern basins. Both phosphorus concentration and phytoplankton abundance are consistently lower in the north than in the south basin (Table 1). The surface water temperature of Windermere has shown a significant increase since the late 1980s (Winfield *et al*., 2008a,b). The fish community includes 16 species, although only Arctic charr (*Salvelinus alpinus* (L.)), perch, pike and, in recent years, introduced roach are abundant (Winfield *et al*., 2008a). Commercial fisheries have not operated for many decades, but a small-scale recreational fishery persists for Arctic charr and catch-and-release angling is practised for pike and some other species (Le Cren, 2001).

Lake Geneva

Lake Geneva, a deep peri-alpine lake located on the border between France and Switzerland is the largest in our study, in terms of surface area (Table 1). In the second half of the 20th century, the lake experienced a rapid increase in nutrient concentrations which switched it from oligotrophy to eutrophy with annual mean total phosphorus (TP) concentrations reaching 89 mg/m^3 (Anneville *et al*., 2000), but these have now been lowered as a result of a reduction in phosphorus loadings (Table 1). Besides changes in trophic status, the effect of climate variability has become evident during the last few decades: the water temperature in the 0-20 m layer has increased markedly during winter and spring and though summer temperatures do not show any warming trend, they are strongly influenced by subtropical Atlantic climate variability (Molinero *et al*., 2007). In addition the phytoplankton community has undergone an important shift in species composition (Anneville *et al*., 2002). Within the zooplankton community, *Daphnia*, one of the preferred prey items of zooplanktivorous fish, showed an overall decrease in abundance between 1986 and 2010 (Laine and Perga, 2015). Fish species caught by professional fishers have included Arctic charr, pike, burbot, roach, brown trout (*Salmo trutta* L.), perch and whitefish (*Coregonus lavaretus* (L.)). The contribution of whitefish to commercial catches decreased from 25% in 1950-1962 to 10% in 1963-1978. From 1979 to the mid-1990s, whitefish contribution remained low and catches were dominated by percids that made up 76% of the total catches (41% for perch and 35% for roach). Since the 1990s, whitefish contributions started to increase again (up to 66% in 2010-2012) accompanied by a decrease in roach contributions while perch remained high ranging from 32% to 70% (Anneville *et al*., 2017).

Lake Maggiore

Lake Maggiore is a large holo-oligomictic and naturally oligotrophic lake (Marchetto *et al*., 2004); the deepest and most oligotrophic in our study (Table 1). In the late 1950s, TP in Maggiore began to rise and by the late 1970s, the lake reached a trophic state close to eutrophy with maximum TP concentrations of 30 mg/m^3 during winter mixing. Since the 1980s, nutrient loads have been significantly reduced and in-lake TP in the whole water column has decreased to 10 mg/m^3 (Obertegger and Manca, 2011). Phytoplankton biomass gradually declined, with a time lag, following the reduction of nutrient loads (Fastner *et al*., 2016). The abundance of *Daphnia longispina - galeata* declined with lake re-oligotrophication, but began to increase again after 1996 (Manca *et al*., 2007). There are 22 native fish species in Lake Maggiore but the lake has experienced extensive intentional introductions of fish species. In the 19th century, *Coregonus wartmanni coeruleus* and *C. schinzii helveticus* introduced from Lake Konstanz apparently hybridized with local whitefish *C. lavaretus*, called lavarello (Berg and Grimaldi, 1965). After World War II, whitefish *C. macrophthalmus* N. (locally called “bondella”) was introduced for commercial purposes from Lake Neuchatel and soon became the most abundant species in catches (Grimaldi, 1972). Since the 1990s, roach, ruffe, pikeperch, crucian carp (*Carassius carassius* (L.)), bitterling (*Rhodeus amarus* L.), and wels catfish (*Silurus glanis* L.) were introduced. After its introduction in 1950, the whitefish bondella became a major target of the commercial fishery alongside bleak (Grimaldi and Numann, 1972).

In recent decades, global warming has affected the autumn - winter mixing in Lake Maggiore resulting in a gradual reduction of the depth reached by convective mixing (Ambrosetti *et al*., 2010).

Data collection

Lake Võrtsjärv

The present analysis is based on yearly statistics of commercial fish catches from the period 1971-2013. During this period, passive fishing gear (fish traps and gill nets) was used and the intensity of fishing remained at a relatively constant level of 300-360 fyke nets and 300-360 gill nets (the number of fyke and gill net licences per year, with fyke nets set in the ice free period, and gill nets from September up to the next spring ice-out, usually in March). In addition, experimental trawling as a sampling method for fish stock monitoring was started in 1981. During the ice-free period (April-November), fish were caught with a bottom otter trawl (mouth width 8 m, height 2.5 m, cod-end mesh size 12 mm). In the pelagic part of the lake 15-20 hauls per year, lasting 15 to 30 minutes each, were made in the daytime at a trawling speed of 4.5 km/h. Catch per unit effort (CPUE) of the trawl was calculated in kilograms per trawl-hour.

Water chemistry, phyto- and zooplankton have been studied since 1964, 1-4 times per month. A series of 1-litre samples was taken with a Ruttner sampler at 1-m intervals from the surface to the bottom and mixed in a tank. For phytoplankton, a subsample of 250 ml was preserved with acidified Lugol's solution and analysed microscopically as described by Nõges et al. (2010). TP was analysed according to Grasshoff *et al*. (1983). Zooplankton samples were taken with a quantitative Juday net (85 µm mesh size), towed from the bottom to the surface (in 1964-2000) or by filtering 20 L of depth-integrated water through a net of 48 µm mesh size (since 2001), preserved with acidified Lugol's solution and counted under a stereomicroscope Nikon (SMZ1500) in a Bogorov chamber at up to 120x magnification. For biomass calculations, the average body length of 10 individuals from each taxon was measured. The length of adult crustaceans was converted to weight according to Balushkina and Vinberg (1979).

Water temperature was measured daily at the outflow and data were provided by the Estonian Meteorological and Hydrological Institute.

Windermere

In the absence of commercial fisheries, the Arctic charr, perch, pike and roach populations have been studied and continuously monitored since the early 1940s using a range of methodologies including gill nets targeted at Arctic charr (Winfield *et al*., 2008a), gill nets targeted at pike (Winfield *et al*., 2008b; Paxton *et al*., 2009) and traps targeted at perch (Paxton *et al*., 2004), augmented by the collection of Arctic charr recreational angling records since the mid-1960s (Winfield *et al*., 2008a) and the use of survey gill nets at 5-year intervals since 1995 targeted at roach (Winfield *et al*., 2008b). This scientific monitoring constitutes the only removal of fish from the lake, with the exception of insignificant numbers of Arctic charr and brown trout removed by recreational anglers. The present analysis is based primarily on basin-specific annual sampling effort, absolute catch by numbers and weight for perch and pike, together with derived numerical CPUE and biomass CPUE for perch and pike monitoring and annual angler numerical CPUE for Arctic charr.

These fish studies have been accompanied by more frequent, typically daily, weekly or fortnightly, monitoring of the lake's abiotic and biotic features including water level, water temperature and phosphorus concentrations (Winfield *et al*., 2008a). The present analysis is based primarily on annual mean inshore surface water temperature, together with basin-specific mean concentrations of TP and Chl *a* during May to October of each year. TP and Chl *a* concentrations were determined from integrated surface water samples collected using a weighted plastic tube according to Mackereth *et al*. (1978) and Talling (1974), respectively. Details of the methodology used to determine water temperature are given by Winfield *et al*. (2008a), those used for TP concentrations and Chl *a* are given by Parker and Maberly (2000).

Lake Geneva

In Lake Geneva, some physical parameters started to be regularly monitored at the end of the 1950s and a standardized long-term monitoring of physical and chemical variables, as well as plankton communities, was launched in 1974. Sampling takes place 1 or 2 times per month in the middle of the lake at its deepest part. Sampling protocols and analytical methods for physical, chemical and plankton variables are described in CIPEL annual reports (http://www.cipel.org/documentation/publications-cipel/) and on the website dedicated to the Observatory of LAkes (OLA) (http://www6.inra.fr/soere-ola). Water temperature was measured at discrete depths with a thermometer until 1998, after which multiprobes were used (Sharma *et al*., 2015). Water for nutrient measurements was collected at discrete depths and TP concentrations were estimated according to a standardized protocol (AFNOR NF EN 1189, Monod *et al*., 1984). Water for estimating phytoplankton as Chl *a* was sampled at discrete depths and filtered through a Whatman GF/C filter (47mm). The pigments were extracted with 90% (v/v) acetone/water, the solution was filtered through a GF/C filter (25mm) and Chl *a* concentration was measured by spectrophotometry (Strickland and Parsons, 1968). Zooplankton was sampled from a depth of 50 m to the surface using a 200 µm mesh plankton net. Samples were preserved in a 5% buffered formaldehyde solution. Zooplankton species were identified and individuals were enumerated in a 0.1 ml sedimented subsample using a dissecting microscope.

Fish abundance data used in this study include commercial landing statistics compiled annually by the cantonal fisheries agency in Switzerland and the Haute-Savoie's Direction Départementale des Territoires (DDT) in France. French and Swiss commercial landing data are available since 1950, data on the number of French professional fishers are available since 1979, and fishing activity has been recorded for the last few years. The fishing activity is thought to be fairly constant since the numbers of commercial fishing permits and nets were kept constant at least until 1988 (Gerdeaux, 1988; Gerdeaux *et al.*, 2006). For recent years, CPUE values (kg/fisherman) have been computed based on French fish statistics provided by the Haute-Savoie's DDT. The available French data allowed CPUE computation per species (Anneville *et al.*, 2017) for the period 1979-2012. As these CPUEs indicated significant correlations with the French landings ($p < 0.005$), we assumed that French catches give a good indication of the abundance of the different targeted fish species from the whole lake. Therefore, French catches were used in this analysis as a proxy of fish abundance.

Lake Maggiore

Data on fish and fisheries in Lake Maggiore remained scattered until the end of the 1970s when the Italian-Swiss Commission for the Fishery (Commissione Italo-Svizzera per la Pesca – CISPP) was created under the International Commission for the Protection of Italian-Swiss water (CIPAIS). Since then, the total annual catch for each species of commercial interest and the number of active commercial fishermen have been recorded annually (Volta *et al.*, 2011). This enabled the calculation of CPUE both for the total catch and for the most important commercial species as the harvest divided by the number of fishers (tonnes/individual per year).

Water temperature has been measured and samples for TP analysis have been collected monthly since 1979 at the deepest point of the lake at 0, 5, 10, 20, 30, 50, 100, 150, 200, 250, 300 and 360 m depth. TP was analysed by spectrophotometry, after mineralization of the samples, according to Valderrama (1981). Mean volume-weighted values were calculated for the epilimnion (0-25 m) and for the whole water column (0-360 m). Mean annual values were calculated as the average of 12 monthly values. Samples for Chl *a* and phytoplankton analysis were collected as integrated water from 0-20 m layer. Chl *a* was determined spectrophotometrically after 90% acetone extraction (Lorenzen, 1967) until 2010, then a fluorimetric *in vivo* method was adopted using a bbe Fluoroprobe instrument. Between 2008 and 2010, when the two methods were compared, a strong correlation was found ($r=0.9$, $n=27$, $p<0.0001$; Morabito, unpublished data). Phytoplankton samples were preserved in acidic Lugol's solution; algal cells were counted under a Zeiss Axiovert 10 microscope, following Lund *et al.* (1958). Zooplankton samples were collected with two Clarke-Bumpus plankton samplers (126 and 76 µm mesh size) towed together at a constant speed of *ca* 3 km/h, along sinusoidal hauls from 0 to 350 meters, within stacked layers, each of them with a 50 meters thickness. The samples were preserved in ethanol and counted entirely, with identification of species and developmental stages.

Prediction of fish standing stock

To assess the impact of fishery activities on standing stocks, we first needed to estimate the fish biomass in each lake basin (i.e. our fish community response variable). To do this, we used an established relationship between areal fish biomass and TP concentration, derived from lake data spanning a similar TP concentration range to our focal lakes (Yurk and Ney, 1989). Based upon this, we used annual TP concentrations in upper/mixed layers (Figure 1A) to predict fish biomass, thus:

$$\log_{10}\text{Fish (kg ha}^{-1}\text{)}=1.07+1.14*\log_{10}\text{TP (mg m}^{-3}\text{)} \qquad (1)$$

Statistical analyses

To assess the evidence for long-term changes in the state of the case study lakes, we applied non-parametric Mann-Kendall tests to detect monotonic trends, and parametric cumulative deviation tests to detect step changes (temporal breakpoints) in our measured variables (eWater toolkit http://www.toolkit.net.au/Tools/TREND, last accessed on 16 June 2016). Details of these methods are described by Kundzewicz and Robson (2004).

Fish occupying different ecological niches and trophic positions are likely to respond differently to environmental pressures. Therefore, when examining correlations between measures of fish stocks and environmental parameters we distinguished between the main plankti/benthivorous (MPB) and main piscivorous (MPi) fish species for each case study lake. Pikeperch was considered the MPi and bream the MPB in Lake Võrtsjärv; pike the MPi and perch the MPB in Windermere (as the body size of this species is generally small in this lake); pike the MPi and whitefish the MPB in Lake Geneva; pikeperch the MPi and coregonids the MPB in Lake Maggiore. To assess these correlations, we used non-parametric Spearman rank order correlation analysis (STATISTICA version 12, StatSoft, Inc.).

RESULTS

Multiple pressures related to fish communities

Fisheries pressure

Mean fish standing stocks calculated from annual TP concentrations varied from 153 kg ha^{-1} in Lake Maggiore up to1034 kg/ha in Võrtsjärv (Table 3). In Lake Geneva and Windermere less than 1% of this theoretical standing stock was removed from the lake annually, while in Võrtsjärv (1.4%) and in Maggiore (7.9%), the fishing pressure was 1-2 orders of magnitude greater (Table 3, Fig. 3).

Environmental pressures

Though subject to marked inter-annual variation, TP concentrations exhibited a long-term decrease in all case study lakes while Chl *a* concentrations decreased only slightly (Geneva and Maggiore) or even increased (Võrtsjärv and Windermere) (Table 2). Increasing trends of water temperature have been recorded in all lakes.

In Lake Maggiore, phytoplankton biomass was significantly positively correlated with TP and negatively with the CPUE of main piscivore, while it was *vice versa* in Lake Geneva. MPB and MPi were positively correlated in Võrtsjärv and Geneva, not correlated in Windermere, and negatively correlated in Maggiore. In Windermere, Geneva and Maggiore, MPi was positively correlated with water temperature (WT). In Geneva MPB was positively correlated with WT and negatively with TP, while in Maggiore it was *vice versa*. *Daphnia* was significantly negatively correlated with Bphyto, MPi and MPB in Lake Geneva but not in other lakes (Figure 4).

Some correlations showed regular changes along gradients of mean depth, TP, and Chl *a* (Fig. 5). For example, the correlation between phytoplankton and MPB was strong and positive in deeper lakes with lower TP concentration (Geneva and Maggiore) but negative and weak in shallower lakes with higher TP concentration (Võrtsjärv and Windermere). The correlation between water temperature and MPi was positive in lakes with low and moderate TP and chlorophyll *a* concentration but turned negative in Võrtsjärv characterised by high TP and Chl *a*.

DISCUSSION

Factors controlling fish communities

Among our case study lakes, Geneva and Maggiore have undergone drastic reductions in nutrient loading and considerable changes in fish communities. During this re-oligotrophication, the total fish CPUE and especially that of coregonids has substantially increased in Lake Geneva (Gerdeaux *et al.*, 2006) but decreased in Maggiore (Volta, 2000). It must be noted, however, that the “starting point” of the re-oligotrophication trend was much higher in Lake Geneva where TP values have only now reached those from which Lake Maggiore started to decline at the end of the 1970s. Hence, these contrasting nutrient ranges may be the reason for the observed different responses of fish communities through food web interactions and differences in reproductive success and survival (Massol *et al.*, 2007).

In Lake Geneva, the decrease in TP concentration was accompanied by a decrease in roach and perch an increase in whitefish CPUE (Gerdeaux, 2004; Anneville *et al*., 2017), thus the observed switch from percid and cyprinid to coregonid dominated community could have been induced by a change in the lake's trophic status (Jeppesen *et al*. 2005). However, changes in species contributions to commercial landings may also reflect changes in the habits of fishers. While the decrease in the contribution of perch to landings suggests a decrease in its abundance due to re-oligotrophication (Dubois *et al*., 2013), the contrasting fishery values of perch and roach and their consequently differing target profiles may complicate interpretation of the observed trends in catches.

The increase in whitefish abundances in Lake Geneva correlated strongly with the decreasing TP concentration and increasing temperature (Fig. 4). Re-oligotrophication potentially increases reproductive success by re-oxygenation of spawning areas, improving egg survival which is strongly influenced by oxygen concentrations at the water-sediment interface (Müller 1992).Warmer spring temperatures allow whitefish larvae to grow faster, and provide a better temporal match with the seasonal development of their prey species

(Anneville *et al*., 2009). In addition, a change in the age structure of the whitefish population in the 2000s also probably contributed to the increase in the population (Anneville *et al*., 2017). Because of the high fishing pressure, the whitefish cohort entering the stock used to be completely harvested (Caranhac and Gerdeaux, 1998). Before the 2000s, catches were made up of only few cohorts, mainly age 2+ or 3+, while fishes older than 4 years were rare in the lake. In contrast, studies have shown that fish caught by French fishers in recent years are older (Anneville *et al*., 2017). Such a change in the age structure of the catches indicates that the stock is not totally harvested anymore. The non-harvested brood stock now survive to spawn for several years and thus can contribute to the expansion of the stock. In Lake Geneva, the percentage of the calculated fish standing stock caught annually was one of the lowest among our case study lakes (0.17%) and this could explain the continuously increasing fish biomass (CPUE) in this lake (Anneville *et al*., 2017). However, phosphorus concentration alone is apparently insufficient to estimate fish stock as the field data indicate that the relationship between phosphorus and fish is not so simple. In Lake Geneva, for example, low phosphorus concentrations are associated with high annual catches dominated by coregonid species (whitefish) which are sensitive to trophic status and whose reproductive success is impaired by eutrophic conditions. So, depending on fish community composition, the model may or may not be appropriate. Furthermore, the model makes the expected and general prediction that eutrophic lakes are more productive for fish than are oligotrophic lakes. However, in the range of phosphorus variations observed in our case study lakes, parameters other than phosphorus such as pressure from fisheries and the balance between predatory and non-predatory fish may explain a considerable part of the observed variability in fish abundance.

In Lake Maggiore, the pressures impacting the coregonid populations (lavarello and bondella) were rather different. The two deep lakes differ in their "trophic history" with Maggiore switching from mesotrophy to oligotrophy and Geneva from eutrophy to mesotrophy. In contrast to Geneva, the change in coregonid harvest in Maggiore (mainly consisting of the bondella) was positively correlated with increasing TP concentration and negatively correlated with epilimnion temperature (Fig. 4), but, according to Massol *et al*. (2007), data from both lakes suggest that coregonids show highest catches at intermediate TP concentrations (15-30 µg L^{-1}). Among our case study lakes, the highest percentage of the calculated fish standing stock was caught annually in Maggiore (up to 25%,exceeding other lakes by 1-2 orders of magnitude) (Table 3). We acknowledge that the fish biomass values calculated from TP concentrations are only crude estimates. However, even with this uncertainty it is still clear that the fishing pressure in Maggiore has been much stronger than in the other lakes. Although re-oligotrophication and the introduction of several fish species have undoubtedly had a strong impact on Lake Maggiore ecosystem, the high fishing pressure is likely to be among the reasons explaining the strong reduction in coregonids, trout and perch CPUE and is thus regarded as an important factor controlling the fish community in this lake.

Strong impacts of fisheries management measures on fish community composition and the balance between predatory and non-predatory fish species have been demonstrated in Võrtsjärv, where the banning of small-meshed fishing gear in the 1970s caused a major change in the age and size structure of fishes and contributed to the establishment of predatory fish control over previously dominant ruffe and roach populations (Nõges *et al*., 2016). Neither of the fish feeding groups' abundances were correlated with *Daphnia* or TP and only temperature was significantly negatively correlated with the main piscivore abundance (Fig. 4).

In Windermere, where commercial fisheries are absent, higher temperature was associated with higher CPUE of the main piscivore (pike) as has also been observed over a longer time scale by Edeline *et al*. (2016). No direct impact was detected on perch, the main planktivore in this lake, during the present study (Fig. 4), even though over a longer time scale this environmental parameter has been shown to have an important effect on recruitment (Paxton *et al*., 2004).

Top-down effects in lake ecosystems

Changes in fish abundance in Lake Geneva may have had strong implications for zooplankton. Long-term changes in whitefish (MPB) abundance were strongly correlated with inter-annual changes in *Daphnia* abundance (Fig. 4). The negative correlation between *Daphnia* abundance and whitefish catches suggests whitefish control of cladoceran population which according to Alric *et al*. (2013) have been under strong top-down pressure during re-oligotrophication of Lake Geneva. Although changes in zooplankton abundance can also be caused by a bottom-up mechanism if changes in phytoplankton species composition alter their palatability and food value for zooplankton (Perga and Lainé, 2013), our results support rather the top-down hypothesis that the effect of the increasing abundance of zooplanktivorous whitefish has contributed to the long-term decrease in *Daphnia*. Re-oligotrophication has brought about only a slight reduction in phytoplankton biomass in this lake (Table 2). As the abundance of *Daphnia* was strongly negatively correlated with both whitefish and phytoplankton abundance (Fig 3), the substantial increase in whitefish feeding pressure on zooplankton could presumably reduce the grazing impact of zooplankton on phytoplankton and so enable phytoplankton biomass to increase despite the reduction in TP levels.

As phytoplankton biomass in Maggiore correlated positively with planktivorous coregonids and negatively with the main piscivore (pikeperch), we can draw a general conclusion of strong cascading effects of fisheries on the ecosystem of this lake. In Maggiore, a simultaneous reduction in TP and phytoplankton took place in the 1980s-1990s, while in the 2000s occasional high phytoplankton peaks occurred, such as that recorded in summer 2011, caused by an exceptional bloom of *Mougeotia* sp. (Fig. 2B). Blooms of this taxon are known to occur in the deep peri-alpine oligo-mesotrophic lakes, although the driving factors are still not completely understood (Tapolczai *et al*., 2015). In Maggiore, the abundance of pikeperch was rather strongly negatively correlated with both whitefish and phytoplankton (Fig. 4) which could reflect a cascading effect of the main piscivore on phytoplankton through the food chain. However, the cascading effect and the phytoplankton response could have been confounded in Lake Maggiore due to the strong nutrient limitation on phytoplankton growth which developed during the re-oligotrophication phase.

In Võrtsjärv, the present correlative analysis and a recent study by Nõges *et al*. (2016) demonstrated that the main predator (pikeperch) could exert control over phytoplankton, reflected by a significant negative correlation between phytoplankton and pikeperch biomasses (Fig. 4) most likely caused by a cascading top-down effect through the food web. Supporting this, Nõges *et al*. (2016) found negative correlations between phyto- and zooplankton biomasses in this lake and a shift in zooplankton size structure relative to pikeperch biomass: higher pikeperch abundances were associated with smaller rotifers and larger copepods. In addition, the individual weight of crustacean zooplankton was smaller in years of high abundance of small fish that stimulated ciliate domination over metazooplankton and enhanced the domination of the microbial food web.

In Windermere, phytoplankton was likely primarily bottom-up controlled by phosphorus as no strong correlation with any of its major fish species was detected in this study, although after taking into account the effects of a pathogen outbreak on perch population structure in the 1970s, Edeline *et al*. (2016) found indications from this longer data set that a pike-dominated intra-guild predation triggered a temperature-controlled trophic cascade passing through pike down to dissolved nutrients.

Across all of our case study lakes, we found a stronger link between phytoplankton and planktivorous fish, and thus a more important cascading top-down effect, in the relatively deeper lakes Geneva and Maggiore (Fig. 5A). The strengths of these connections mean that, for such lakes, careful ecosystem-based fishery management is of utmost importance for maintaining high water quality and related ecosystem services such as recreational value and suitability as drinking water.

Our results also demonstrated that at certain levels of phosphorus loading, increasing water temperature might favour piscivores (Fig. 5D) and thus enhance the potential for a cascading top-down control over phytoplankton. Such an effect could counteract the commonly envisaged impact of climate change supporting elevated phytoplankton development and cyanobacterial blooms (Paerl and Huisman, 2008). Indeed, higher temperatures may be expected to reinforce top-down control in food chains dominated by ectothermic top predators such as fish by increasing consumption rates faster than primary production (Vasseur and McCann, 2005; Ohlberger *et al*., 2011). Trophic amplification by climate change – the intensification of trophic interactions and pathways through the food web (Kirby and Beaugrand, 2009; Van Looy *et al*., 2016) – can result in totally different effects compared to laboratory or microcosm experiments with strongly simplified biotic structure. An increase in fish predation pressure on zooplankton and higher importance of nutrient loading in warm southern lakes was found also by an experimental study undertaken along a latitudinal gradient in Europe (Moss *et al*., 2004). A higher degree of omnivorous feeding by fish and less piscivory in subtropical and tropical lakes than in temperate lakes has been found to limit the success of fish-based biomanipulation methods in warmer climates (Jeppesen *et al*., 2005). In agreement with these findings, our results showed that at high P loadings and Chl *a* concentrations the correlation between water temperature and piscivores turned negative (Figs. 5C,D). This finding means that in eutrophic lakes the loss of piscivores in warmer waters might amplify the generally anticipated warming effect of increased frequency of phytoplankton blooms. As a result, this cascading effect also has considerable potential to cause a much greater and wider loss of ecosystem services beyond those directly associated with commercial and recreational fisheries.

CONCLUSIONS

The assessment of long-term concurrent effects of fisheries, changing trophic state and changing climate upon lake ecosystems in five European lake basins of differing trophic states (Lake Võrtsjärv, two basins of Windermere, Lake Geneva and Lake Maggiore) revealed that:

- Decreasing phosphorus concentrations (re-oligotrophication) and increasing water temperatures in all five lake basins have coincided with no or slight decreases in phytoplankton concentrations.
- Parameters other than phosphorus, including fisheries pressure and the relative abundances of predatory and non-predatory fish species, explained a significant part of the observed overall variability in fish abundance.
- A stronger link between phytoplankton and planktivorous fish was observed in Lakes Geneva and Maggiore, suggesting a more important cascading top-down effect in these relatively deeper lakes which makes their careful ecosystem-based fisheries management extremely important for maintaining high water quality.
- Our analyses indicated that increasing water temperature may favour piscivores at low phosphorus loadings, but suppress them at high phosphorus loadings and may thus either enhance or diminish the cascading top-down control over phytoplankton with strong implications for water quality.

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Tables

Table 1

General characteristics of the case study lakes. TP and chlorophyll *a* (Chl *a*) concentrations in the upper/mixed water columns are the mean values of the latest year of the time series presented in Fig. 2 and Table 2.

Lake basin	**Latitude Longitude**	**Altitude (m.a.s.l.)**	**Lake area (km^2)**	**Mean depth (m)**	**Max. depth (m)**	**Annual mean TP mg m^{-3} Latest year**	**May-Oct mean Chl *a*, mg m^{-3} Latest year**
Võrtsjärv	58°17′N 26°02′E	34	270	2.8	6	42	47
Windermere North Basin	54°21′N 2°56′W	39	8.1	25.1	64	16.6	8.2
Windermere South Basin	54°21′N 2°56′W	39	6.7	16.8	39	19	11.3
Geneva	46°27′N 6°32′E	372	582	153	309	14	4.5
Maggiore	46°5′N 8°43′E	193	212	177	370	6.4	2.4

Table 2
Time series lengths, upward (↑) and downward (↓) trends and breakpoints, and mean concentrations of total phosphorus (TP) and chlorophyll *a* (Chl *a*) in the upper/mixed water layer of the case study lakes. Average values of TP and Chl *a* were calculated for the entire period, as well as before and after any temporal breakpoints detected. In Windermere NB – North Basin, SB – South Basin. no = no detected breakpoint.

Lake, water layer	Time series length	Mann-Kendall trend	Breakpoint by Cumulative deviation test	Average before breakpoint	Average after breakpoint	Average for whole period
	Annual TP	**Annual TP**	**Annual TP**	**Annual TP, mg m^{-3}**	**Annual TP, mg m^{-3}**	**Annual TP, mg m^{-3}**
Võrtsjärv, 0-3m	1983-2014	↓ $p<0.01$	↓1990, $p<0.1$	67	45	50
Windermere NB, 0-7m	1980-2012	no	no			14
Windermere SB, 0-7m	1980-2013	↓ $p<0.01$	↓ 1992, $p<0.01$	25	19	21
Geneva, 0-20m	1974-2011	↓ $p<0.01$	↓ 1988, $p<0.01$	49	19	31
Maggiore, 0-25m	1979-2014	↓ $p<0.01$	↓ 1989, $p<0.01$	13	8	10
	May-October Chl *a*	**May-October Chl *a***	**May-October Chl *a***	**May-Oct Chl *a* mg m^{-3}**	**May-Oct Chl *a* mg m^{-3}**	**May-Oct Chl *a* mg m^{-3}**
Võrtsjärv, 0-3m	1982-2013	↑ $p<0.01$	↑ 1997, $p<0.01$	33.5	49.6	41.5
Windermere NB, 0-7m	1966-2012	↑ $p<0.1$	↑ 1980, $p<0.05$	7.3	8.8	8.3
Windermere SB, 0-7m	1966-2012	no	no			12.9

Geneva, 0-20m	1976-2012	↓ $p<0.05$	no			5.6
Maggiore, 0-25m	1984-2013	↓ $p<0.01$	↓ 1997, $p<0.05$	4.8	3.7	4.2

Table 3

Annual theoretical fish standing stocks calculated on the basis of annual average total phosphorus (TP) concentration in the upper/mixed water column (see Fig. 2A) from the relationship $\log_{10}$Fish (kg ha^{-1}) = 1.07+1.14*$\log_{10}$TP (mg m^{-3}) published by Yurk and Ney (1989); annual catch values based on fishery statistics and % of the annual catches from the calculated fish standing stocks in case study lakes in 1980-2011. NB - North Basin, SB – South Basin.

		Years	n	Mean	Minimum	Maximum	Std.Dev.
Võrtsjärv	Annual TP mg m^{-3}	1983-2014	32	50	22	129	20
	Fish stock, kg ha^{-1}	1983-2014	32	1034	400	2993	475
	Fish stock, tonnes lake^{-1}	1983-2014	32	27913	10804	80805	12825
	Annual catch tonnes lake^{-1}	1980-2011	32	340	183	720	124
	Annual catch, % of stock	1980-2011	29	1.4	0.6	4.1	0.7
Windermere NB	Annual TP mg m^{-3}	1980-2012	33	14	11	17	1.4
	Fish stock, kg ha^{-1}	1980-2012	32	229	180	287	25
	Fish stock, tonnes lake^{-1}	1980-2012	32	185	146	233	21
	Annual catch tonnes lake^{-1}	1980-2012	33	0.51	0.09	0.97	0.23

	Annual catch, % of stock	1980-2012	32	0.28	0.04	0.56	0.14
Windermere SB	Annual TP mg m^{-3}	1980-2012	33	21	13	31	4
	Fish stock, kg ha^{-1}	1980-2012	33	383	225	592	82
	Fish stock, tonnes lake^{-1}	1980-2012	33	257	151	396	55
	Annual catch tonnes lake^{-1}	1980-2012	33	0.4	0.1	0.6	0.1
	Annual catch, % of stock	1980-2012	32	0.1	0.0	0.2	0.0
Geneva	Annual TP mg m^{-3}	1974-2011	38	31	14	56	16
	Fish stock, kg ha^{-1}	1974-2011	32	504	235	1130	294
	Fish stock, tonnes lake^{-1}	1974-2011	32	293103	136888	657512	171362
	Annual catch tonnes lake^{-1}	1980-2011	32	363	169	708	126
	Annual catch, % of stock	1980-2011	32	0.17	0.03	0.51	0.12
Maggiore	Annual TP mg m^{-3}	1979-2014	36	10	6	19	3
	Fish stock, kg ha^{-1}	1979-2014	32	153	93	284	46
	Fish stock, tonnes lake^{-1}	1979-2014	32	3241	1978	6022	977
	Annual catch tonnes lake^{-1}	1980-2010	31	265	39	723	197
	Annual catch, % of stock	1980-2010	31	7.9	1.3	25.2	5.2

Figures

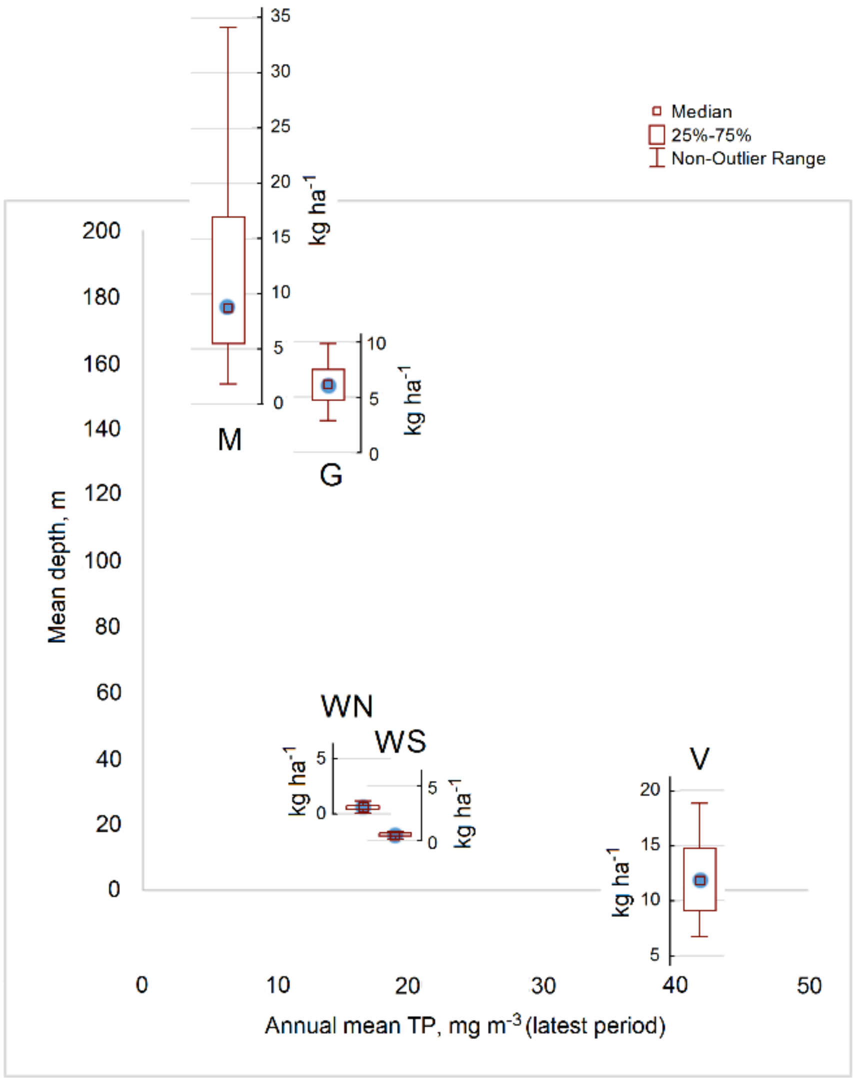

Fig. 1. Ecological gradients among the study lakes. The lake basins are plotted in a plane defined by their mean depth and annual mean total phosphorus concentration, with summaries of annual fish catches indicated by boxplots. The location of the median fish catch indicates the position of each lake basin in the TP – mean depth plane. Maggiore (M), Geneva (G), Windermere North Basin (WN), Windermere South Basin (WS), and Võrtsjärv (V).

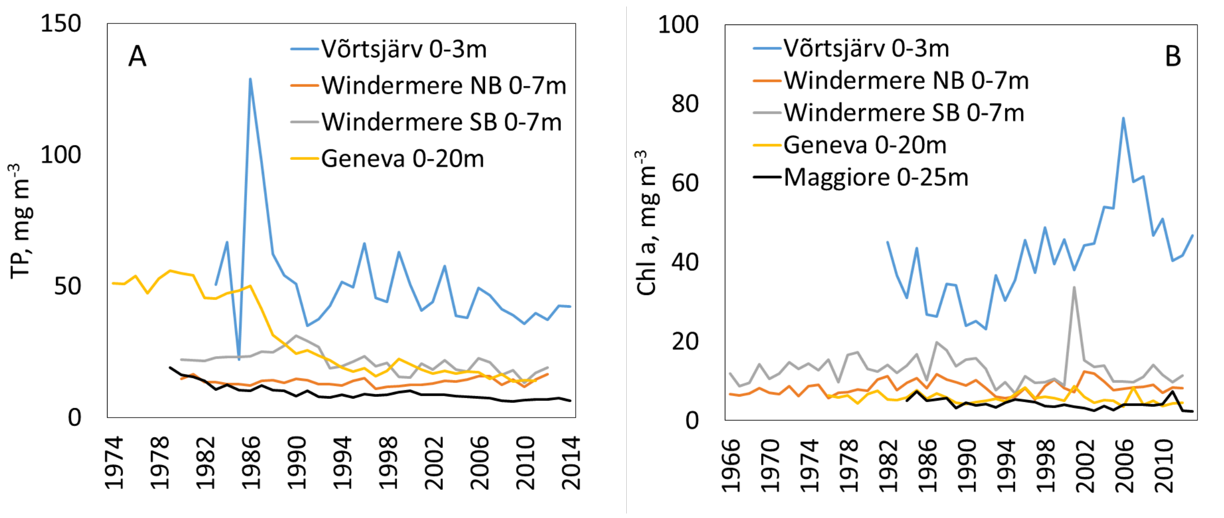

Fig. 2. Time series of annual average total phosphorus (TP: A) and May-October average chlorophyll *a* concentration (Chl *a*: B) in the upper/mixed water column of case study lakes In Windermere NB – North Basin, SB – South Basin.

Fig. 3

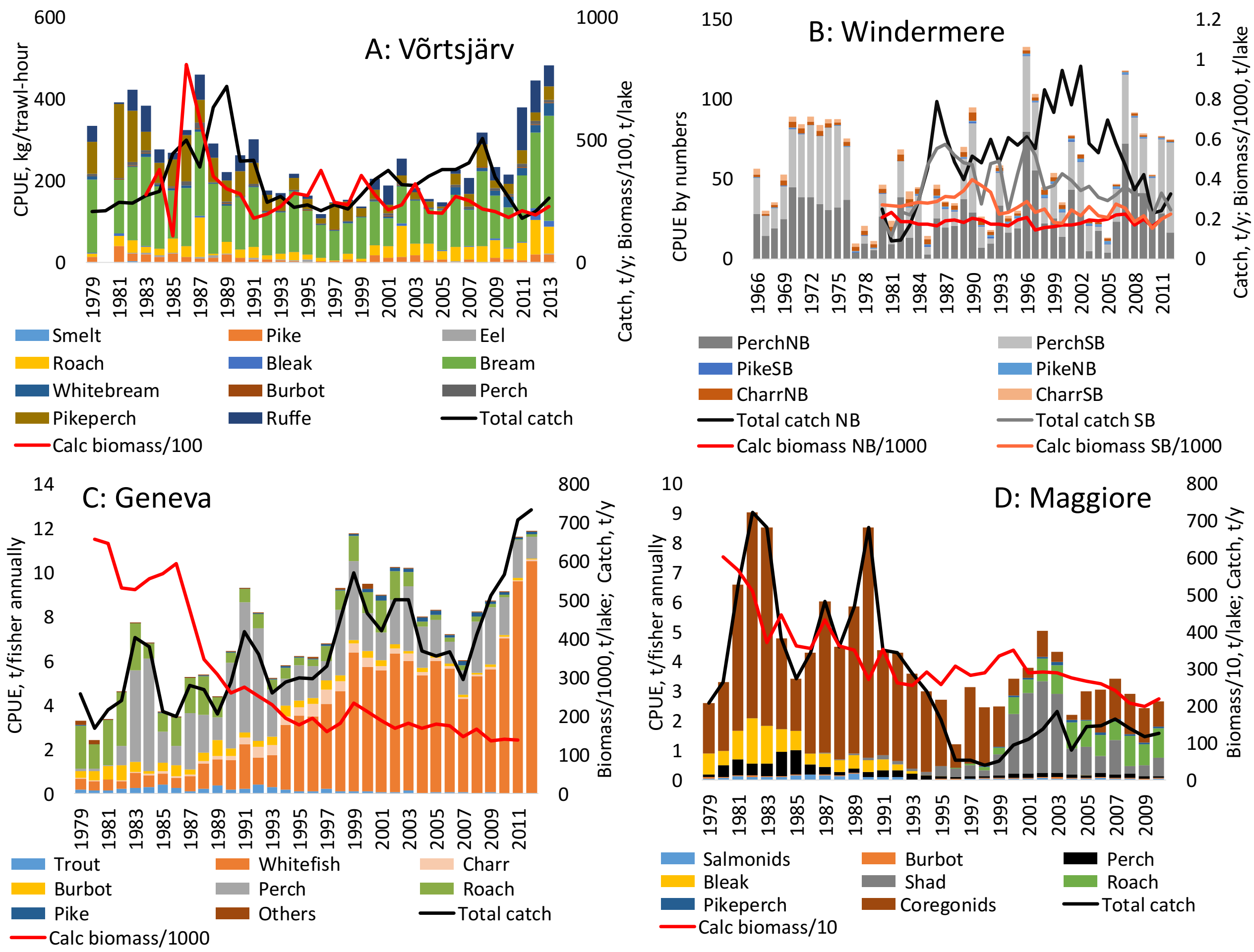

Fig. 3. Catch per unit effort (CPUE) by fish species, calculated fish biomass based on total phosphorus (TP) concentration (see Table 3) and total catch data in Lake Võrtsjärv in 1979-2013 (A); CPUE by fish species, calculated fish biomass based on TP in Windermere in 1966-2012: NB - North Basin, SB – South Basin (B); CPUE by fish species, calculated fish biomass based on TP and total catch data in Lake Geneva in 1979-2012 (C) and CPUE by fish species (Salmonids = trout + Arctic charr; Coregonids = lavarello + bondella), calculated fish biomass based on TP and total catch data) in Lake Maggiore in 1979-2010 (D), t -metric tonnes.

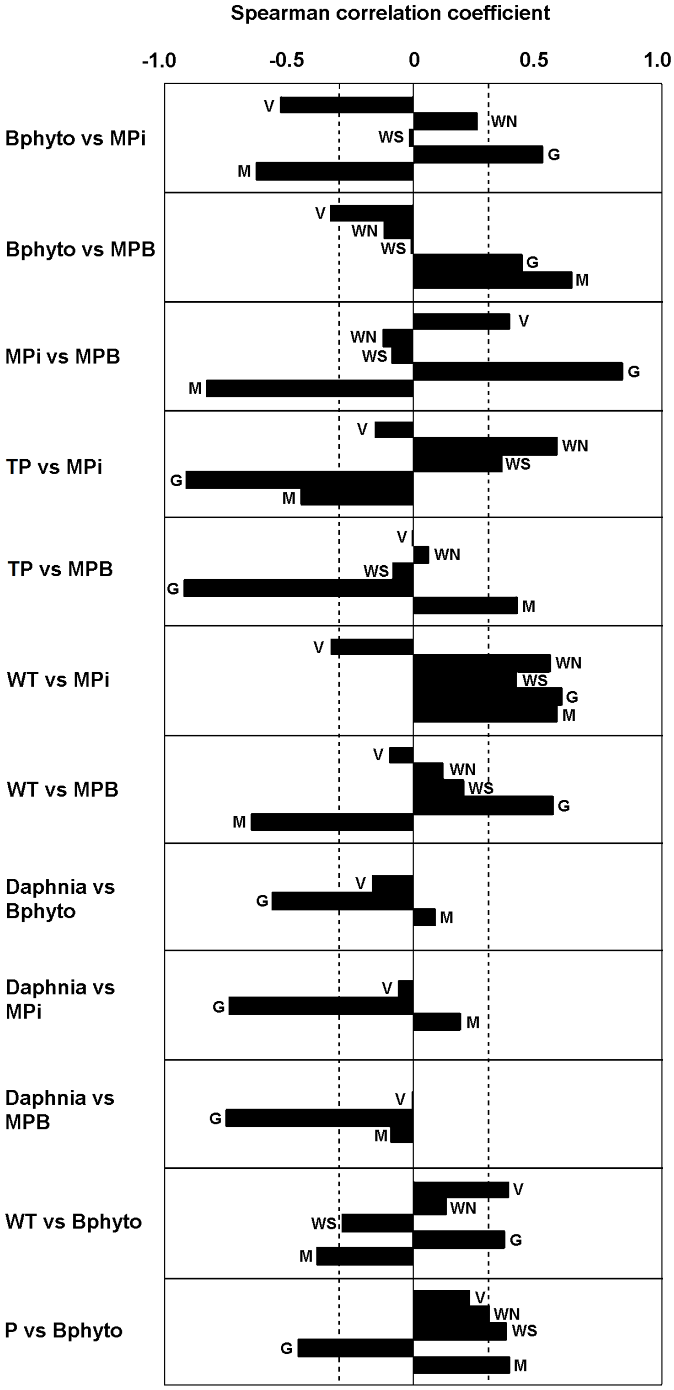

Fig.4. Spearman correlation coefficients of fish feeding groups (catch per unit effort of main piscivorous (MPi) and main plankti/benthivorous (MPB) fish species; see explanation in text) with phytoplankton biomass (Bphyto), *Daphnia*, total phosphorus (TP) and water temperature (WT) in case study lakes Võrtsjärv (V), Windermere North Basin (WN), Windermere South Basin (WS), Geneva (G), and Maggiore (M). Dashed lines denote the significance level at p=0.05.

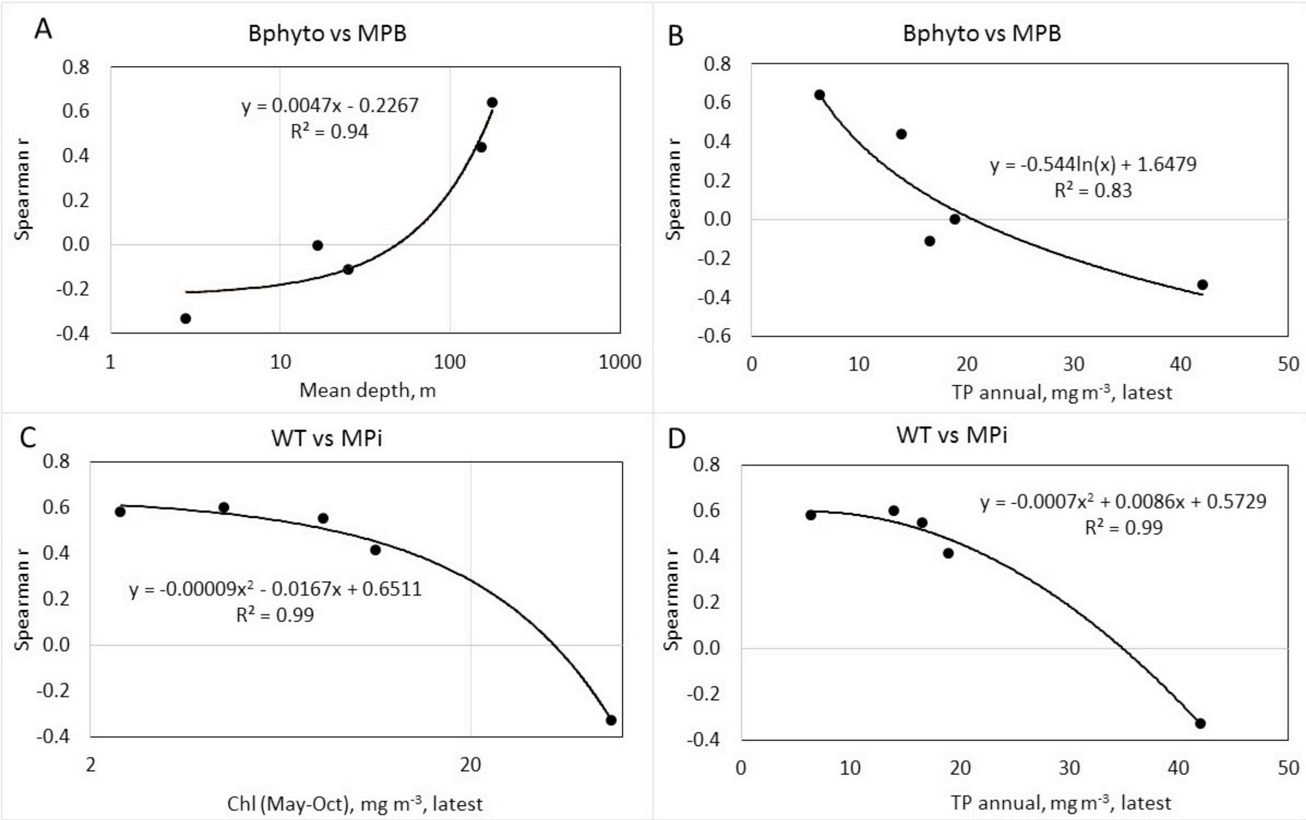

Fig. 5. Changes in the strength of the correlation between phytoplankton and main plankti/benthivorous fish (MPB) along gradients of lake depth (A) and annual average total phosphorus (TP) concentration of the latest year that our dataset includes, as shown in Table 1 (B); Changes in the strength of the correlation between water temperature (WT) and main piscivorous fish (MPi) along gradients of chlorophyll *a* (Chl*a*) concentration (C) and TP concentration in the latest year of the time series (D). Each point represents one lake basin.

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MARS PROJECT

Managing Aquatic ecosystems and water Resources under multiple Stress

Deliverable 4.2-5: Multiple-stressor risks for pathogens

Lead beneficiary: **NERC**

Contributors: Daniel S. Read, Mike J. Bowes, Soon Gweon, Anna Oliver, Lindsay Newbold, Glenn Rhodes, Laurence Carvalho **(NERC)**

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Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Content

Non-technical summary

A wide variety of disease-causing pathogenic microorganisms, including bacteria, are spread and transmitted via water. There are many potential sources of waterborne diseases in freshwater environments, although the main recognised routes are via direct contamination of water by faeces from both humans and animals, as well as by pathogens that can survive and are distributed by sewage treatment processes. Understanding the dynamics of both pathogenic bacteria and bacteria associated with faeces and sewage infrastructure can be helpful in determining the sources of bacteria and how they interact with freshwater environments. Many freshwaters are experiencing a high degree of degradation in the form of interacting stressors such as elevated nutrients, chemical pollution, modified morphology and flows, as well as increases in algal bloom frequency and intensity. It is important to understand how these multiple stressors, particularly those associated with climate change, interact to affect pathogen abundance and dynamics. This study used DNA sequencing to investigate the microbial composition of a lowland river, the river Thames, UK, that is experiencing multiple stressors, at a weekly resolution over a two-year period. It aimed to examine whether new molecular approaches, such as high throughput sequencing of the 16S rRNA bacterial gene, could provide more targeted indication of pathogenic bacteria loads rather than proxies, such as Faecal Indicator Organisms (FIOs). Families and genera of bacteria were selected to act as indicators of pollution from faeces, sewage infrastructure and a group of potentially pathogenic bacteria. Strong seasonal dynamics in abundance were observed, with most faecal and sewage indicators increasing in abundance during the winter months. Of the potential pathogen indicators, no confirmed pathogens were identified to species level, but closely related genera were present in low abundance, or if in higher abundance, were more likely associated with pathogens or parasites of natural populations of aquatic wildlife. These results indicate that whilst faecal and sewage indicators show that contamination via these routes is occurring in the river Thames, it is not associated with high loads of pathogen species. Future studies covering a wider range of sites are recommended to identify potential hotspots associated with agriculture or sewage treatment.

Pathogen dynamics in the river Thames (UK) over a two year period.

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Introduction

Outbreaks of waterborne disease is a global issue that occurs in both developed and developing countries (Brookes *et al.* 2004). Disease causing microorganisms, such as *Vibrio cholera* (the causative agent of cholera), *Campylobacter*, and *Salmonella* have long been associated with water (Koelle 2009; Van Dyke *et al.* 2010) and such pathogens are frequently transmitted to humans via drinking water. As a result, the demand for water, primarily driven by increasing populations, recreational use and agricultural practices makes clean, drinkable water a valuable resource (Ferguson *et al.* 2003). Even in developed countries where drinking water treatment processes can reliably remove pathogens (Bennett 2008), there is a need to reduce surface water contamination and pathogen loads from agriculture and storm overflows to prevent the spread of disease amongst livestock, wild animals and participants of recreational activities involving water such as swimming, boating and fishing (Dubinsky *et al.* 2016), as well as improving efficiency and reducing the risk of water treatment failures.

Contamination of watercourses with faecal matter is one of the main sources of entry for waterborne pathogens, and contributes to the spread and dissemination of numerous human and animal diseases (Ferguson *et al.* 2003). Animal and human faeces can contain a mixture of both pathogenic and commensal microorganisms, including bacteria, viruses and protozoa (Odagiri *et al.* 2016), all of which can persist and be transported considerable distances through freshwater catchments (Ferguson *et al.* 2003). Sources of faecal contamination are diverse and can include discharges from anthropogenic sources, primarily through effluent from sewage treatment plants (Ye & Zhang 2011), but also runoff from urban areas contaminated with domestic and wild animal faeces (Belt *et al.* 2007), sewage misconnections (Newton *et al.* 2013) and combined storm overflows (CSOs). Animal sources include livestock with access to water, surface runoff from grazed land, as well as land with manure applications and discharges from stored manure and slaughterhouses (Pandey *et al.* 2014). Finally wild animals may pose a significant reservoir of pathogenic microbes (Daszak *et al.* 2000), particularly wild birds such as ducks, geese and swans that are associated with and defecate in surface waters (Hubálek 2004; Jamali *et al.* 2014).

There are numerous challenges to understand the factors that control, and ultimately predict the prevalence and composition of faecal contamination in freshwaters. As already mentioned, the sources and processes by which microbes may be transported into water are diverse. Once in water, survival, retention and transport rates influence how microbes are spread and persist, and these are profoundly influenced by the physical and biological configuration of the freshwater environment. Freshwater catchments, and the rivers and streams within them, are highly dynamic systems that are exposed to multiple stressors that can interact both spatially and temporally, affecting pathogen abundances. These include chemical stressors in the form of nutrients (Bowes *et al.* 2015) and organic pollutants (Petrie *et al.* 2015), physical modification via canalisation, weirs, dams, and dredging (Newson & Newson 2000), land use change such as urbanisation and agriculture (Tong & Chen 2002), as well as long term climate change that influences water temperature and flow (van Vliet *et al.* 2013). Understanding how these stressors interact with the prevalence and composition of faecally derived organisms is necessary to predict how future environmental change and increasing demand on both terrestrial and freshwater resources may impact microbial water quality.

A secondary challenge to understand the nature of freshwater disease transmission is that traditional approaches to characterise faecal contamination have relied upon proxies of pathogens, such as the quantitative cultivation of specific indicator organisms including *Escherichia coli*, enterococci or thermotolerant coliforms (TTCs). A number of studies have shown that the correlation between these indicators and the actual prevalence of pathogenic species is often poor (Marti *et al.* 2017), because these groups may have different dynamics in the environment. For example, *Escherichia coli* has been shown to survive and reproduce in the freshwater environment, potentially uncoupling the link between pathogenic species and indicators which may behave in a different manner (Anderson *et al.* 2005). An additional drawback of culturing based approaches is the fact that they lack host specificity and therefore do not indicate the source of pollution (Newton *et al.* 2013). An alternative approach that has been used to meet some of these challenges is the use of species and strain specific qPCR assays. A range of host-specific assays have been developed, particularly focussing on species from the phylum Bacteroidales, and in particular the genus *Bacteroidetes* (Reischer *et al.* 2013). However, the specificity of these assays is sometimes questionable (Boehm *et al.* 2013) and they require an *a priori* assumption of the source of contamination.

High throughput sequencing of the 16S rRNA gene is an alternative approach for characterising the composition of whole bacterial communities, not just specific pathogens or faecal indicators (Fisher *et al.* 2015). We have used this approach to examine a two year, weekly time series of samples collected from the middle reaches of the River Thames, UK. By linking the prevalence of potentially pathogenic and faecally derived organisms with a multi-season, high resolution dataset of physicochemical parameters including nutrients, flow and temperature, as well as cell numbers of bacteria and major phytoplankton groups, we can start to reveal the drivers of faecal contamination in the environment.

Materials and Methods

Study site

The River Thames is the second longest river in the United Kingdom, 354 km long from its source in the Cotswold Hills to its tidal limit at Teddington Lock in south west London. It has a catchment area of 9948 km^2 (Bowes *et al.* 2016). The Thames basin contains the city of London, and other major urban centres, including Swindon, Oxford, Slough and Reading (Figure. 1). The basin has a population density of approximately 960 people km^{-2} (Merrett 2007). The upper Thames basin is relatively rural, with approximately 45% of land area being classified as arable, 11% woodland, 34% grassland, and only 6% classed as urban and semi-urban development (Fuller *et al.* 2002). The River Thames at Reading has a mean annual flow of 38.9 m^3 s^{-1}, a base flow index of 0.68 and a mean annual rainfall of 744 mm (Bowes *et al.* 2016). The sampling site for this study is the River Thames at Wallingford, situated in the middle reaches of the Thames, downstream of the city of Oxford.

Sampling and contextual environmental variables

At each weekly sampling point, a water sample was collected by lowering a clean bucket into the centre of the river channel, transferred into 1 L autoclaved polypropylene bottles and stored in the dark until taken back to the lab for filtering witthin 24 hours. Samples were pre-filtered using bleach and acid washed vacuum filtration devices through 1.6 µm glass fibre GF/A filters (Whatman, Buckinghamshire, UK) to separate particle and eukaryote associated microbes, and then through a 0.22 µm Durapore membrane filter (Merck Millipore, Watford, UK) to collect the remaining microbial cells. Distilled water blanks were included as controls for filtration equipment contamination. All filters were stored in sterile containers at -80 °C for later analysis.

Water chemistry parameters, collected as described in (Bowes *et al.* 2012), included pH, alkalinity, suspended sediment, soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), total phosphorus (TP), ammonia (NH_4), dissolved reactive silicon, fluoride (F), chloride (Cl), nitrite (NO_2), nitrate (NO_3), total dissolved N (TDN), sulfate (SO_4) and dissolved organic carbon (DOC). Additionally, the concentrations of a suite of metals, including sodium (Na), boron (B), iron (Fe), magnesium (Mg), zinc (Zn), copper (Cu) and aluminium (Al) were measured by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Bowes *et al.* 2012).

DNA extraction and sequencing

DNA extraction from both membrane and glass fibre filters was carried out using the MoBio (Qiagen) PowerWater kit, following the manufacturer's instructions. As controls, filter blanks (clean unfiltered filters) and DNA extraction blanks (no sample added) were included. Amplification of the 16S rRNA gene used primers 341F (5'- CCTACGGGAGGCAGCAG -3') (Muyzer *et al.* 1993) and 806R (5'- GGACTACHVGGGTWTCTAAT -3') (Caporaso *et al.* 2011) employing the dual-index approach described in (Kozich *et al.* 2013). PCRs contained 2 µl of (10-20 ng/µl) template DNA, 1× PCR buffer containing 2 mM $MgCl_2$, 10 µM dNTPs, 0.2 U Q5 high-fidelity DNA polymerase (New England Biolabs, Hitchin, UK) and 0.5 µM of each primer, with each reaction made up to 25 µL with PCR water. PCR reactions used an initial denaturation of 95 °C for 2min, followed by 30 cycles of 95 °C for 20 s, 55 °C for 15 s, 72 °C for 5 min, and a final extension step of 72 °C for 10 min. PCR blanks (no DNA added to PCR) were included. The library, including all control samples, was sequenced at a concentration of 6 pM with a 10% PhiX control on an Illumina MiSeq using the 2x 300 bp V3 chemistry (Illumina Inc., San Diego, CA, USA).

Data processing and analysis

Sequenced paired-end reads were joined using PEAR (Zhang *et al.* 2014), quality filtered using FASTX tools (Hannon, http://hannonlab.cshl.edu) and chimeras were identified and removed with ChimeraSlayer (Haas *et al.* 2011). The sequences were clustered into operational taxonomic units with UCLUST (Edgar 2010) as part of the QIIME package (Caporaso *et al.* 2010) and representative sequences were selected (pick_rep_set.py, QIIME). The taxonomy of representatives was determined by QIIME's UCLUST consensus taxonomy assigner (assign_taxonomy.py, QIIME) using the Greengenes database release 13_2 (McDonald *et al.* 2012). The raw sequence data have been deposited in the EMBL-EBI European Nucleotide Archive (ENA), accession number XXXX. Sequencing barcodes are in Supplementary Table S1. All statistical analyses and visualisations were carried out in R (v.3.3.3) (R Core Team 2017) using the package 'Vegan' v2.0-10 (Oksanen *et al.* 2013). Proportional abundance data was converted into estimated cell counts by mapping relative taxa abundance onto bacterial cell counts as described in (Props *et al.* 2017).

Identification of indicator organisms (sewage infrastructure, faeces and potential pathogens)

Peer reviewed, government and health organisation literature was reviewed to identify pathogenic and faecal organisms that are known to be associated with freshwater. Organisms were selected to represent three categories of bacterial contamination of water; potential bacterial pathogens with a known association with water, sewer infrastructure (i.e. non-faecal, but sewage associated) bacteria and commensal organisms associated with mammalian guts. The full list of groups and their references are shown in Table 1. The species-level phylotype table, representing the two year, weekly dataset, was then searched for these taxa and the relative abundance of these groups plotted using R (R Core Team 2017).

Results and Discussion

The river Thames at Wallingford exhibited strong seasonal fluctuations in physicochemical conditions throughout the two-year sampling period. Both the winter of 2012/13 and 2013/14 experienced high rainfall and extensive flooding over both agricultural and urban land in the catchment above the sampling site (Hannaford *et al.* 2013; Parry *et al.* 2014). This is reflected in the flow rates, measured at Day's Lock, approximately three miles upstream the sampling point (Figure 2). Temperature peaked in summer at +20 °C, falling to around 5 °C in winter. Both Soluble Reactive Phosphorus (SRP) and Total Dissolved Phosphorus (TDP) peaked in September 2013. Neither ammonium (NH_4) and Nitrate (NO_3) showed clear seasonal patterns over the two-year sampling period, with ammonium peaking in April 2013 before falling to a lower level for the rest of the sampling period, and nitrate showing a clear peak in winter 2013/14.

The bacteria found in faeces, particularly human, comprise some of the best characterised microbial communities, due to their link to human health. We identified nine families of bacteria that have the potential to act as indicators of faecal contamination, due to their prevalence in human and animal faeces. These were *Bacteroidaceae*, *Porphyromonadaceae*, *Clostridiaceae*, *Lachnospiraceae* and *Ruminococcaceae, Lactobacillaceae, Prevotellaceae, Veillonellaceae, and Erysipelotrichaceae* (Table 1). Taxa from the order Bacteroidales (including the family *Bacteroidaceae*) have widely been used in PCR or qPCR based faecal indicator assays, due to the presence of host (species) specific marker taxa within these groups (Kildare *et al.* 2007; Vogel *et al.* 2007), including humans, cows, ruminants, gulls and pigs (Boehm *et al.* 2013). The emergence of high throughput sequencing of the 16S rRNA gene to characterise microbial communities has meant that a fuller picture of the faecal microbiome has emerged in recent years. As a result, previous studies have identified a number of other potential faecal marker groups, including *Lachnospiraceae* (Newton *et al.* 2011; Mclellan *et al.* 2013; McLellan & Eren 2014; Fisher *et al.* 2015), *Porphyromonadaceae* (Newton *et al.* 2013), *Clostridiaceae* (Mclellan *et al.* 2013; Newton *et al.* 2013) and *Ruminococcaceae* (McLellan *et al.* 2010; Newton *et al.* 2013). The families *Lactobacillaceae, Prevotellaceae, Veillonellaceae, and Erysipelotrichaceae* have previously been used as faecal markers to determine contamination in drinking water in Kathmandu, Nepal (Karkey *et al.* 2016).

Indicators of faecal contamination

Over the course of two years, faecal indicators species comprised a relatively small proportion of the total sequences, with a maximum of 8.2 % of the total reads, a minimum of 0.0063 % and a mean of 1.3 % (SD = 1.5). Despite this, members of all indicator taxa were found to be present over the two-year sampling period (Figures 3A and 3B). A clear seasonal pattern in the relative abundance was apparent, with peaks in the total abundance of indicator taxa occurring in both the winter of 2012/13 and 2013/14. Although many faecally associated taxa were detected, only a few were driving this seasonal pattern, including *Phascolarctobacterium* sp. (*Veillonellaceae*), *Veillonellaceae* spp., *Ruminococcaceae* spp., *Clostridium* sp., *Lachnospiraceae* spp., *Prevotella copri*, *Paludiobacter* sp., *Bacterioides plebeius*, and *Bacteriodes* sp. These taxa have clear associations with faeces, and there are currently no reports of them being found in non-faecal environments.

Sewage is a diverse environment, due to the broad definition of what defines sewage treatment and the diverse range of sources that make up sewage influent. However, there is a relatively consistent core of organisms that constitute a sewage community. For example, (McLellan *et al.* 2010) found members of the genera *Aeromonas*, *Acinetobacter* and *Trichococcus*, as well as *Pseudomonas* spp. (which are commonly found in a range of environments and thus not a good marker), to be the most abundant in influent from two major Waste Water Treatment Plants (WWTPs) in the US. This was later confirmed by (Vandewalle *et al.* 2012) who reported that these genera represent a sewage signal, distinct from the faecal bacteria found in sewage influent. Members of the genus *Arcobacter*, a member of the family *Campylobacteraceae*, are also commonly found in abundances of 5–11% in the sewage bacterial community (Fisher *et al.* 2014), but in low abundance (<0.001%) in human faecal samples (Koskey *et al.* 2014) making them good candidates as indicators of sewage infrastructure. It has been reported that these five genera (*Aeromonas*, *Acinetobacter* and *Trichococcus*, *Pseudomonas* and *Arcobacter*) make up 35 % of most sewage communities (McLellan *et al.* 2015).

Sewage associated taxa

The diversity of sewage associated taxa that were detected were low. Over the course of two years, sewage indicators species comprised a a maximum of 8.2 % of the total reads, a minimum of 0.032 % and a mean of 1.8 % (SD = 1.9). Three *Acinetobacter* species, *Acinetobacter lwoffi*, *A. johnsonii*, and *Acinetobacter* sp., and a single *Trichococcus* sp. were detected at low abundance. *Acinetobacter lwoffi* is part of the normal skin and oropharynx microbiome in humans, although it has been identified as a potential cause of nosocomial infections such as gastroenteritis (Regalado *et al.* 2009). Because of this, it is possible that this species is not a sewage marker and may in fact represent faecal contamination. No representatives of the genus *Aeromonas* were detected, which is surprising given their frequency in sewage. However, two species of *Arcobacter; A. cryaerophilus* and *Arcobacter* sp. were recorded in abundance throughout the two-year sampling period (Figure 4). A strong seasonal pattern in abundance was observed for the sewage community, peaking in January in both years. *Arcobacter* has previously been identified as being associated with sewage infrastructure (Fisher *et al.* 2014), although recent work has identified this group as potential pathogens that may have been underestimated in importance due to their similarity to *Campylobacter* (Figueras *et al.* 2014). Given the high volumes of sewage effluent entering the Thames (there are 103 sewage treatment works (STWs) in Oxfordshire alone, and over 200 with the Thames catchment) and that the sampling site is downstream of major STWs in Swindon, Oxford, Didcot and Abingdon, it is surprising that so few sewage indicators were detected. One possible explanation is that the survival rate of these species in the river environment is low, and once in the river rapid die-off occurs, although this needs to be investigated further.

In most cases it is not possible to identify pathogenic species using 16S rRNA sequences alone, as the taxonomic resolution is not sufficient to determine specific phenotypic characteristics such as pathogenicity. This is especially true for organisms that have closely related strains that contain species that have different host specificity, for example in *Campylobacter* (Sheppard *et al.* 2011; Read *et al.* 2013) as well as species complexes that have both pathogenic and commensal strains that can be found in the gut, as for *Escherichia coli* (Rasko *et al.* 2008; Tenaillon *et al.* 2010). However, groups of organisms can be identified at the family or genus level that contain known pathogenic species or strains, to act as an indicator for the presence of these organisms. We identified ten families of bacteria that have previously been shown to be associated with transmission via water, including *Campylobacteraceae*, *Vibrionaceae*, *Enterobacteriaceae*, *Mycobacteriaceae*, *Legionellaceae*, *Leptospiraceae*, *Pseudomonadaceae*, *Francisellaceae*, *Aeromonadaceae*, and *Staphylococcaceae* (Cliver & Fayer 2007; Risebro & Hunter 2007; Aw & Rose 2012; Lee *et al.* 2016; Lenaker *et al.* 2017; Lamb *et al.* 2017).

Pathogenic Indicator Species

Apart from the potentially pathogenic family *Francisellaceae*, representatives of all families were detected (Figure 5). Over the two-year sampling period, pathogen indicator species comprised a maximum of 15.1 % of the total reads, a minimum of 1.5 % and a mean of 5.7 % (SD = 2.4). Most these were in low abundance, with only *Leptospira* sp., *Pseudomonas* sp. and member of the *Legionellaceae* present at higher abundances. No confirmed pathogens were identified to species level, with only related organisms identified, either due to their absence from the samples or a lack of taxonomic certainty due to the limitations of relatively short reads of the 16S rRNA gene. As a group ("pathogen indicators") there was a seasonal pattern, with increases observed in winter, but less so in contrast to the faecal and sewage indicators. However, this is partly because the most abundant species, *Rickettsiella* sp. peaked in abundance in the summer months. Species belonging to this family have been identified as host associated as a parasites of crustaceans and insects (Kleespies *et al.* 2014; Wang & Chandler 2016). This leads to the possibility that the prevalence of this species is a natural phenomenon, and intriguingly may be linked to the seasonal abundance of its host organism in freshwater environments. Two other potentially pathogenic species exhibited peaks in abundance during the warmer months; *Leptospira* sp. and *Mycobacterium* sp. Both these genera contain serious pathogens of humans. Species from the genus *Lepitospira* are the causative agent of Weil's disease (Vijayachari *et al.* 2008) but also contains opportunistic pathogens as well as non-pathogenic species. Similarly, *Mycobacterium* contains a range of pathogen species, including the *Mycobacterium tuberculosis* complex, the causative agent of tuberculosis. However, the genus *Mycobacterium* also contains species known to live non-pathogenically in soils (Ho *et al.* 2012), and other environmental reservoirs may exist, including in freshwater sediments. A few other member of the family *Legionellaceae* were present, exhibiting higher abundances in winter, including *Aquicella* sp., and some higher-level classifications including *Legionellaceae* spp., *Coxiellceae* spp. and *Legionellales* spp. Member of the genus *Aquicella* have been found to infect protozoa, and therefore likely represent a natural component of the freshwater ecosystem. The family *Legionellaceae* contains the species *Legionella pneumophila*, a water associated species that causes Legionnaires' disease (Fraser *et al.* 1977). These results indicate that, at least for this study period and this point on the river Thames, levels of pathogens are relatively low and no species with a known pathogenicity to humans were identified.

There are two possible reasons for the presence of faecal and sewage infrastructure indicators but also a lack of pathogenic species. It is possible that faecal and sewage contamination, whilst present in the Thames, is not a source of pathogens. However, sewage effluent has been shown to be a source of pathogenic bacteria that can survive the sewage treatment process (Pandey *et al.* 2014), and animal faeces are frequently found to be a primary cause of environmentally transmitted disease (Galan *et al.* 2016). More likely is that the persistence and dynamics of these species differs, where some sewage and faecal indicator species can survive and be transported within the catchment, whilst other pathogenic species do not. Further work, at a greater range of sites, to identify hotspots of microbiological contamination (such as downstream of sewage effluent sources or areas with high agricultural or wildlife population densities), would help understand the link between indicator species and pathogens, as well as better determine how these interact with the freshwater environment.

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Table 1. Families of bacteria used as indicators of sewage, faecal compartments, as well as common waterborne pathogens.

Habitat	Indicator Family	Example Species	Reference
Sewage infrastructure associated	Acinetobacter	*Acinetobacter baumanii*	(Vandewalle *et al.* 2012)
	Aeromonas	*Aeromonas caviae*	(Monfort & Baleux 1990)
	Arcobacter	*Arcobacter cryaerophilus*	(Fisher *et al.* 2014)
	Trichococcus	*Trichococcus flocculiformis*	(Vandewalle *et al.* 2012)
Faecal associated	Bacteroidaceae	*Bacteroides fragilis*	(Wexler 2007)
	Porphyromonadaceae	*Porphyromonas gingivalis*	(Rinttilä *et al.* 2004)
	Clostridiaceae	*Clostridium difficile*	(Lopetuso *et al.* 2013)
	Lachnospiraceae	*Blautia sp.*	(Eren *et al.* 2015)
	Ruminococcaceae	*Ruminococcus flavefaciens*	(Wu *et al.* 2011)
	Lactobacillaceae	*Lactobacillus casei*	(Cox *et al.* 2010)
	Prevotellaceae	*Prevotella copri*	(Scher *et al.* 2013)
	Veillonellaceae	*Veillonella parvula*	(Kummen *et al.* 2016)
	Erysipelotrichaceae		(Kaakoush 2015)
Potential pathogen	Campylobacteraceae	*Campylobacter coli* *Helicobacter pylori*	(Van Dyke *et al.* 2010) (Aziz *et al.* 2015)
	Vibrionaceae	*Vibrio cholerae*	(Vezzulli *et al.* 2010)
	Enterobacteriaceae	*Escherichia coli,* *Shigella dysenteriae* *Citrobacter freundii* *Salmonella enterica* *Klebsiella pneumoniae*	(Woodford *et al.* 2014) (Kittinger *et al.* 2016b)
	Mycobacteriaceae	*Mycobacterium tuberculosis*	(Primm *et al.* 2004)
	Legionellaceae	*Legionella pneumophila*	(Delgado-Viscogliosi *et al.* 2009; Wunderlich *et al.* 2016)
	Leptospiraceae	*Leptospira kmetyi*	(Ganoza *et al.* 2006)
	Pseudomonadaceae	*Pseudomonas aeruginosa*	(Kittinger *et al.* 2016a)
	Francisellaceae	*Francisella tularensis*	(Forsman 1995)
	Aeromonadaceae	*Aeromonas hydrophila*	(Olaniran *et al.* 2015)
	Staphylococcaceae	*Staphylococcus aureus*	(Concepcion Porrero *et al.* 2014)

Figure Legends

Figure 1. Temporal variation in physicochemical conditions over the two-year sampling period.

Figure 2. A map of the study site, showing the River Thames catchment, major urban areas and the sampling site (red dot) on the River Thames at Wallingford.

Figure 3A and 3B. Heatmaps showing the relative abundance of bacterial taxa with a known association to human and animal faeces over the two-year sampling period.

Figure 4. Heatmap showing the relative abundance of bacterial taxa with a known association to sewage infrastructure over the two-year sampling period.

Figure 5. Heatmap showing the relative abundance of bacterial taxa that contain species known to be pathogenic to humans and have an association with water, over the two-year sampling period.

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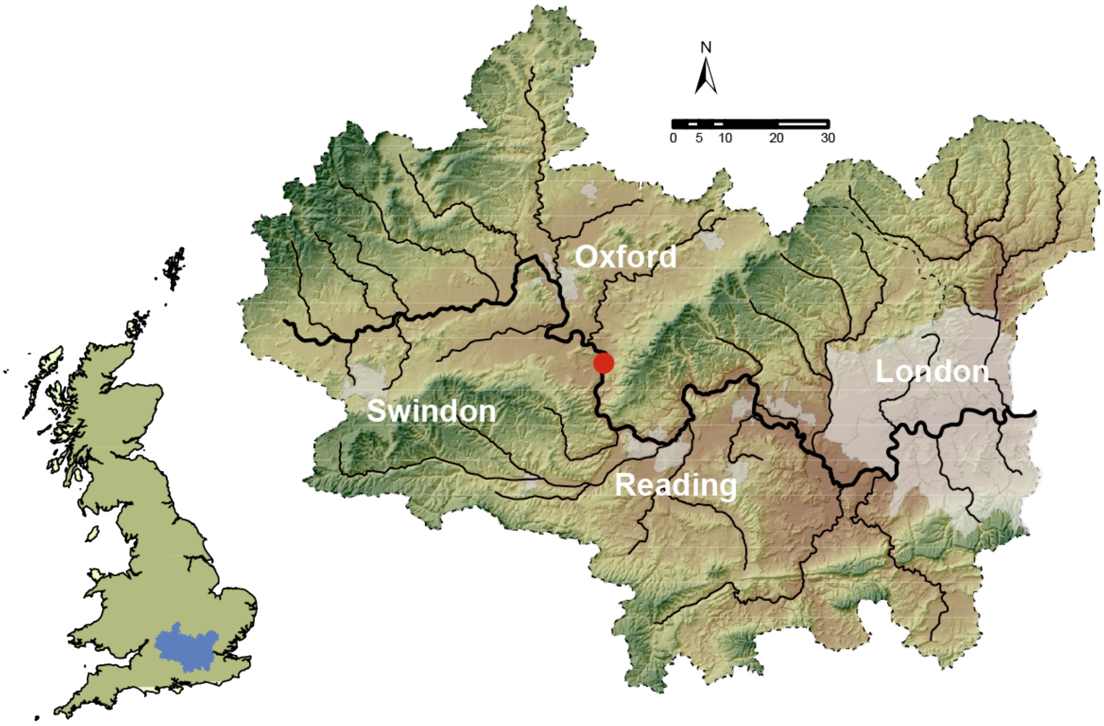

Figure 2. Temporal variation in physicochemical conditions over the two-year sampling period.

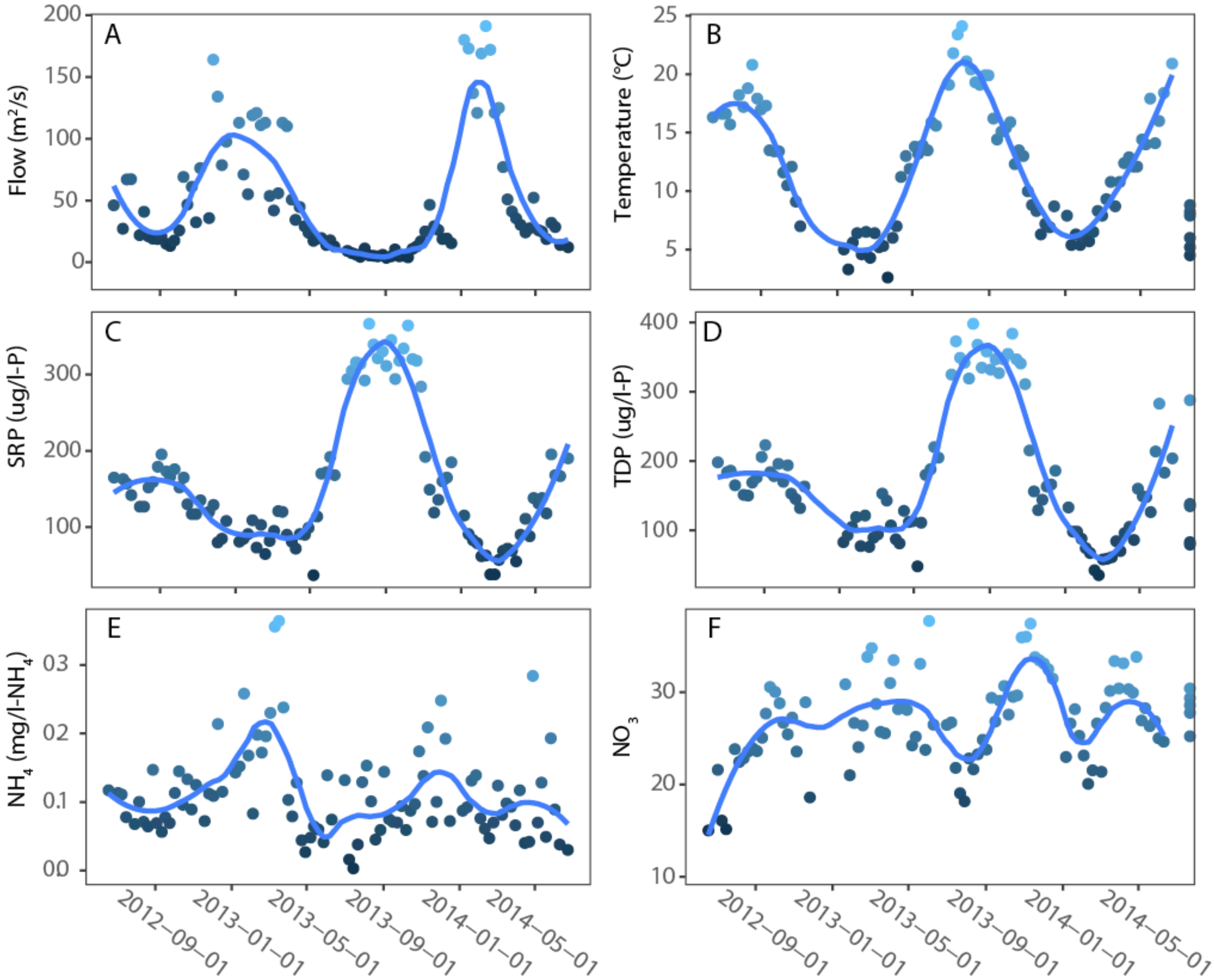

Figure 3A. Heatmap showing the relative abundance of bacterial taxa with a known association to human and animal faeces over the two-year sampling period.

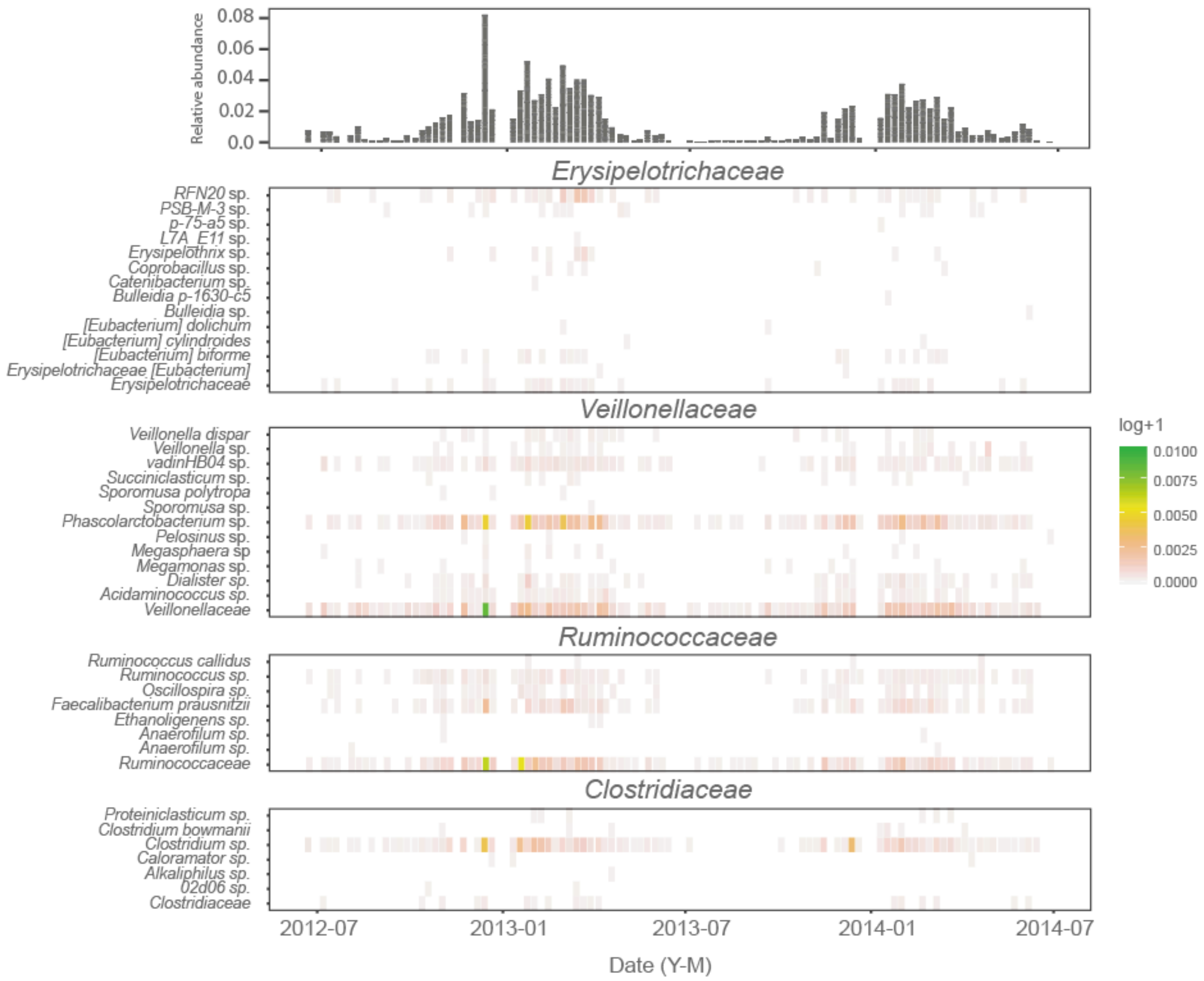

Figure 3B. Heatmap showing the relative abundance of bacterial taxa with a known association to human and animal faeces over the two-year sampling period.

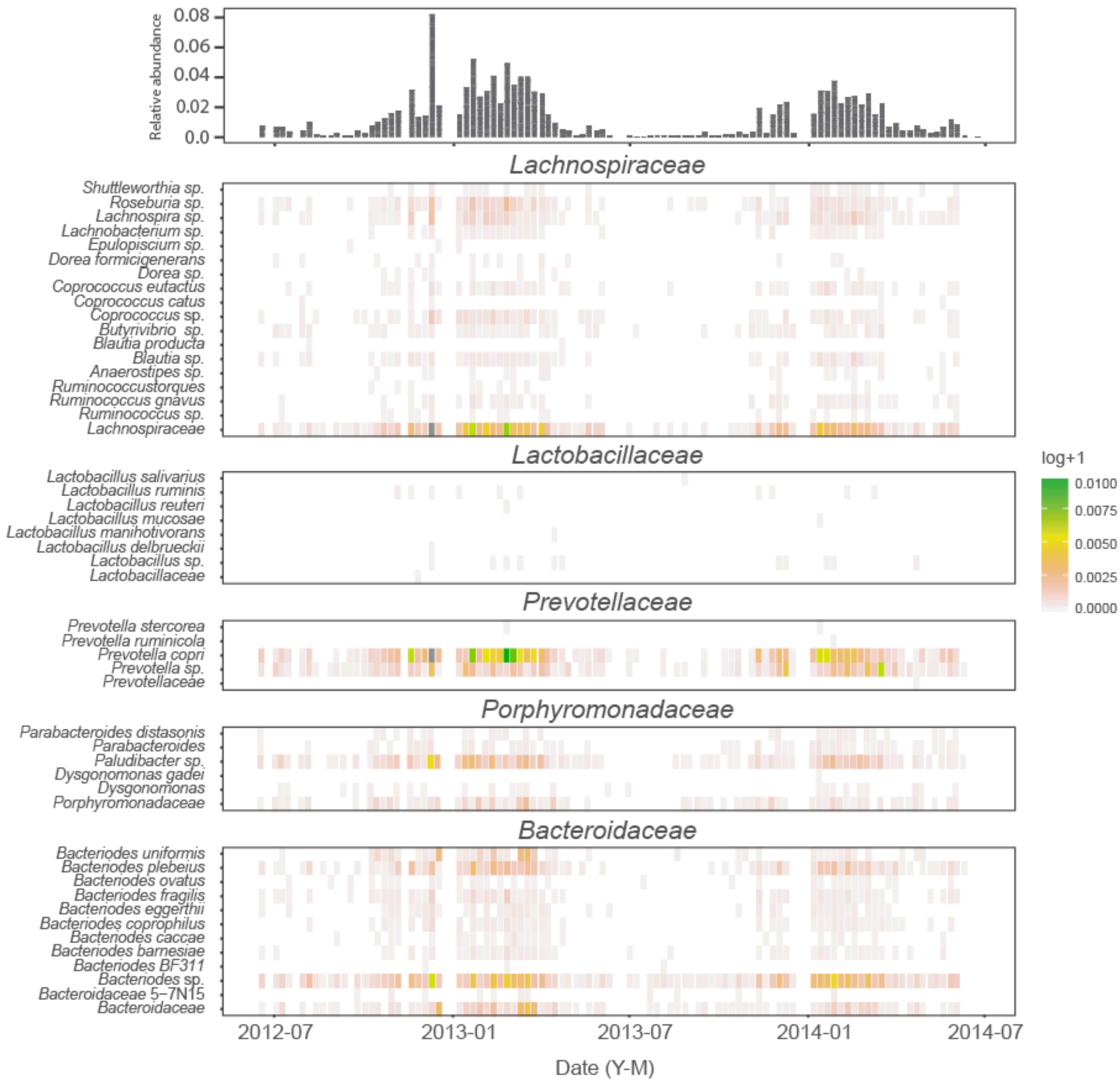

Figure 4. Heatmap showing the relative abundance of bacterial taxa with a known association to sewage infrastructure over the two-year sampling period.

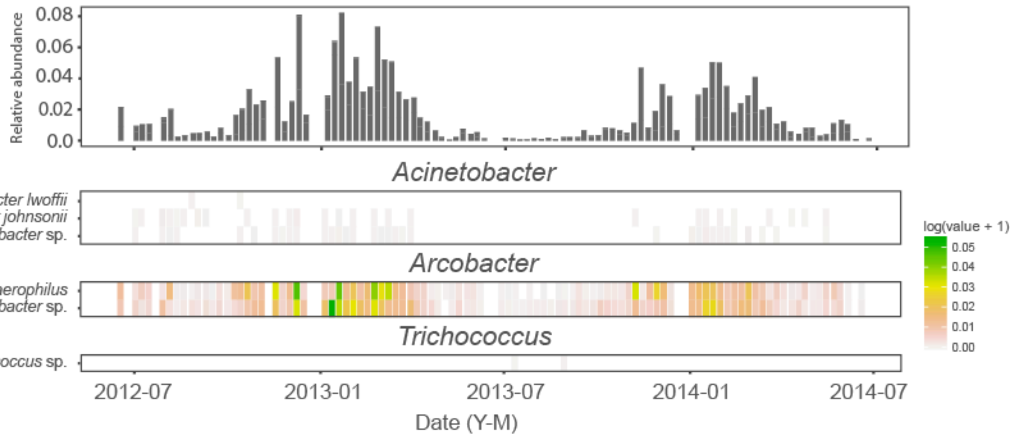

Figure 5. Heatmap showing the relative abundance of bacterial taxa that contain species known to be pathogenic to humans and have an association with water, over the two-year sampling period.