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Prioritization of water quality stressors according to their relative impact on ecological quality of rivers using large-scale field data: salinity first?

E. Berger, Senckenberg Gesellschaft / Department Quantitative Landscape Ecology; R. Schäfer, University Koblenz-Landau; P. Haase, A. Sundermann, Senckenberg

The political aim of achieving good ecological quality of all European water bodies requires knowledge on how to prioritize stressors and human pressures for management based on their relative impact. A challenge thereby is the frequent co-occurrence of multiple stressors. We applied eco-epidemiological approaches to large scale monitoring data from Saxony, Germany, to investigate the relative contribution of different water quality and land-use gradients to ecological change. Two approaches were applied: First, water quality gradients (e.g. oxygen, conductivity, phosphorous and micropollutants) and land-use gradients (e.g. % arable and urban catchment land cover, position of wastewater treatment plants) were used as predictor variables in multiple linear regression analysis and hierarchical partitioning with ecological quality indices based on invertebrates (% EPT, MMI, ASPT, BMWP, GSI, SPEAR %) as response variables. Secondly, individual taxon responses with respect to different water quality gradients (including also major ions such as potassium, sodium, chloride etc.) were assessed using Threshold Taxa Indicator Analysis (TITAN). The method is based on change point and indicator species analysis and allows the identification of ecological change points that may be used to derive environmental quality criteria. Both regression analysis and TITAN results indicate a high impact of oxygen and salinity, which were associated with arable and urban catchment land cover. Although observed associations may not be direct causes of ecological impairment, it may be worthwhile to implement legally binding quality standards for these variables. Of the 324 analyzed taxa 23% had change points far below the German orientation value for chloride (200 mg/L) that should not be exceeded to achieve good ecological status according to the water framework directive. Thus, lowering of orientation values for salinity and associated ions should be considered to protect and restore stream biodiversity. Moreover, the results suggest that preventing release of poorly treated wastewater should be prioritized over up-grading of well-functioning treatment plants.

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Estimating protective potassium concentrations for freshwater mussels, a taxon of global conservation concern

T. Augspurger, U.S. Fish and Wildlife Service / Ecological Services
Globally, there are about 620 species of freshwater mussels (Family Unionidae), and IUCN lists 28 species as extinct and 106 as endangered or critically endangered. Mussels are among the most sensitive freshwater water organisms to toxicity from chloride and potassium, and the environmental relevance of these is increasing with sea level rise and brine discharges. Average potassium concentrations in relatively unpolluted streams of North Carolina (USA) range from 0.2 to 2 mg/L. An industrial effluent with potassium averaging 504 mg/L and proposed for discharge to a stream with endangered mussels necessitated derivation of protective potassium limits (because there are no State standards or USA water quality criteria for potassium). From the literature, we compiled potassium 96-h EC50s (with endpoints of lethality or immobilization) for mussels and retained those with > 90% control survival, measured test chemical concentrations, and acceptable test water quality. Five EC50s ranged from 31 to 48 mg/L at a water hardness of 100 mg/L as CaCO₃, and we applied the North Carolina guidance of one-third of the lowest EC50 to define an acceptable concentration to avoid acute toxicity. We adjusted the 10 mg/L acute limit to a water hardness of 18 mg/L which is the 5th percentile of the proposed receiving stream (protective most of time because potassium is less toxic as hardness increases). The hardness-adjusted acute water quality guideline of 7 mg/L potassium was recommended as an instantaneous concentration not to be exceeded. Chronic toxicity data for potassium and mussels were available for two studies from 28 to 300-d. Because mussels can live for decades, we used the 300-d test. The geometric mean of the test NOEC (1 mg/L) and LOEC (7 mg/L) yield a chronic value of 2.6 mg/L potassium which was recommended as a monthly average guideline not to be exceeded more than once every three years. We used 32 years of receiving stream flow data to derive estimates of instream waste concentration and effluent limits. We identify uncertainties in guideline derivation and discuss recommendations for quarterly mussel toxicity tests, instream monitoring, and research to narrow uncertainties. There are several means by which stream-specific and mussel-specific potassium guidelines could be derived. This method tracks North Carolina water quality standards and definitions and is reasonable with available data.

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LIFE LAGOON REFRESH - Coastal lagoon habitat (1150*) and species recovery by restoring the salt gradient increasing fresh water input. Management measures in the northern Venice Lagoon (NE, Italy)

F. Cacciatore, ISPRA-Institute for Environmental Protection and Research / Mitigation and Prevention of Impacts; A. Bonometto, A. Feola, E. Ponis, ISPRA Institute for Environmental Protection and Research; A. Sfriso, University Ca Foscari of Venice; B. Matticchio, IPROS; M. Lizier, Regione del Veneto; V. Volpe, Provveditorato OO. PP. Veneto, Trentino Alto Adige e Friuli Venezia Giulia; M.

Ferla, R. Boscolo Brusà, ISPRA - Institute for Environmental Protection and Research

The northern Venice Lagoon (SCI IT3250031) holds several Annex I-listed habitats of the Habitats Directive, such as the habitat type 1150* (Coastal lagoons). Recent monitoring activities showed that conservation status of the habitat 1150* is improving within SCI IT3250031, but it is still unfavourable in the inner landward areas, due to lack of ecotonal buffer areas, favouring self-regulation processes, between lagoon and mainland. In the past, the project area was occupied by reedbeds in large amounts, now significantly receded due to increasing of lagoon water salinity, caused by historical human activities (e.g. diversion of rivers with reduction of freshwater supply, inlet and channel excavation). With reduction or disappearance of reedbeds, their contribution to ecosystem services, like supporting numerous biological communities and species, are minimised. The LIFE LAGOON REFRESH project, started on Sept 2017, foresees the restoration of favourable conservation status of habitat 1150* in the northern Venice Lagoon and the recreation of favourable habitats for faunal species of community interest. The project actions involve: diversion of a freshwater flow from the Sile river into the lagoon (necessary for the recreation of the typical salt gradient of buffer areas between lagoon and mainland); restoration of intertidal morphology through the implementation of structures properly arranged to slow down the freshwater dispersion and to favour reed development; planting of *Phragmites australis* to accelerate the development of the reedbeds; transplantation of small dumps of seagrass species of the habitat 1150*, suitable to accelerate the recolonization by aquatic plants of low-salinity environments. The project aims to exploit the ecosystem services resulting from the recreation of a typical estuarine system to: counteract the depletion of lagoon bottom and fish communities; reduce eutrophication through reedbed phytoremediation function, favouring the presence of sensitive species and high ecological value aquatic plants; improve conservation status of bird species, including those listed in Annex I of the Birds Directive; increase the presence of fish species, listed in Annex II of the Habitats Directive. The restoration of salinity gradients will also contribute to increase biodiversity in the Natura 2000 network site, and to help achieving targets defined by the 2020 Biodiversity Strategy.

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Comparing the growth of fescue and clover plants in petroleum industrial effluents and solutions of similar salinity

P. Srikhumsuk, University of Strathclyde / Department of Civil & Environmental Engineering; C. Knapp, J. Renshaw, University of Strathclyde / Civil and Environmental Engineering

Effluents (produced and flow-back waters) from the petroleum industry have been investigated for their potential toxicity to the environment, particularly in regards to chemical composition and salinity. The purpose of this study was to investigate whether their toxicity is any greater than exposure to solutions of similar salinity. The tolerance of *Festuca rubra* L. (fescue) and *Trifolium pratense* L. (clover) were investigated for 8 weeks under hydroponic conditions to compare their growth in brine effluents from tertiary recovery operations. Experiments further compared serially-diluted effluents and synthetic solutions (e.g., NaCl/CaCl₂) of similar salinities. There were different growth responses to the wastewater and saline solution among both plant species. *F. rubra* was exhibited a significant higher survival percentage than *T. pratense*. After four weeks of exposure, *T. pratense* exhibited greater sensitivity and lethality. Interestingly, biomasses of both plants were greater from industrial wastewater than the comparable brine solution. Although salinity limited plant growth, the tertiary wastewaters contained abundant inorganic and organic substances that may have triggered plant survival and salt-tolerance. *F. rubra* grew under salts stress, and presented a mechanism to crystallize salt on their leaves. Hence, plant uptake, under certain conditions, may be promoted as an alternative treatment for high salt concentrations.

WE277

Contribution to the salinization risk assessment, under drought conditions, in the Alqueva irrigation area (South Portugal)

A. Tomaz, S. Fialho, A. Lima, Instituto Politécnico de Beja; A. Penha, H. Novais, M. Potes, M. Iakunin, G. Rodrigues, Instituto de Ciências da Terra; P. Alvarenga, LEAF Centro de Investigação em Agronomia, Alimentos, Ambiente e Paisagem, Instituto Superior de Agronomia, Universidade de Lisboa; M. Costa, M. Morais, R. Salgado, Instituto de Ciências da Terra; P. Palma, Instituto Politécnico de Beja / Department of Technologies and Applied Sciences

In Mediterranean regions, climate changes have enlarged water limitation for crops, leading to an increased demand for irrigation water. During the hydrological years of 2016 and 2017, Portugal experienced a drought season that has extended throughout almost the entire mainland territory reaching a severe drought level. Under water scarcity conditions and high atmosphere evaporative demand, the risk of land salinization is one of the major threats to the sustainability of irrigated agriculture. Therefore, it is very important to assess the quality of irrigation water and the risks of salinity for crop production, in order to adopt appropriate management practices in irrigated areas. This study is focused on the salinity risks for the production of the most representative crops grown in the Alqueva irrigation area. This is a large irrigation scheme with a total area of 120 000 ha centered in the Alqueva reservoir. For the purpose of the study, a chemical assessment of some

major inorganic ions (Na⁺, Ca²⁺, Mg²⁺, K⁺, SO₄²⁻ and Cl⁻), pH and electric conductivity (EC_w), was conducted throughout 2017, on water samples collected on four platforms sited in the reservoir. Water quality for irrigation was evaluated considering both the Portuguese regulations and the FAO guidelines. Sodium adsorption ratio (SAR) and soil salinity (ECe) were estimated, in order to assess potential sodium-related soil permeability and crusting problems, as well as, potential yield reductions in the most significant crops of the Alqueva perimeter. Higher ion concentrations and water salinity were quantified with the increase of atmosphere evaporative demand. Sodium hazard assessment showed slight to moderate risk of reduced infiltration rates, a result that should be taken into account when surface or sprinkler irrigation systems are used. Furthermore, relative yield reductions may be mainly found in horticultural crops, classified as moderately sensitive to sensitive in the salt tolerance scale.

Systems ecotoxicology: application of OMICS data across multiple level of biological organization in research and risk assessment (P)

WE279

Investigating wildlife diets using high-tech DNA sequencing

J. Ludwigs, Rifcon GmbH; I. Katzschner, RIFCON GmbH Goldbeckstr Hirschberg Germany; G. Weyman, ADAMA; A. Winkler, J. Kalinowski, Center for Biotechnology (CeBiTec) Universität Bielefeld

In wildlife risk assessments according to EFSA (2009), the ingested diet is one of the core factors to define exposure, using default diet compositions in the first tier risk assessment. The so-called PD factor (composition or portions of diet) is one of the standard refinement parameters which intend to add realism to higher tier risk assessments. Publically available dietary data are often used to refine PD in wildlife risk assessments; however, such data are often variable and/or not representative for the specific risk assessment scenario. Besides such literature data, specifically registration-relevant PD field studies can be conducted. PD values gathered from such studies are based on collected faeces, stomach samples, or stomach flushing. In these studies samples (or parts thereof), are investigated visually by microscope and food items are identified based on comparison with comprehensive reference data libraries and collections of potential diet items. For plant material, the results are mainly presented on a fairly basic taxonomic level and are often related to wildlife risk assessment defined diet fractions which have different default residue levels (i.e. dicotyledonous plants or monocotyledonous plants only). However, this is rather time-consuming and imprecise. Recently, DNA sequencing techniques are increasingly applied for diet composition analyses in ecological science. We initiated an approach using 'next-generation' DNA amplicon sequencing to quantitatively assess the diet composition of wild herbivorous mammals, taken from faeces samples collected on arable fields. Data on the relative abundance of each plant species were derived by enrichment and sequencing of a specific DNA region (ITS2 region of the ribosomal DNA) and by comparison to comprehensive plant species DNA databases. The approach has proved to be very useful on identification of relative abundances of plant species from faecal samples. This new genomics approach, its needs and limitations for refined risk assessment will be presented and discussed.

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Design of a Real-Time PCR array to analyze the gene expression in *Physella acuta* (Gastropoda) in chemical stress and starvation

M. Novo, J. Martínez-Guitarte, UNED / Física Matemática y de Fluidos
Molecular endpoints are nowadays under study for their inclusion in test toxicity tests. While vertebrate species are usually well-known; there is a lack of information on invertebrates. The study of the latter is complex since their body shape, behavior, and ecology are very diverse, and great differences can be found even within the same animal group. In order to improve our knowledge in putative molecular endpoints and to evaluate some genes as biomarkers, a Real-Time PCR array has been designed for *Physella acuta*. This species of freshwater snail is used in environmental toxicology studies and it has been proposed as an adequate species for toxicity tests because of its sensitivity to different toxicants and ease of culture. A transcriptome for this species was assembled, by sequencing cDNA libraries from individuals of different developmental stages and exposed to different toxicants. Comparison with database allowed the identification of genes involved in pathways related with the response to toxicants. We selected 42 of these genes plus six genes used as reference to design an array for Real-Time PCR analysis. Stress response, detoxification mechanisms, endocrine system, or epigenetics were some of the pathways analyzed in the array. In order to validate the toxicological and ecological interest of this approach, individuals were treated with an antibiotic, tetracycline, for seven days or were left starving for 7 and 10 days. The results obtained for these experiments are presented, showing the interest of designing specific arrays to perform more detailed analysis of molecular endpoints that can be related with toxicant mode of action and stress situation. We hope that the methodology presented here can serve as an example for the study of other species in order to improve our knowledge of their biology. This work has been funded by the *Ministerio de Economía y Competitividad*, CICYT (SPAIN), CTM2015-64913-R.

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Effects of temperature on the transcriptome of the marine copepod *Temora longicornis*

L. Semmouri, Ghent University (UGent) / Animal Sciences and Aquatic Ecology; J. Asselman, Ghent University / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; C.R. Janssen, Ghent University / Applied Ecology and Environmental Biology; K. De Schampelaere, Ghent University (UGent) / Applied Ecology and Environmental Biology

Over the past decades, the world's oceans and seas have been influenced by several human induced impacts, including climate change. In the North Sea region, the average sea surface temperature of the water has already risen with 1-2 °C over a time-period of twenty-five years and is likely to rise further. Understanding the impacts of this changing environmental condition in zooplankton communities is crucial, as alterations in the zooplankton communities can affect entire marine ecosystems. Here, we focus on the potential effects of an increase in temperature on the calanoid copepod species, *Temora longicornis*, the dominant zooplankton species of the southern part of the North Sea. Since responses to environmental stress are genome-driven, a genetic study on the physiological responses to thermal stress can provide an increased mechanistic understanding and help predict potential responses to climate change in this copepod species. Therefore, we sequenced the whole transcriptome (using RNA-sequencing technology) in *T. longicornis*, after being exposed to thermal stress, to investigate gene expression differences as a response to temperature fluctuations. As such, this dataset will provide us with new insights on how exposure to increased sea water temperatures may affect the fitness of the most dominant zooplankton species of the southern part of the North Sea.

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A traditional approach to modern endpoints - quantitative assessment of stress gene expression response to a range of copper concentrations in the freshwater mussel *Anodonta anatina*

G.M. Ekelund Ugge, Lund University / Biology; A. Jonsson, University of Skövde / Department of Bioscience; O. Berglund, Lund University / Dept of Biology

In the field of ecotoxicology, modern *transcriptomics* technologies have the potential to improve and complement current toxicity assessment methods and biomonitoring protocols. Early warnings of general stress and specific toxic modes of action could in theory be used as biomarkers of pollutant exposure or adverse effects. However, necessary base level understanding is currently lacking considering how gene expression may vary under realistic exposure scenarios. Therefore, we adopted an approach of quantitative assessment as an alternative to more descriptive methods. We chose the freshwater mussel *Anodonta anatina* as our model organism. Being a stationary filter feeder, it shows promise for use in exposure studies under both laboratory and field conditions. Furthermore, it is the most abundant freshwater mussel species in Sweden, and occurs in freshwater ecosystems over most of Europe. For the present study, mussels were collected locally in Vinne å (southern Sweden), on a location free from point source pollution. After two weeks of acclimatization to laboratory conditions, mussels were exposed for 96 h to one of three copper treatments (nominal concentrations of 1, 10 and 100 µg/l Cu²⁺), or a control treatment (n= 5 per treatment). Using RT-qPCR, relative expression of a selection of general stress genes will be quantified in extracted digestive gland and gill tissue. Preliminary data will be presented, testing the hypotheses that the amplitude (fold-change) of relative expression differs (i) between treatments of the same gene, and (ii) between genes in the same treatment. Results from this initial experiment will be used to design a follow-up experiment, in order to test the dose-dependence of gene expression responses. Gradually, successively increased exposure scenario complexity (e.g. duration, chemical composition) will help us to better understand how expression patterns potentially vary under environmental exposure. By subsequent incorporation of biochemical and physiological biomarkers, we also aim to link stress gene expression patterns to effects at higher biological levels. Ultimately, a more thorough understanding of natural and pollution-induced variation in gene expression may allow *transcriptomics* to be usefully and successfully incorporated into various ecotoxicological assessment protocols.

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Validating a contamination assessment tool from lab to the field: *Folsomia candida* exposed to a fungicide-based formulation

T.F. Simoes, S.C. Novais, Polytechnic Institute of Leiria / MARE IPLeia; T. Natal da Luz, University of Coimbra / Department of Life Sciences, University of Coimbra; J. Renaud, CFE - Centre for Functional Ecology; J. Sousa, University of Coimbra / Department of Life Sciences; J. Römbke, ECT Oekotoxikologie GmbH; D. Roelofs, Vrije Universiteit / Department of Ecological Science; N. van Straalen, Association of Retired Environmental Scientists ARES / Department of Ecological Sciences; M.F. Lemos, Instituto Politécnico de Leiria / MARE IPLeia
Folsomia candida is a widespread arthropod that occurs in soils throughout the world and has been used as a standard test organism in past decades for estimating the effects of pesticides and environmental pollutants on non-target soil arthropods. This species is among the most sensitive representatives of its taxon, being selected as a genomic model organism for soil toxicology studies. Although laboratory