Bull Environ Contam Toxicol (2011) 86:591–595
DOI 10.1007/s00128-011-0283-2

Effects of Iron on Benthic Macroinvertebrate Communities in the Field

Adam Peters · Mark Crane · William Adams

Received: 5 October 2010 / Accepted: 15 April 2011 / Published online: 24 April 2011

Abstract Matched chemical and ecological monitoring data were used to assess the effects of iron on benthic macroinvertebrate communities. Three measures of iron exposure: dissolved, total, and particulate iron were assessed. Ecological responses were normalised to an unimpacted reference condition to make site-specific predictions of the reference condition. Ecological data were expressed as an Ecological Quality Index (EQI), indicating quality relative to the reference condition. Quantile regression analysis was used to derive thresholds as an EQI value equivalent to the cut-off between good and moderate ecological status for water quality classification. Thresholds for Good Ecological Status ranged from 1.25 to 2.46 mg L^{-1} depending on the measure of exposure and ecological response.

Keywords Quality standard · Iron · Benthic invertebrates · RIVPACS

Ecological monitoring data can be a valuable source of information on toxicant effects. The effects of iron on benthic macroinvertebrate assemblages were previously assessed in two studies (Linton et al. 2007; Crane et al. 2007). Crane et al. (2007) analysed monitoring data from the UK and derived water quality criteria between 43 and 250 μg L^{-1} on the basis of dissolved iron, for comparison against a Predicted No Effect Concentration (PNEC) of 16 μg L^{-1} derived from laboratory ecotoxicity test data and an assessment (Environment Agency 2007). A comparable study in the USA (Linton et al. 2007) derived a criteria value of 210 μg L^{-1} on the basis of total iron for the most sensitive taxon assessed (*Leptophlebiidae*-a mayfly [Ephemeroptera] family) and 1,740 μg L^{-1} for overall community protection.

Assessments of ecological monitoring data (such as measurements of benthic macroinvertebrate communities) should preferably be expressed relative to a reference condition. Crane et al. (2007) included assessments based on an Ecological Quality Index (EQI). The EQI used in the UK is an example of such a metric, and is calculated using monitoring data and a prediction of the community that would be present at the site if it were in an unstressed condition (Wright et al. 2000; Clarke et al. 2006). Assessment in this way normalises the data for differences in habitat which result from the presence of different ecological assemblages at different sites. It also takes account of the fact that many high scoring (but relatively rare) taxa are not expected to be present in significant numbers at the majority of sites. Assessments which are not normalised in this way will always be limited to some extent by uncertainties about whether a particular taxon would have been present at a site regardless of contaminant concentrations.

In the UK the EQI values for macroinvertebrates used by environmental regulatory agencies are based on the Biological Monitoring Working Party (BMWP) scoring system, and may be expressed in several ways (Clarke et al. 2003a). There are 85 BMWP scoring macroinvertebrate families and within this system each family is assigned a specific score. A few of the BMWP scoring taxa include more than one family, and whole community metrics are

A. Peters (✉) · M. Crane
WCA Environment Ltd., Brunel House, Volunteer Way,
Faringdon, Oxfordshire SN7 7YR, UK
e-mail: adam.peters@wca-environment.com

W. Adams
Rio Tinto, South Jordan, UT 84095, USA

calculated based on the presence of a taxon in the sample, not its abundance. There are typically between 15 and 35 different taxa present at unimpacted sites, depending upon habitat. Prediction of the expected presence or absence of particular taxa for a habitat in an unimpacted state allows several metrics to be calculated. The number of scoring taxa (N-Taxa) is the number of BMWP scoring taxa present in the sample, and the BMWP score is the sum of the BMWP scores for all scoring taxa. N-Taxa and the total BMWP score which would be expected for a site if it were in an unimpacted condition can both be calculated from River Invertebrate Prediction and Classification System (RIVPACS) software and compared with field observations to calculate EQI values (Clarke et al. 2003b). The EQI values are calculated as the ratio of the Observed and Expected scores. A further metric, the Average Score Per Taxon (ASPT), may also be calculated as the total BMWP score divided by N-Taxa. The EQI values are calculated as the observed score, divided by the RIVPACS predicted score, for each of the three indices.

Previous assessments of the toxicity of iron to aquatic organisms in laboratory tests (e.g. OECD 2007) have been unable to identify definitively whether chemical toxicity or precipitate formation is responsible for the observed effects. A key advantage of deriving a PNEC or Environmental Quality Standard (EQS) from field data is that both chemical toxicity and physical effects can be taken into account (Linton et al. 2007). The specific objective of this study is to define a threshold for iron concentrations above which good ecological status is unlikely to be achieved, without constraining the analysis to a single measure of iron exposure.

Methods

A set of monitoring data with comprehensive ecological survey information for benthic macroinvertebrates and associated water chemistry parameters for river and stream sampling sites in England and Wales was provided by the Centre for Intelligent Environmental Systems (CIES), Staffordshire University, UK, from work they had undertaken for the Environment Agency of England and Wales. The dataset contains 1,830 samples with monitoring data for both total and dissolved iron concentrations, and EQI BMWP, EQI N-Taxa, and EQI ASPT from 1995 to 2004. The analyses of the dataset considered three different metrics of iron exposure: total iron, dissolved iron, and particulate iron (which is defined as the difference between the total and dissolved iron concentrations). Dissolved iron was defined as the fraction passing a 0.45 µm filter membrane. Given the behaviour of iron in freshwaters it is likely that the operationally defined "dissolved" and "particulate" iron both include suspended particles. Monitoring data for benthic macroinvertebrate fauna are reported in the dataset as log abundance values on an individual taxon basis. Summarised results, such as the number of taxa (N-Taxa), BMWP scores, and ASPT, are calculated by RIVPACS (Version 3+).

Quantile regression was used to derive field-based limit values. The quantile regression approach can be used to derive a limiting function and is particularly useful in cases where it is not possible to remove all of the potentially confounding factors (e.g. Cade et al. 1999). The approach taken in this study was similar to that applied in previous assessments of field data (Linton et al. 2007; Crane et al. 2007), although in this case all assessments were made using ecological data expressed relative to a reference condition. The statistical analyses were performed using R (Version 2.9.1; http://cran.r-project.org). Quantile regression was applied to the 90th quantile of the data using a log linear model (log(response) = a + b.(exposure)). Higher quantiles are potentially more susceptible to outliers in the data and may also be subject to greater uncertainties (Pacheco et al. 2005), although both 90th quantiles (Linton et al. 2007) and 99th quantiles (Crane et al. 2007) have been successfully applied to analyses of iron. The principle of the quantile regression approach to derive a limiting function is illustrated in Fig. 1. Data that show unimpacted responses at low exposures to the substance of interest serve as "controls", and impacted responses at low exposures are not considered in the analysis, because a limiting function is derived which describes the 90th percentile of the maximum achieved ecological quality at a given iron exposure concentration. A decline in the 90th percentile response with increasing exposure is assumed to be due to the substance of interest, although other toxicants which

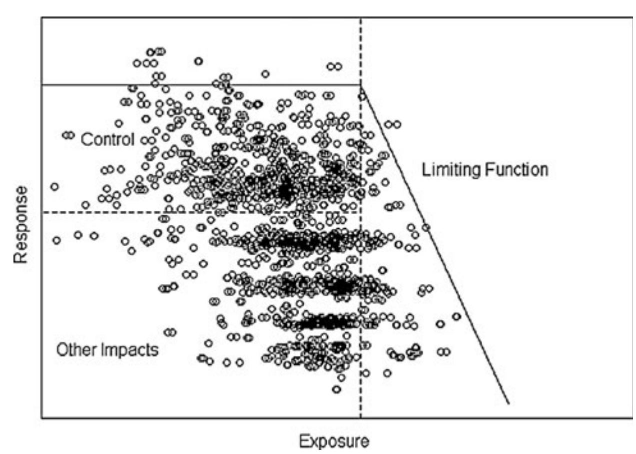

Fig. 1 Illustration of the interpretation of combined chemical and ecological field data. Ecological quality is not affected at sites above the *horizontal dashed line*, and is limited by iron exposure at sites to the right of the *vertical dashed line*

co-vary with iron would result in a steeper decline. If this were the case it would lead to more stringent thresholds derived by this approach than if they were derived from controlled exposures in laboratory tests.

Thresholds for the effects of iron on benthic macroinvertebrate communities were defined as a decline in the maximum EQI score, based on the 90th quantile regression model to a level which represents the cut-off between Good and Moderate ecological status under the water quality classification system used in the UK. This threshold is set at an EQI value of 0.7 for EQI BMWP and EQI N-Taxa, and 0.9 for EQI ASPT. EQI thresholds were calculated after setting the maximum predicted response to a value of 1. Confidence intervals were calculated by bootstrapping using 2,000 iterations.

Results and Discussion

All three measures of ecological quality (EQI BMWP, EQI N-Taxa, and EQI ASPT) showed a significant decline in response to increasing exposure to each form of iron (dissolved, total, and particulate) at the 90th quantile (Fig. 2). The EQI threshold values are shown in Table 1. In all of the analyses the sensitivity of the ecological metrics to iron exposure is similar. EQI BMWP is slightly more sensitive than either EQI N-Taxa or EQI ASPT, and this is most pronounced where the exposure is expressed as particulate iron. The fact that the thresholds derived on the basis of both total and "dissolved" iron are very similar suggests that total iron may be the most appropriate basis for assessing compliance against an EQS for iron.

This approach to deriving quality standards from field data may not be able to identify effects on particularly sensitive taxa if their absence does not result in a significant difference in the EQI metrics, because these are based on summaries of the whole benthic macroinvertebrate community. Taxa-specific assessments, such as those undertaken by Linton et al. (2007) may be able to identify particularly sensitive species or groups of species. However, relatively rare families, such as Leptophlebiidae, would only be expected to occur at a relatively small fraction of the sites, so their presence or absence at a particular site may be affected to a greater extent by the local habitat than by iron concentration. The exclusion of locally rare taxa from ecological assessments has been suggested (Van Sickle et al. 2007), although this does result in a reduction in the sensitivity of the overall assessment (Clarke and Murphy 2006). The approach taken in this study, where the ecological data are expressed relative to a reference condition, should be able to take account of this issue to some extent, whereas taxa-specific analyses similar to those of Linton et al. (2007), which are expressed relative to a predicted reference condition, may identify any taxa which are more sensitive to iron exposures than the whole community (when expressed as EQI metrics).

Leptophlebiidae were identified by Linton et al. (2007) as being particularly sensitive to iron, although their assessment was based on their abundance, and was not expressed relative to a reference condition. A criterion value of 0.21 mg L^{-1} total iron was derived for this taxon, on the basis of a 20% decline in the 90th quantile of the data ($EC20_{90th\%ile}$). This criterion value is considerably lower than any of the threshold values derived on the basis of community metrics for the UK dataset considered in this study. Three other taxa identified by Linton et al. (2007) as sensitive to total iron exposure also had $EC20_{90th\%ile}$ values below the lowest $EC10_{90th\%ile}$ value derived on the basis of total iron exposures in this study. This suggests that there may be some sensitive taxa which would not be adequately protected by quality standards based on whole community responses.

Correlations were analysed between the different metrics of iron exposure used in this study and some other potential chemical pressures, such as trace metals and indicators of sanitary pollution. Both dissolved iron concentrations and particulate iron concentrations were closely correlated with total iron concentrations, with r^2 values of 0.82 and 0.90 respectively. Positive, although weaker, correlations were also observed between dissolved iron concentrations and the concentrations of dissolved copper, nickel and zinc, with correlation coefficients (r^2) of 0.44, 0.39, and 0.42 respectively. The relationships were statistically significant ($p < 0.001$ in all cases). The bioavailabilities of these metals are known to vary with dissolved organic carbon (DOC) concentrations (De Schamphelaere and Janssen 2004; Deleebeeck et al. 2008; Heijerick et al. 2005), with higher concentrations of DOC resulting in reduced toxicity. Although no information on DOC concentrations at the sampling sites was included in the dataset, matched data for dissolved (0.45 μm filtered) iron and DOC concentrations (also 0.45 μm filtered) in a variety of surface waters throughout Great Britain were positively correlated ($r^2 = 0.74$, $p < 0.001$, Fig. 3).

Some studies have suggested that the effects of iron may be influenced by water quality parameters such as DOC (Vuorinen et al. 1999; Peruanen et al. 1994). Recent chronic studies with *Ceriodaphnia dubia* have shown the bioavailability of iron to be significantly reduced by both DOC and hardness (*Patricio Rodriguez, Centre for Investigation of Metals and Minerals, Santiago, Chile, pers. comm.*).

Ionic forms of iron (Fe^{2+} and Fe^{3+}) are unstable in the environment and are rapidly converted to corresponding hydroxides, especially at pH values greater than 7.0. In this pH range any toxicity would be due to the presence of

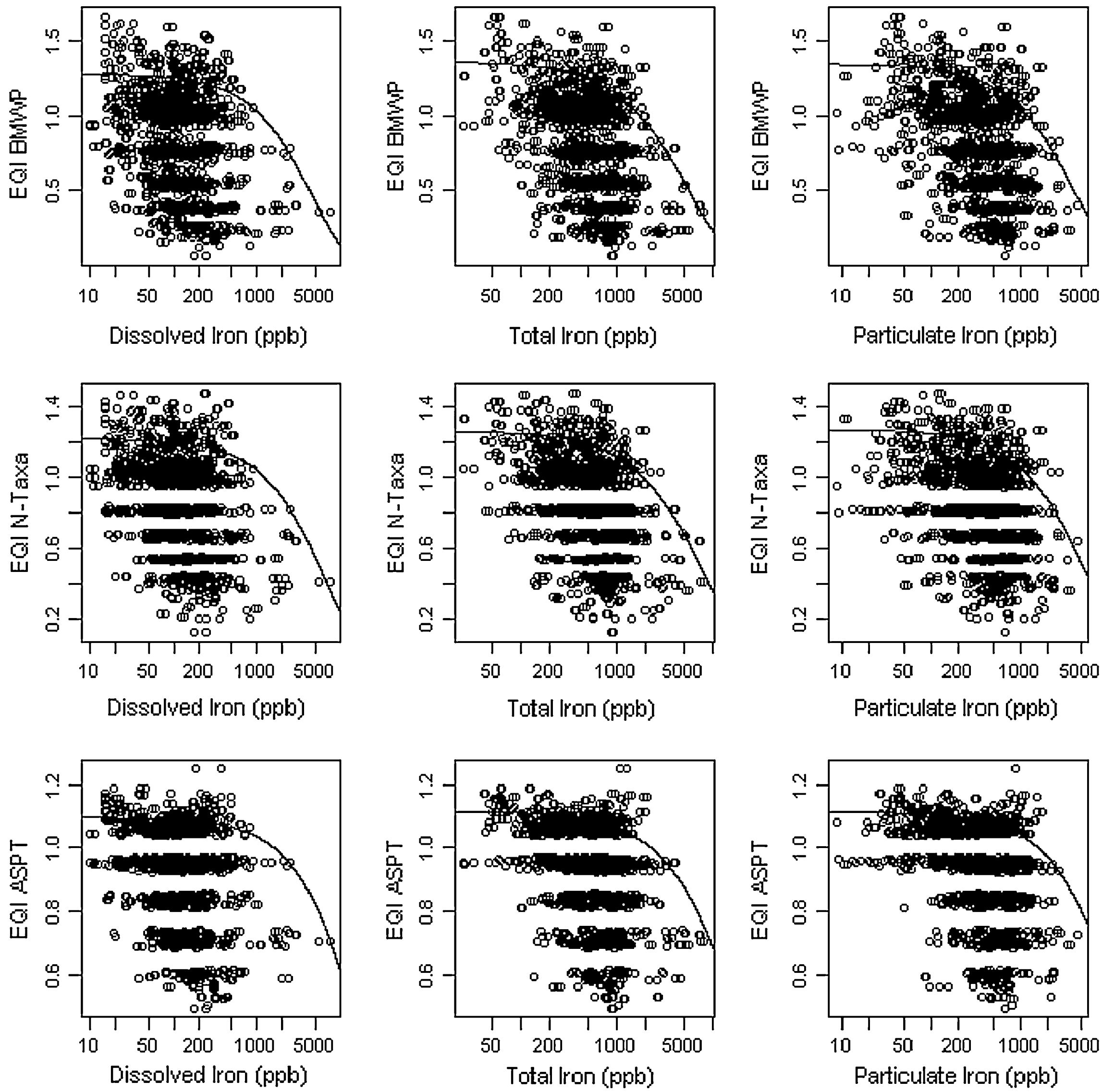

Fig. 2 Effect of iron exposure (expressed as dissolved, total, or particulate iron) on ecological responses (expressed as EQI BMWP, EQI N-Taxa, and EQI ASPT)

polymeric iron hydroxides. The majority (approximately 80%) of sites within the dataset have pH values which are above 7.0. In order to assess the influence of pH on the derivation of EC10 values the dataset was split into samples with pH values below and above either pH 6.5 or 7, and data for total iron and EQI BMWP were reanalysed. This analysis revealed that the threshold values derived for the separate portions of the data were not significantly different from each other, suggesting that pH in natural surface waters for the existing data set may have only a limited influence on the response of benthic macroinvertebrate communities to iron exposure. The thresholds derived here are appropriate for iron exposures at pH values of above 7, but may not be appropriate for exposures under lower pH conditions.

The EQI threshold values derived here (Table 1) are aligned with current ecological assessments of water quality in the UK, although are not entirely consistent with guidance under the Water Framework Directive. There may be sensitive species or taxonomic groups potentially affected at lower levels of iron exposure. Future studies should aim to identify and assess the effects of iron on such sensitive taxa, and any effects of water quality conditions on ecological responses. These studies should also, where possible, express the ecological responses relative to a reference condition in order to minimise the effects of any potentially confounding factors, such as habitat differences between sites. The use of field evidence for the derivation of chemical quality standards which are more closely aligned with ecological quality standards should ensure a

Table 1 Summary of EQI threshold values (mg L^{-1}) for different measures of iron exposure and ecological response

Exposure metric	Ecological metric[a]	EQI threshold	LCL[b]	UCL[b]
Dissolved Iron	BMWP	1.37	1.22	1.87
Dissolved Iron	N-Taxa	1.89	1.34	2.13
Dissolved Iron	ASPT	1.85	1.56	1.93
Total Iron	BMWP	1.25	1.12	1.41
Total Iron	N-Taxa	1.65	1.34	2.08
Total Iron	ASPT	1.59	1.24	2.05
Particulate Iron	BMWP	1.73	1.68	1.92
Particulate Iron	N-Taxa	2.46	2.19	3.25
Particulate Iron	ASPT	2.23	1.83	2.67

[a] BMWP, total Biological Monitoring Working Party score; N-Taxa, number of BMWP scoring taxa; ASPT, average score per taxon

[b] lower (LCL) and upper (UCL) 95% confidence limits

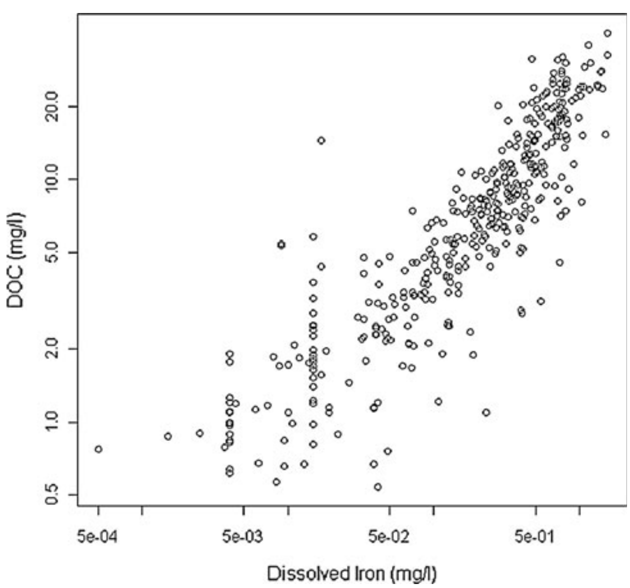

Fig. 3 Dissolved iron and dissolved organic carbon concentrations in 407 samples from the United Kingdom

more consistent level of environmental protection under the Water Framework Directive in Europe.

Acknowledgments This work was sponsored by Rio Tinto. The authors are grateful to Paul Whitehouse at the Environment Agency, Wallingford, UK, Dave Trigg at Staffordshire University for the provision of the dataset, and two anonymous peer reviewers for comments on the draft manuscript.

References

Cade B, Terrel J, Schroeder R (1999) Estimating effects of limiting factors with regression quantiles. Ecology 80:311–323

Clarke R, Murphy J (2006) Effects of locally rare taxa on the precision and sensitivity of RIVPACS bioassessment of freshwaters. Freshw Biol 51:1924–1940

Clarke RT, Furse MT, Davy-Bowker J (2003a) Analysis of 1995 survey data: phase 1 post-survey appraisal. Unit I: changes in biological condition. Environment Agency, R&D Technical Report E103, Environment Agency, Bristol

Clarke RT, Wright JF, Furse MT (2003b) RIVPACS models for predicting the expected macroinvertebrate fauna and assessing the ecological quality of rivers. Ecol Model 160:219–233

Clarke R, Davy-Bowker J, Sandin L, Friberg N, Johnson R, Bis B (2006) Estimates of comparisons of the effects of sampling variation using "national" macroinvertebrate sampling protocols on the precision of metrics used to assess ecological status. Hydrobiologia 566:477–503

Crane M, Kwok KWH, Wells C, Whitehouse P, Lui GCS (2007) Use of field data to support European Water Framework Directive quality standards for dissolved metals. Environ Sci Technol 41:5014–5021

De Schamphelaere KAC, Janssen CR (2004) Development and field validation of a biotic ligand model predicting chronic copper toxicity to *Daphnia magna*. Environ Toxicol Chem 23:1365–1375

Deleebeeck NME, De Schamphelaere KAC, Janssen CR (2008) A novel method for predicting chronic nickel bioavailability and toxicity to *Daphnia magna* in artificial and natural waters. Environ Toxicol Chem 27:2097–2107

Environment Agency (2007) Proposed EQS for Water Framework Directive Annex VIII substances: iron (total dissolved). SC040038/SR9 Environment Agency, Bristol

Heijerick DG, De Schamphelaere KAC, Van Sprang PA, Janssen CR (2005) Development of a chronic zinc biotic ligand model for *Daphnia magna*. Ecotoxicol Env Saf 62:1–10

Linton TK, Pacheco MAW, McIntyre DO, Clement WH, Goodrich-Mahoney J (2007) Development of bioassessment based benchmarks for iron. Environ Toxicol Chem 26:1291–1298

OECD (2007) SIDS initial assessment profile: iron salts category. (available from: http://cs3-hq.oecd.org/scripts/hpv/accessed 02/12/09)

Pacheco M, McIntyre D, Linton T (2005) Integrating chemical and biological criteria. Environ Toxicol Chem 24:2983–2991

Peruanen S, Vuorinen P, Vuorinen M, Hollender A (1994) The effects of iron, humic acids and low pH on the gills and physiology of brown trout (*Salmo trutta*). Ann Zool Fennici 31:389–396

Van Sickle J, Larsen D, Hawkins C (2007) Exclusion of rare taxa affects the performance of the O/E index in bioassessments. J N Am Benthol Soc 26:319–331

Vuorinen P, Keianen M, Peuranen S, Tigerstedt C (1999) Effects of iron, aluminium, dissolved humic material and acidity on grayling (*Thymallus thymallus*) in laboratory exposures, and a comparison of sensitivity with brown trout (*Salmo trutta*). Boreal Environ Res 3:405–419

Wright JF, Sutcliffe DW, Furse MT (2000) Assessing the biological quality of fresh waters: RIVPACS and other techniques. Freshwater Biological Association, Ambleside