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Reference:
de Jonge Maarten, Belpaire C., Van Thuyne G., Breine J., Bervoets Lieven.- *Temporal distribution of accumulated metal mixtures in two feral fish species and the relation with condition metrics and community structure*
Environmental pollution - ISSN 0269-7491 - 197(2015), p. 43-54
DOI: http://dx.doi.org/doi:10.1016/j.envpol.2014.11.024

Temporal distribution of accumulated metal mixtures in two feral fish species and the relation with condition metrics and community structure

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Abstract

The present study investigated temporal influences on metal distribution in gudgeon (*Gobio gobio*) and roach (*Rutilus rutilus*), and its relation to condition metrics and fish community structure. Fish communities were sampled in two seasons (autumn and spring) during two successive years and the Index of Biotic Integrity (IBI) was calculated. Cadmium, Cu, Pb, Zn and As concentrations were measured in gill, liver, kidney and muscle, and condition factor (CF) and hepatosomatic index (HSI) were measured. Cadmium (max. 39.0 µg g^{-1} dw) and Zn (max 2,502 µg g^{-1} dw) were most strongly stored in kidney and liver and periodical influences on metal accumulation were observed. CF appeared to be a stable metric related to accumulated metal-mixtures and was best related to hepatic levels, while the HSI was less useful. Relations between single metal accumulation and IBI were influenced by sample period, however, when taking into account multiple metals periodical influences disappeared.

Key words: Metal accumulation; Condition Factor (CF); Hepatosomatic Index (HSI); Index of Biotic Integrity (IBI); Fish communities

Capsule: Fish condition factor is a stable metric to assess the toxicity of accumulated metal mixtures in feral fish populations.

1. Introduction

Metal contamination in aquatic ecosystems can pose a severe threat to resident biological communities (e.g. fish, macroinvertebrates, phytobenthos), eventually resulting in the loss of species diversity (Bervoets et al., 2005; De Jonge et al., 2008). In natural watercourses, metals may occur in mixtures of different concentrations, in which they can interfere with each other both at uptake sites and at the site of toxic action, hampering easy discrimination between single and mixture effects of metals in the field (Borgmann et al., 2008; Norwood et al., 2003).

In order to assess possible impacts related to metal pollution, taking into account bioavailability aspects, bioaccumulation can be measured (Belpaire and Goemans, 2007; Bervoets and Blust, 2003). Quantifying metal concentrations in tissue of resident species represents an integrated and ecologically-relevant image of site-specific metal bioavailability, and may be a valuable alternative for the numerous physical-chemical measurements associated with the monitoring in environmental compartments such as surface water and/or sediment (Bervoets et al., 2005; De Jonge et al., 2012, 2013). Since many fish species are at a high trophic level, they can easily accumulate metals via different exposure routes (via surface water, food and sediment ingestion) and thus represent possible health risks for other fish species, piscivorous birds and mammals including humans (Bervoets and Blust, 2003; Couture and Rajotte, 2003). Moreover, accumulated metal levels in fish tissue can provide an indication of metal-induced toxicological effects (Couture and Rajotte, 2003; Bervoets et al., 2005). Various studies already investigated relations between accumulated metal levels in fish tissue and condition metrics (de la Torre et al., 2000; Bervoets and Blust, 2003; Couture and Rajotte, 2003; Maes et al., 2005; Pyle et al., 2005, 2008; Reynders et al., 2008; Bervoets et al., 2009, 2013) as well as fish community metrics (Bervoets et al., 2005; Van Ael et al., 2014). For example the study of Bervoets et al. (2005) observed that accumulated metal mixtures in gudgeon (*Gobio gobio*) liver were negatively related to the integrity of the fish community structure, measured using the Index of Biotic Integrity (IBI) (Belpaire et al., 2000). Following these relations, metal accumulation in fish tissue can be used to predict impacts of metal mixtures on natural fish communities. Similar approaches have been successfully applied for aquatic invertebrates (see e.g. the studies of Rainbow et al., 2012 and De Jonge et al., 2013).

Nevertheless, metal accumulation in fish tissue can be very variable due to seasonal influences such as shifts in diet items, temperature or activity-driven alterations in metabolic rate and decreasing body mass due to reproduction (Belpaire and Goemans, 2007; Pyle et al., 2008; Couture et al., 2008). Pyle et al. (2008) concluded in their study that relations between metal contamination and condition metrics of yellow perch (*Perca flavescens*) should be interpreted taking into account seasonal and regional influences to avoid drawing erroneous conclusions from one-time fish biomonitoring programs. Therefore it is crucial to account for seasonal variation when interpreting relations between metal accumulation and fish condition and/or fish community indices.

The aims of the present study were twofold: (1) to study temporal influences (i.e. two seasons, autumn and spring, during two successive years) on metal distribution in gill, liver, kidney and muscle tissue of gudgeon (*Gobio gobio*) and roach (*Rutilus rutilus*), and (2) to investigate relations between accumulated metal mixtures and condition metrics as well as community responses for both fish species, and assess whether seasonality can influence these relations. Both gudgeon and roach are widespread feral fish species which have been previously shown to reflect environmental metal contamination in their tissues (Bervoets and Blust, 2003; Reynders et al., 2008).

2. Material and methods

2.1. Study area and fish sampling

The River Dommel is a 145 km long lowland river of second to third order, located in the northeastern part of Flanders (Belgium) flowing from Peer to ‘s Hertogenbosch (The Netherlands). The river is part of the Meuse basin and is mainly fed by rainwater (Groenendijk et al., 1999). Near Neerpelt, the small tributary Eindergatloop has discharged metal-contaminated water in the Dommel for many years until the beginning of the 1990s, due to the presence of metallurgic industry (Ivorra et al., 2000; De Jonge et al., 2008). In order to remediate these historical polluted sediments the Dommel was dredged from February 2007 until the end of April 2007, starting from the input of metal contamination in Neerpelt (D3) till the Belgium-Dutch border (D6) (De Jonge et al., 2012).

The present study assessed eight sites on the River Dommel (D1-D8), starting from Kleine-Brogel till Valkenswaard (Figure 1). The first two sites (D1 and D2), which are located upstream of Neerpelt, contain only low levels of metals (De Jonge et al., 2008, 2012). The other sites (D3-D8) are downstream of Neerpelt and are severely metal-contaminated (De Jonge et al., 2008, 2012). Sites D1 to D8 correspond to the sites of previous studies of De Jonge et al. (2008, 2012). Although the present study did not investigate spatial variation in metal accumulation, the selected sites provide a lot of variation in metal contamination pressure (ranging from highly contaminated to non-contaminated), allowing us to better compare relations between metal accumulation and fish condition and community metrics between periods. River width of the Dommel varies from 5.0 m at site D1 to 9.2 m at site D8 while stream depth varies from 0.5 to 1.1 m (De Jonge et al., 2012).

Fish communities were sampled at all sites during four periods (two seasons, autumn and spring, during two successive years): i.e. November 2006, April 2007, November 2007 and April 2008. Sampling occurred by electrofishing using a 5 kW generator (Deka 7000) with an adjustable output voltage ranging from 300 to 500 V. The pulse frequency was 480 Hz. Electric fishing was carried out along both banks over a distance of 100 m. Furthermore paired-fyke nets (height of first ring 1 m; length 6.4 m) were placed along the banks for two successive days. All fish were identified to species level and counted. Subsequently total length was measured (± 1 mm) and weight was determined using a Kern 442.43 balance (± 0.1 g). When present, individuals of gudgeon (n varies from 0 to 17) and roach (n varies from 0 to 16) of the same size were sacrificed using the anesthetic ethyl meta-amino-benzoate methanesulphonic acid (MS222) and transported to the lab on ice. Upon arrival, all samples were immediately frozen at -20 °C.

2.2 Metal analysis in fish tissue

After thawing, fish tissues (gill, liver, kidney and muscle) were dissected and liver weight was determined using a Mettler AT 261 balance (Metller-Toledo; ± 0.001 g). Samples were dried under a constant temperature at 60 °C in a laboratory furnace. Subsequently they were weighed on a Mettler AT 261 balance and transferred to acid-cleaned and pre-weighed 14 mL polypropylene vials. Samples were microwave digested in a nitric acid-hydrogen peroxide (H_2O_2; 30%) solution (3:1, v/v) by a step-wise method in which samples were microwave treated for four times, each time increasing the microwave power by 10% (Blust et al., 1988). For each series of 50 samples additionally 5 blank and 5 reference material samples (mussel BCR-668) were included for quality control. Recoveries were within 10% of the certified values. After the digestion procedure the digest was diluted with ultra-pure water (Milli-Q) to obtain a solution of 2% acid and the vials were reweighed to accurately determine the final sample volume. Trace metals (Cd, Cu, Pb and Zn) together with the metalloid As were analysed using a quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Varian UltraMass 700, Victoria, Australia). Metal concentrations in fish tissue are expressed as µg g^{-1} dry weight.

In order to relate accumulated metal mixtures to observed effects, metal concentrations (As, Cd, Cu, Pb and Zn) in tissue were summed, taking into account differences in background concentrations between metals. The relative metal load in tissue of both gudgeon and roach was calculated following the formula (Bervoets and Blust, 2003; Van Praet et al., 2014):

$$ML_{Tissue} = \frac{[\sum_i (C_{ij}/CR_i)]}{N}$$

In which ML_{Tissue} is the metal load of the summed metal mixture in tissue (gill, liver, kidney or muscle) at a site, C_{ij} is the measured tissue concentration (µg g^{-1} dw) of metal *i* at site *j*, CR_i represents tissue concentrations (µg g^{-1} dw) of both gudgeon and roach for metal *i* from non-contaminated watercourses. Regarding CR_i in gudgeon, tissue concentrations (gill, liver, kidney and muscle) of Cd, Cu, Pb and Zn were adopted from Bervoets and Blust (2003), while As was taken from the reference locations (D1-D2) of the present study. For CR_i in roach, tissue concentration of Cd, Cu and Zn were taken from Reynders et al. (2008), whereas As and Pb levels were taken from reference locations (D1-D2) of the present study. *N* is the number of metals measured.

2.3 Fish condition and community assessment

Fish condition factor (CF) was calculated according to the following formula (Bagenal and Tesh, 1978):

$$CF = \frac{W}{L^b}\, x\, 100$$

Where W represents the wet weight of the fish (g) and L is the total length (mm). The exponent b is derived from the length-mass relationship of fish from reference sites, which is described by W = a L^b, and was previously derived for gudgeon (b=3.295; R^2=0.991; p<0.001; n=1231) and roach (b=3.367; R^2=0.987; p<0.001; n=124) from non-contaminated watercourses (Knaepkens et al., 2001).

The Hepatosomatic index (HSI) is the relation between the fish liver and the total body weight and is calculated as follows (Bagenal and Tesh, 1978; Bervoets et al., 2009, 2013):

$$HSI = \frac{W_L}{W} x\ 100$$

Where W_L is the wet liver weight (g) and W is the total wet weight of the fish (g).

The index of biotic integrity (IBI) was calculated according to Belpaire et al. (2000). For the type of watercourse under investigation, the IBI is comprised of eight metrics each scored from 1 to 5. Fish metrics that were determined are total species number, mean tolerance, mean typical species value, relative presence of type species, trophic composition, the presence of non-native species, total biomass and relative natural recruitment (Belpaire et al., 2000; Breine et al., 2004). The overall IBI score for a given sample site was calculated as the mean of scores for all metrics and ranges from 0 (poor quality) to 5 (excellent quality).

2.4 Statistics

Prior to analysis all data were log (x+1) transformed in order to meet the condition of normality. Pearson correlations were used to investigate relations between accumulated metal levels in fish tissue. The latter was done using SigmaPlot version 11.0 (Systat Software, Inc., USA).

Linear mixed models (LMM) were used (1) to explore differences in accumulated metal levels per period and tissue, (2) to compare metal accumulation between gudgeon and roach, and (3) to relate accumulated metal levels in tissue to calculated fish condition metrics and IBI. Since in the present study the number of sampled fish largely differed per period and per site (see Table S1), the dataset was largely unbalanced, justifying the use of LMM to analyze the data (Zuur, 2009). The mixed model can be considered as a linear regression with both fixed and random, hence mixed, regression coefficients. In the first model, sample period and tissue were considered as fixed factors while site constituted the random factor. In the second and third model, metal accumulation in gudgeon was related to levels in roach (second model), while metal accumulation in fish tissue (both singular and in mixtures of different combinations using the ML_{Tissue} approach) was related to fish condition and IBI (third model), taking period as fixed factor and site as random factor. All models used the restricted maximum likelihood estimator (REML). Mixed models are not interpreted in terms of explained variance (R^2), but model factors are evaluated by the *t*-statistic and corresponding *p*-value. LMM were executed using the package lme4 of the statistical software R (Bates, 2010).

3 Results

3.1 Metal concentrations in fish tissue

An overview of individual gudgeon and roach caught per site and per season is presented in Table S1. Individuals of gudgeon were caught at all sites except at D4 (Nov 2006 and Apr 2007), D5 (Nov 2007), D6 (Nov 2007 and Apr 2008) and D8 (Apr 2007). No individuals of roach could be sampled at D1 (Nov 2006, Apr 2007 and Nov 2007), D2 (Nov 2007), D3 (Apr 2007 and Apr 2008), D4 (Nov 2006 and Apr 2008), D5 (Apr 2008) and D7 (Apr 2007). Average length of gudgeon was 103 ± 28 mm (ranging from 44 to 234 mm) while it was 117 ± 63 mm (ranging from 38 to 515 mm) for roach.

Although the present study only investigates temporal trends in metal accumulation in fish, spatial patterns in As, Cd, Cu, Pb and Zn accumulation per tissue and period are presented in table S2-S6 for both gudgeon and roach.

3.1.1 Gudgeon

Significant interactions between period and tissue were observed for As, indicating significant lower As levels in liver (t=-2.92; p=0.004) and muscle (t=-2.25; p=0.025) in November 2007, compared to the other periods (Table 1). In general As distribution in gudgeon tissue followed the pattern: $[As]_{gill} > [As]_{kidney} > [As]_{muscle} > [As]_{liver}$. Significant correlations were found between [As] in all tissues resulting in R values ranging from 0.490 (p<0.05) to 0.883 (p<0.001) (Table 2). For Cd, significant interactions between period and tissue were observed, indicating significant higher Cd levels in kidney in November 2006 (t=2.27; p=0.024) and significantly lower Cd levels in liver (t=-4.75; p<0.001), kidney (t=-2.63; p=0.009) and muscle (t=-2.05; p=0.041) in November 2007 (for liver also in April 2008: t=-2.18; p=0.030) compared to the other periods (Table 1). In general Cd distribution in gudgeon tissue followed the pattern: $[Cd]_{kidney} > [Cd]_{liver} = [Cd]_{gill} > [Cd]_{muscle}$. Significant correlations were found between [Cd] in most tissues, except between muscle and gill, resulting in R values ranging from 0.534 (p<0.01) to 0.859 (p<0.001) (Table 2).

For Cu, significant interactions between period and tissue were observed, indicating significant lower Cu levels in liver (t=-4.25; p<0.001) in November 2007 and in liver (t=-3.08; p=0.002), kidney (t=-1.99; p=0.047) and muscle (t=-2.89; p=0.004) in November 2006, compared to the other tissues (Table 1). In general Cu distribution in gudgeon tissue followed the pattern: $[Cu]_{liver} > [Cu]_{kidney} > [Cu]_{gill} > [Cu]_{muscle}$. Significant correlations were found between [Cu] in most tissues, except between liver and gill, resulting in R values ranging from 0.471 (p<0.05) to 0.681 (p<0.001) (Table 2).

For Pb, significant interactions between period and tissue were observed, indicating significant lower Pb levels in liver (t=-4.29; p<0.001) and muscle (t=-4.89; p<0.001) in November 2006 (Table 1). Lead distribution in gudgeon tissue followed the pattern: $[Pb]_{gill} > [Pb]_{kidney} > [Pb]_{liver} > [Pb]_{muscle}$. Significant correlations were found between [Pb] in most tissues, except between muscle and gill, resulting in R values ranging from 0.576 (p<0.01) to 0.820 (p<0.001) (Table 2).

For Zn, significant interactions between period and tissue were observed, indicating significant higher Zn levels in kidney (t=3.06; p=0.002) in November 2006 and significant lower Zn levels in liver (t=-4.36; p<0.001) and muscle (t=-2.33; p=0.020) in November 2007 (Table 1). Zinc distribution in gudgeon tissue followed the pattern: $[Zn]_{kidney} = [Zn]_{gill} > [Zn]_{liver} > [Zn]_{muscle}$. Significant correlations were found between [Zn] in most tissues, except between liver and kidney, resulting in R values ranging from 0.513 (p<0.01) to 0.702 (p<0.001) (Table 2).

3.1.2 Roach

Significant interactions between period and tissue were observed for As, indicating significant lower As levels in muscle in November 2006 (t=-2.03; p=0.043) and November 2007 (t=-3.94; p<0.001), compared to the other periods (Table 1). Arsenic levels were comparable between tissues. Significant correlations were found between [As] in all tissues resulting in R values ranging from 0.604 (p<0.01) to 0.919 (p<0.001) (Table 2).

For Cd, significant interactions between period and tissue were observed, indicating significant lower Cd levels in liver (Nov 2007: t=-3.46; p<0.001; Apr 2008: t=-2.89; p=0.004), kidney (Nov 2007: t=-5.78; p<0.001; Apr 2008: t=-3.11; p=0.002) and muscle (Apr 2008: t=-2.88; p=0.004) compared to the other periods (Table 1). In general Cd distribution in roach tissue followed the pattern: $[Cd]_{kidney}$ > $[Cd]_{liver}$ = $[Cd]_{gill}$ > $[Cd]_{muscle}$. Significant correlations were found between $[Cd]_{liver}$ in liver with both muscle and kidney (Table 2).

For Cu, no significant interactions between period and tissue were observed, but Cu levels in all tissue were significantly higher in November 2007 (Table 1). Copper distribution in roach tissue followed the pattern: $[Cu]_{liver}$ > $[Cu]_{kidney}$ > $[Cu]_{gill}$ > $[Cu]_{muscle}$. Significant correlations were found between $[Cu]_{muscle}$ and both kidney and gill (Table 2).

For Pb, significant interactions between period and tissue were observed, indicating significant lower Pb levels in liver (t=-4.00; p<0.001), kidney (t=-2.93; p=0.004) and muscle (t=-2.19; p=0.029) in November 2006 (Table 1). Lead distribution in roach tissue followed the pattern: $[Pb]_{gill}$ > $[Pb]_{kidney}$ > $[Pb]_{liver}$ > $[Pb]_{muscle}$. Significant correlations were found between $[Pb]_{muscle}$ and both kidney and gill, and between $[Pb]_{muscle}$ and $[Pb]_{gill}$ (Table 2).

For Zn, no significant interactions between period and tissue were observed and Zn distribution in roach tissue followed the pattern: $[Zn]_{kidney}$ > $[Zn]_{gill}$ > $[Zn]_{liver}$ > $[Zn]_{muscle}$ (Table 1). Only a significant correlation was found between $[Zn]_{liver}$ and $[Zn]_{gill}$ (Table 2).

3.1.3 Comparison between gudgeon and roach

Significant relations in metal accumulation between gudgeon and roach, including significant interactions with sample period, were observed for $[As]_{liver}$ (Apr 2007, Nov 2007 and Apr 2008), $[Cd]_{gill}$, $[Cd]_{kidney}$ (Nov 2006 and Apr 2008), $[Cd]_{muscle}$ and $[Zn]_{gill}$ (Nov 2007) (Figure 2). Overall, significantly higher Cu (only liver: t=-2.32; p=0.027) and Zn levels (all tissues; gill: t=-2.90; p=0.018; liver: t=-7.05; p<0.001; kidney: t=-9.13; p<0.001; muscle: t=-2.18; p=0.037) were observed in roach compared to gudgeon, while for all other metals, tissue concentrations did not differ significantly between both fish.

3.2 Fish condition and community metrics

Condition factor of gudgeon varied from 0.41 (November 2007) to 0.75 (April 2007) (Table 3). A significant lower CF was observed in November 2006 (t=-3.44; p<0.001) compared to all other periods. Gudgeon HSI varied from 0.69 to 2.52 (both November 2007). Compared to the other periods, a significant higher HSI (t=2.07; p=0.040) was observed in April 2008, while a significant lower HSI (t=-2.99; p=0.004) was observed in November 2007. For roach, CF varied from 0.42 to 0.68 and HSI from 0.36 to 1.91. A significant lower HSI compared to all other periods was observed in November 2006 (t=-2.37; p=0.020) and November 2007 (t=-3.12; p=0.002). The Index of Biotic Integrity (IBI) ranged from 1.47 (insufficient quality) (April 2008) to 3.08 (moderate quality) (April 2007). No significant differences in average IBI, nr. of species and nr. of individuals were observed between the different periods.

Metal levels in gudgeon were generally poorly related to CF and HSI. Best relations were observed between Cd accumulation and CF, and more specifically between CF and $[Cd]_{kidney}$ (t=-1.50; p=0.15). However, none of the constructed models appeared to be significant (Figure 3). Adding Pb improved the Cd models, resulting in a significant negative relation between $[Cd]_{kidney}$ and CF (t=-2.45; p=0.025) (Figure 3). No significant interactions with period were observed.

Significant negative relations were observed between CF of roach and both As and Cd accumulation (Figure 4, Table 4). For As, strongest relations were observed between $[As]_{muscle}$ and CF (t=-3.58; p=0.002) (Table 4), while for Cd, best relations with CF were found using $[Cd]_{liver}$ (t=-5.48; p<0.001) and $[Cd]_{kidney}$ (t=-3.42; p=0.003) (Figure 4). The relation between CF and $[Me]_{liver}$ improved and was strongest when taking into account mixtures of both $ML[As+Cd]_{liver}$ (t=-6.03; p<0.001) and $ML[As+Cd+Zn]_{liver}$ (t=-6.07; p<0.001) (Figure 4). A significant effect of sample period on the relation between tissue concentrations and CF was only observed for $[As]_{kidney}$ and $ML[As+Cd]_{gill}$ (Table 4).

A significant negative relation between $[Zn]_{liver}$ in gudgeon and IBI, which was significantly different between sample periods, was observed in November 2006 (t=-2.90; p=0.010, November 2007 (t=-2.37; p=0.030) and April 2008 (t=-2.62; p=0.017) (Figure 5). According to this relation, a threshold value of 132 µg Zn g^{-1} dw in gudgeon liver could be derived at which IBI values were always below 2. No significant relations were observed between IBI and tissue concentrations for any other metal. Taking into account all accumulated metals in gudgeon, muscle tissue resulted in a significant negative relation between $ML[As+Cd+Cu+Pb+Zn]_{muscle}$ and IBI (Figure 6), however, without a significant interaction with period. For roach, no significant negative relations between tissue concentrations (both singular and in different mixtures) and IBI were observed.

4 Discussion

4.1 Temporal metal distribution in gudgeon and roach

Levels of cadmium and zinc in gudgeon reported in the present study were very high compared to concentrations measured in populations from unpolluted watercourses by Bervoets and Blust (2003) (For Cd: difference with factor 10, 171, 77 and 2 for gill, liver, kidney and muscle respectively; for Zn: difference with factor 6, 4, 18 and 1.5 for gill, liver, kidney and muscle respectively). The large discrepancy in the extent of metal accumulation between Cd and Zn is due to the essential nature of Zn, implicating that this metal is regulated in fish tissue in contrast to the non-essential Cd (Bervoets and Blust, 2003; Kraemer et al., 2006; Couture et al., 2008). In general Cd and Zn concentrations in gudgeon and roach tissue are in line with concentrations measured in populations from other Flemish watercourses which are severely contaminated with Cd and Zn (Scheppelijke Nete, Molse Nete and Grote Nete river system) (Bervoets and Blust, 2003; Van Campenhout et al., 2003; Knapen et al., 2007; Reynders et al., 2008; Bervoets et al., 2013). Moreover, Cd and Zn tissue distribution for these fish species follows the same pattern as was observed before in literature (kidney > liver > gill > muscle) (Bervoets and Blust, 2003; Reynders et al., 2008). Kidney and liver are target tissues for metal accumulation and storage in fish exposed to metal contaminated environments (Giguere et al., 2004; Kraemer et al., 2005). In fact metal accumulation in fish kidneys is driven in large part by toxicokinetic factors such as high renal blood flow and plasma protein binding (e.g. to sulfhydryl groups) (Schlenk and Benson, 2001). Strong relations between [Cd] in kidney and liver were observed both for gudgeon and roach, however not for Zn. Our results are in agreement with the study of Couture et al. (2008) which also observed significant relations between renal and hepatic Cd levels in yellow perch (*Perca flavescens*) but not for Zn. Pyle et al. (2005) found that hepatic Zn levels in yellow perch were strongly related to muscle concentrations, which suggested a possible transport pathway for Zn from liver to muscle tissue at elevated environmental Zn levels. In the present study a similar relation between $[Zn]_{liver}$ and $[Zn]_{muscle}$ was observed for gudgeon, but not for roach. Accumulation and tissue distribution of Cu and Pb in the present study is comparable with levels measured in gudgeon by Bervoets and Blust (2003) and in roach by Reynders et al. (2008) corresponding to levels observed in fish from unpolluted or mildly polluted watercourses (Allen-Gil et al., 1997; Bervoets et al., 2001; Bervoets and Blust, 2003). For As, levels in muscle tissue of roach were much higher (on average factor 7) compared to concentrations found in roach from French lakes (Noël et al., 2013) and watercourses in the Czech Republic and Slovenia (Řehulka, 2002; Petkovšek et al., 2012). Arsenic accumulation in gill tissue of both species was however comparable with As levels in gill tissue of various trout species from mining-impacted headwaters in Montana, USA (Farag et al., 2007). In general, our measured fish tissue concentrations confirm the pollution status of the River Dommel with respect to chemical measurements in surface water and sediment, indicating severe contamination with Cd, Zn and

As, and only mild contamination with Cu and Pb (Groenendijk et al., 1999; Ivorra et al., 2000; De Jonge et al., 2008, 2012).

No significant increase in tissue concentrations related to the dredging activity on the River Dommel in April 2007 (De Jonge et al., 2012) could be observed for any of the measured metals. Except for Cu and Zn in roach, influences of sampling period on metal accumulation were observed for all metals in both fish species and differed between tissues. No generalization could be made with respect to differences in tissue concentrations between seasons (spring vs autumn). For example, Cd levels in kidney of gudgeon were significantly higher compared to all other tissues and periods in November 2006, while they were significantly lower in November 2007. Corresponding to our results, Audet and Couture (2003) did not observe seasonal differences in tissue Cd concentrations of wild yellow perch from a Canadian Lake. However, the study of Couture et al. (2008) observed seasonal differences of Cu accumulation in kidney as well as Cd, Cu and Zn accumulation in liver of yellow perch of different metal-contaminated Canadian Lakes, although influences differed between the lakes and no general conclusion with respect to season could be drawn from the results. Also Kraemer et al. (2006) reported seasonal variation in hepatic Cd levels in yellow perch. Köck et al. (1996) found higher Cd concentrations in tissue of Arctic char (*Salvelinus alpinus*) during summer compared to spring, and ascribed this finding to a temperature-driven increase in metabolic rate and resulting metal uptake rate, rather than increased Cd exposure.

Significant relations between As, Cd and Zn levels in tissue of gudgeon and roach were observed, including significant influences of sample period. Roach significantly accumulated more Cu and Zn compared to gudgeon. The study of Bervoets et al. (2013) generally observed similar Cd, Cu and Zn levels between gudgeon and roach. However, observed differences were dependent of exposure concentration (i.e. significant differences between species were observed when environmental concentrations were very high).

4.2 Relation between metal accumulation and fish condition and community metrics

The fish condition factor (CF), based on the length vs weight relationship, is frequently used to determine the overall well-being of fish from natural populations and may represent recent feeding activity (Bagenal and Tesh, 1978; Bervoets and Blust, 2003; Froese, 2006; Couture and Pyle, 2008; Pyle et al., 2008). In general fish with a high condition have more biomass compared to their length, which corresponds to increased energy storage (i.e. fat deposition) from abundant food resources and less energetic requirements. In contrast, fish with low condition deposit less fat due to reduced food availability and/or increased physiological demands for energetic resources. The latter may be the case in metal-contaminated watercourses, where fish allocate their energetic resources toward metal detoxification (Smith et al., 2001; Couture and Pyle, 2008). The present study observed a significant negative relation between accumulated Cd and Pb mixtures in gudgeon kidney and condition factor, while no significant relations were found based on single accumulated metal levels. Similarly, Bervoets and Blust (2003) and Bervoets et al. (2013) did not found significant relations between metal accumulation in gudgeon tissue and CF based on single metals. However, Bervoets and Blust (2003) did observe threshold concentrations corresponding to a certain metal load (ML; relative sum of Cd, Cr, Cu, Ni, Pb and Zn in liver, kidney or gill) above which gudgeon condition was always low. For roach significant relations were observed between CF and accumulated As and Cd (in liver, kidney and for As also in muscle; both singular and in mixture with Zn), which were in general most significant for liver and stronger compared to the relations found for gudgeon. The fact that relations were strongest for liver can be attributed to the central function of fish liver in organismic homeostasis, environmental acclimation and metal detoxification (Schlenk and Benson, 2001). Similarly Bervoets et al. (2013) observed a negative relation between roach condition and $[Cd]_{liver}$. In contrast, Reynders et al. (2008) did not observe significant relations between Cd, Cu and Zn accumulation in roach and CF. With respect to freshwater fish other than gudgeon and roach, strong relations between CF and hepatic Cd in brown trout were observed by Clements and Rees (1997). Pyle et al. (2005) observed negative relations between $[Cd]_{liver}$ in yellow perch and condition factor along a metal pollution gradient. Similar relations were observed by Maes et al. (2005) for European eel (*Anguilla anguilla*) and by Farkas et al. (2003) for bream (*Abramis brama*). Bervoets et al. (2009) found significant relations between condition of caged common carp (*Cyprinus carpio*) and the relative metal load in kidney. In the present study a significant effect of sample period on the relation between tissue concentrations and CF was only observed for $[As]_{kidney}$ and $ML[As+Cd]_{gill}$, but not for the relations using $[Me]_{liver}$, which were generally much stronger. The study of Pyle et al. (2008) observed significant correlations between hepatic and renal Zn levels and condition of yellow perch, which appeared to be stable between seasons. Eastwood and Couture (2002) found negative relations between hepatic Cu concentrations in yellow perch and its scaling coefficient, which is a descriptor of the growth pattern of fish populations that is determined from length vs. weight relationships of a certain population and is used in the calculation of the fish condition index. In the latter study neither fish condition nor scaling coefficient were affected by season. Our results, together with the above-mentioned findings from literature, indicate that condition factor is relatively stable toward influences of sample period confirming the robustness of CF as a metric to assess toxicity of accumulated metal-mixtures in feral fish populations.

The HSI represents the ratio of liver mass to body mass. Short-term stress generally decreases the HSI either because of depressed feeding or because of an increased energy drain. Under conditions of chronic stress liver cells may undergo an adaptive hyperplasia (increase in cell proliferation) and/or hypertrophy (increase in organ volume), resulting in an increase of HSI (Schlenk and Benson, 2001). The present study observed decreased HSI for roach and gudgeon during autumn (November 2006 and 2007) and increased HSI for gudgeon during spring (April 2008), which can be related to differences in food availability between both seasons. No significant relations between metal accumulation in fish tissue and HSI, neither for gudgeon nor for roach, were observed. Similarly Bervoets et al. (2013) did not observe significant positive correlations between metal accumulation in liver and HSI for both gudgeon and roach. In contrast Ozmen et al. (2006) and Bervoets et al. (2009) found a significant positive relation between hepatic Cd levels and HSI in common carp. Studies of both Eastwood and Couture (2002) and Pyle et al. (2005) did not observe significant relations between metal accumulation in tissue of yellow perch and HSI. In their review paper, Couture and Rajotte (2003) stated that, in over five years of research, they did not found consistent indications that HSI of yellow perch was related to metal contamination. Based on our results together with findings from literature, we cannot support the use of HSI to assess the toxicity of accumulated metal mixtures in feral fish populations.

Fish community structure has been widely applied to assess the ecological status of aquatic ecosystems. However, it has only seldom been used to assess ecological impacts of metal pollution (e.g. Hartwell, 1997; Dyer et al., 2000; Bervoets et al., 2005). The Index for Biotic Integrity (IBI), developed by Karr (1981), provides a scoring system to qualify fish community characteristics such as species diversity, trophic position, biomass and condition and was adapted to Flemish rivers by Belpaire et al. (2000). In the present study a significant negative relation was observed between IBI and Zn in gudgeon liver. Similarly, the study of Moraes et al. (2003) observed decreased fish diversity and density associated with elevated levels of Cd, Pb and to a lesser extent Zn in the liver of catfish *Rhamdioglanis frenatus*. Based on the observed relation between Zn in gudgeon liver and IBI, a threshold value of 132 µg Zn g^{-1} dw was derived above which IBI scores were always below 2, corresponding to an insufficient quality of the sampled fish community. Based on relations between IBI and metal levels in muscle tissue of European eel in Flanders, the study of Van Ael et al. (2014) derived threshold values of 33.3 µg Zn g^{-1} ww above which a good ecological status (≈ IBI ≥ 3) was never reached. In the present study Zn in muscle tissue of both gudgeon and roach frequently exceeded the threshold value of 33.3 µg Zn g^{-1} (on dry weight basis) and IBI scores ≥ 3 were never observed. The latter example illustrates the usefulness of deriving critical metal concentrations in fish tissue corresponding to metal-induced effects on fish community structure.

In the present study relations between IBI and hepatic Zn differed between sampling periods. No significant differences in IBI scores were found between sample periods, while significant influences of period were observed for Zn accumulation in gudgeon liver. Therefore we can assume that periodical influences on hepatic tissue concentrations dominantly affected the relations with IBI, rather than influences on IBI itself. Summing all accumulated metals in gudgeon muscle using the relative metal load approach resulted in a significant negative relation with IBI, which however was not influenced by sample period. Although seasonal influences were not included, Bervoets et al. (2005) observed strong negative relations between IBI and accumulated metal mixtures (Cd, Co, Cr, Cu, Ni, Pb and Zn) in gudgeon liver. The study of Dyer et al. (2000) did not found relations between IBI and tissue toxic units (Al, As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag and Zn). However, this large dataset was based on tissues of 43 different fish species which were sampled at different periods between 1990 and 1996. Fish communities are generally affected by multiple stressors instead of single metals alone (Dyer et al., 2000; Bervoets et al., 2005; Posthuma and De Zwart, 2006). Based on the limited dataset of the present study it seems that by taking into account tissue accumulation of multiple metals, periodical influences on relations between tissue concentrations and IBI can be of less importance.

5 Conclusions

The present study observed influences of sample period on metal accumulation in tissue of gudgeon and roach. However, no clear trends with respect to season (autumn vs spring) could be found. Cadmium and Zn concentrations in both fish species were most strongly stored in kidney and liver. Fish condition factor turned out to be a stable metric to assess toxicity of accumulated metal-mixtures in feral fish populations, and was best related to hepatic metal levels. In contrast, the hepatosomatic index appeared to be less useful in the present study. Relations between single metal accumulation in fish tissue and IBI were influenced by sample period. However, when taking into account multiple metals periodical influences disappeared.

In general, taking into account metal mixtures seems to provide better relations between metal concentrations in fish tissue and both organismal (condition factor) and community (IBI) metrics, levelling out periodical influences which can hamper interpretations of biomonitoring results using feral fish populations.

Acknowledgement

We like to thank the fishing team of the Institute for Nature and Forest Research for the fish sampling. Furthermore we thank Dr. Valentine Mubiana and Steven Joosen for sample processing and metal analyses and Prof. Stefan Van Dongen for his help with the statistical analyses. Yves Maes is acknowledged for making the map of the sample sites. This study was financially supported by the Flemish Environment Agency (TWOL). Maarten De Jonge is funded by a post-doctoral research grant of the Research Foundation Flanders (FWO).

Supporting information

Tables S1 to S6 are provided as supporting information.

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Table captions

Table 1: Metal concentrations (in µg g^{-1} dw) in gill, liver, kidney and muscle of *G. gobio* (Average over all sites) and *R. rutilus* (Average over all sites) on the River Dommel (D1-D8) during four sampling periods (November 2006, April 2007, November 2007 and April 2008). Average concentrations ± standard deviations, minimum and maximum (between brackets) concentration are reported.

Table 2: Pearson correlation between metal levels in fish tissue of *G. gobio* (n=26) and *R. rutilus* (n=22). Correlation coefficients (R-values) and significance level (*p*-values) are presented.

Table 3: Seasonal variation in fish condition metrics and ecology (Average of all sites; n=8). Average values (D1-D8) and minimum and maximum values (between brackets) are presented.

Table 4: Results of the linear mixed models studying the relationship between accumulated metal mixtures in *R. rutilus* and condition factor (*n*=22). In case of a significant interaction between tissue and period, only the significant models are presented. Significant models are highlighted in bold.

Figure captions

Figure 1: The River Dommel and its location in Flanders (Belgium). The eight sample sites (D1-D8) are indicated.

Figure 2: Relation between metal levels in *G. gobio* and *R. rutilus*, presented per tissue (n=17). The full line represents the 1:1 relationship.

Figure 3: Relation between *G. gobio* condition factor (CF) and (A) Cd accumulation in kidney; (B) $ML[Cd+Pb]_{kidney}$ (*n*=26). Model diagnostics (*t* and *p* values) for other tissues are presented.

Figure 4: Relation between *R. rutilus* condition factor (CF) and (A) As accumulation in liver, (B) Cd accumulation in liver, (C) $ML[As+Cd]_{liver}$ and (D) $ML[As+Cd+Zn]_{liver}$ (*n*=22). Model diagnostics (*t* and *p* values) are presented.

Figure 5: Relation between IBI and Zn accumulation in liver of *G. gobio* (*n*=26). Model diagnostics (*t* and *p* values) are presented per sample period.

Figure 6: Relation between IBI and $ML[As+Cd+Cu+Pb+Zn]_{muscle}$ for *G. gobio* (*n*=26). Model diagnostics (*t* and *p* values) are presented.