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Research Article

Tolerance of native and non-native fish species to chemical stress: a case study for the River Rhine

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Abstract

Freshwater ecosystems can be impacted by invasive species. Non-native species can become invasive due to their high tolerance to environmental stressors (e.g., pollution and habitat modifications). Yet, tolerance of native and non-native fish species exposed simultaneously to multiple chemical stressors has not been investigated. To quantify tolerance of native and non-native fish species in the Delta Rhine to 21 chemical stressors we derived Species Sensitivity Distributions (SSDs). Differences in tolerance between the two species groups to these stressors were not statistically significant. Based on annual maximum water concentrations of nine chemical stressors in the Delta Rhine the highest contribution to the overall Potentially Affected Fraction (PAF) of both species groups was noted for ammonium, followed by azinphos-methyl, copper, and zinc. PAFs of both groups for metals and ammonium showed a significant linear decrease over the period 1978–2010. Deriving a PAF for each species group was a useful tool for identifying stressors with a relatively highest impact on species of concern and can be applied to water pollution control. Species traits such as tolerance to chemical stress cannot explain the invasiveness of some fish species. For management of freshwater ecosystems potentially affected by non-native species, attention should be given also to temperature, hydrological regimes, and habitat quality.

Key words: species sensitivity distributions; invasive species; tolerance; metals; pesticides

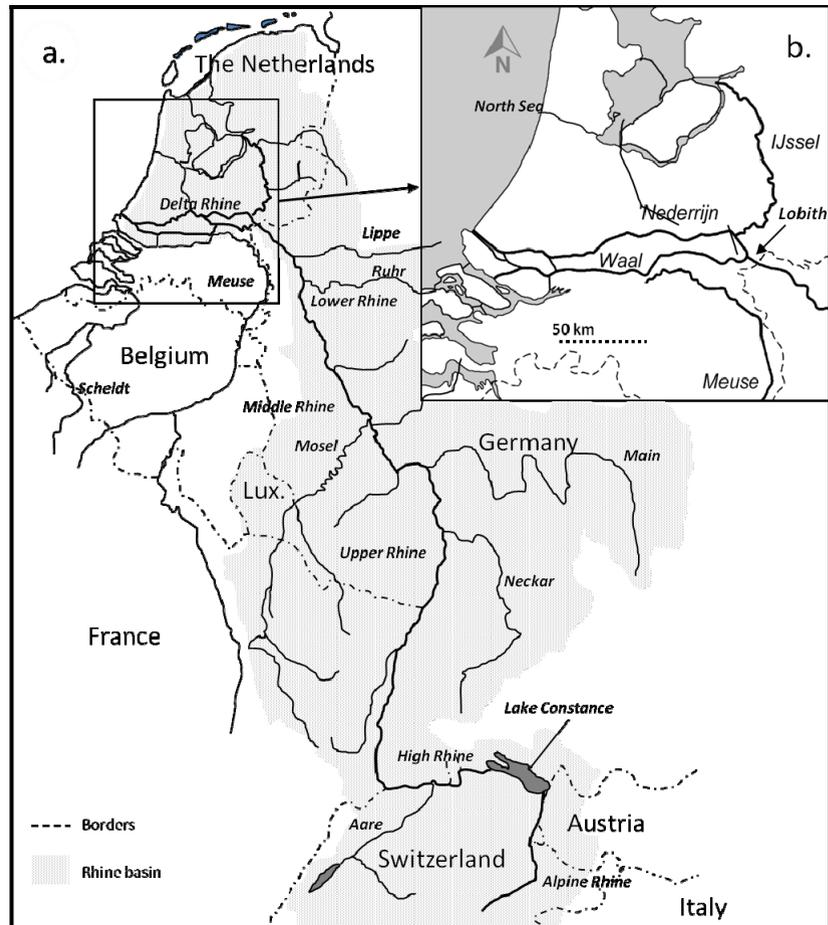
Introduction

Large numbers of species have been introduced in habitats outside their native areas (Lodge 1993). About ten percent of these non-native species can become highly invasive (Jeschke and Strayer 2005; Ricciardi and Kipp 2008). One of the characteristics considered to make a non-native species become invasive is a higher tolerance to environmental stressors, both natural and human induced, compared with that of native species (Karatayev et al. 2009; Leuven et al. 2011; Verbrugge et al. 2012). Yet, research on relative tolerances of non-native species to physico-chemical stressors is severely limited for three main reasons. Firstly, most of the studies focus

on a single non-native species, usually an invertebrate (Piola and Johnston 2006; Karatayev et al. 2009; Weir and Salice 2012). Secondly, most attention is given to a single physico-chemical stressor at a time, for example temperature, organic waste, salinity, single chemical compounds, or nutrient pollution (Menke et al. 2007; Früh et al. 2012). Finally, there is a lack in comparative and quantitative assessment of tolerance of native and non-native species to (multiple) stressors (Vila-Gispert et al. 2005; Scott et al. 2007; Alonso and Castro-Diez 2008).

The number of non-native fish species in the River Rhine strongly increased during the 20th century due to the unintentional and deliberate introductions of species (Leuven et al. 2011). During this century, pollution of the river has

Figure 1. The River Rhine (a) and location of the Lobith gauging station (b).



also increased and reached maximum levels in the period 1960–1980. After the environmental rehabilitation of the Rhine, an improvement in water quality has been observed (ICPR 2012). Yet, pollution with organic substances from agricultural activities and with metals from historically polluted sediments continues to be problematic (Nienhuis et al. 2002; ICPR 2012). A comparison of the potential impact of water pollution on native and non-native fish species has not yet been performed.

The aim of our study was three-fold. Firstly, we examined quantitatively whether native and non-native fish species differed in tolerance to a broad range of chemical stressors occurring in their habitat. Secondly, we investigated the overall effect of multiple chemical stressors on native and non-native species and ranked the chemical stressors according to their potential impact on each species group. Thirdly, we determined the trends in impact of chemical

stressors in the river for both species groups over time, calculated as the potentially affected fraction (PAF) of species. To address this aim, we focused on the native and non-native fish species in the distributaries of the River Rhine in the Netherlands (Delta Rhine, i.e. rivers Waal, IJssel and Nederrijn; Figure 1). We quantified effects of multiple chemical stressors on fish species by combining Species Sensitivity Distributions (SSDs) derived from acute toxicity tests and environmental concentrations measured in the Delta Rhine in the period 1978–2010. We constructed SSDs for native and non-native fish species of the Delta Rhine to analyze their tolerance to chemical stressors. Then, we combined the SSDs with monitoring data on chemical distribution in the river to estimate the effects of environmental exposure for each fish species group. Relevance of our quantitative approach for management of native and non-native fish species and for water pollution control is discussed.

Methods

Deriving Species Sensitivity Distributions from acute toxicity tests

A list of native and non-native fish species occurring in the freshwater sections of the River Rhine distributaries Waal, Nederrijn and IJssel in the Netherlands (Delta Rhine) was derived from Leuven et al. (2011). In total, 60 fish species were recorded in the Delta Rhine over the years 1900–2010, of which 36 were native species and 24 were non-native species (Table 3).

Species Sensitivity Distributions (SSDs) were derived to analyze the variation in tolerance of native and non-native fish species to multiple chemical stressors based on acute toxicity data from laboratory studies. Data on the tolerance to chemical stressors (Table 1) of fish species of the Delta Rhine were collected from the RIVM e-toxBase and the US-EPA ECOTOX database (<http://www.e-toxbase.com>; <http://cfpub.epa.gov/ecotox/>). Both databases comprised acute median Lethal Concentration (LC_{50}) values, i.e., concentrations with mortality effect for 50% of the test organisms. Chronic No Observed Effect Concentrations, i.e., a level of exposure which does not cause observable harm to the organism (Posthuma et al. 2002), were not considered in the current study due to the lack of data. To obtain sufficient toxicity data, test results for different life stages of fish were included. If multiple LC_{50} values were reported for one species and different life stages, the geometric mean for the same life stage with the lowest LC_{50} was taken for further analysis, as suggested by US-EPA (TenBrook et al. 2009). The geometric mean represents the best estimate of a toxicity value (TenBrook et al. 2009). For 21 chemicals, toxicity data were available for at least four different fish species and were included in this study to derive SSDs. This number of test species was sufficient since our study focused on a single taxonomic group (fish) and not on the whole aquatic community (ranging from bacteria to mammals). The variation in sensitivity to chemical stressors within a single taxonomic group is generally lower than over a community (Von der Ohe and Liess 2004).

The SSDs were derived for each stressor for each species group, plotted as the cumulative log-normal distribution of LC_{50} test concentrations. In the log-normal distributions, the standard deviations (SD) describe the variation in tolerance among species. The Hazardous Concentration for

50% (HC_{50}) and 5% (HC_5) of the species, commonly used for regulatory purposes, were calculated according to Aldenberg and Jaworska (2000). If log10-transformed toxicity values from both species groups were normally distributed according to Shapiro-Wilk tests, potential differences in tolerance to stressors between native and non-native fish species were compared with Independent t-tests (D'Agostino and Pearson 1973; Razali and Wah 2011). The difference in variation between species groups was compared by Levene's tests. Differences were considered statistically significant at $p < 0.05$.

Environmental concentrations of chemicals

Monitoring data on water concentrations in the Delta Rhine were available only for nine stressors out of those for which toxicity data were collected (Table 2). The monitoring data were obtained for the period 1978–2010 for metals and ammonium and for the period 1992–2010 for pesticides from the International Commission for the Protection of the Rhine (ICPR 2012, <http://maps.wasserblick.net:8080/iksr-zt/>). The data represent the concentrations of each chemical measured monthly in the surface water of the main River Rhine channel at gauging station Lobith near the Dutch-German border (Figure 1). Since we used effect concentrations based on acute toxicity tests, maximum annual concentrations of each chemical were selected for further analysis.

Effect assessment

In environmental risk assessment, SSDs are used to estimate the Potentially Affected Fraction (PAF) of species at a certain level of exposure (Traas et al. 2002). As such the PAF represents the fraction of species potentially affected above their LC_{50} level at measured environmental concentrations, depending on the mean toxicity, the variation in sensitivity among species, and the environmental concentration of the stressor.

Monitoring data on nine stressors were available to estimate the PAFs of the native and non-native species group as the fraction of each species group exposed beyond the LC_{50} end point at the specific river location (Lobith) (Aldenberg and Jaworska 2000). The PAFs of species at the measured exposure concentration can be considered a quantification of the severity of effect. The PAFs were calculated for each stressor per year for the period 1992–2010 to facilitate

Table 1. The Hazardous Concentration (log-transformed, mg/L) at 5% (HC₅) and 50% (HC₅₀) of chemicals and the standard deviations (SD) derived from Species Sensitivity Distributions for non-native and native fish species occurring in the Delta Rhine. Also, the number of species (*n*) for which toxicity data were available per chemical is provided.

Chemical stressor	Non-native species				Native species			
	Log HC ₅	Log HC ₅₀	SD	<i>n</i>	Log HC ₅	Log HC ₅₀	SD	<i>n</i>
Zinc	-0.54	0.58	0.64	7	-0.15	0.51	0.39	13
Ammonium	-1.00	0.64	1.04	9	-0.66	0.86	0.92	10
DDT	-3.00	-1.40	1.14	7	-2.70	-1.4	0.84	8
Malathion	-0.85	0.45	0.76	8	-2.00	-0.16	1.23	8
Lindane	-2.00	-0.78	0.74	7	-2.00	-1.22	0.55	8
Trichlorfon	-1.10	0.85	1.11	7	-1.40	0.47	1.09	8
Copper	-1.40	-0.05	0.79	7	-1.52	-0.51	0.58	7
Phenol	0.31	1.25	0.53	6	0.72	1.32	0.35	8
Cadmium	-0.59	0.58	0.68	7	-2.00	0.28	1.32	6
Pentachlorophenol	-2.00	-0.80	0.72	5	-1.70	-0.92	0.42	8
Endosulfan	-4.00	-2.07	1.18	7	-3.00	-1.98	0.55	6
Deltamethrin	-2.40	-1.55	0.46	4	-3.00	-1.16	1.27	8
Chlorpyrifos	-2.40	-0.94	0.81	4	-3.00	-1.44	0.84	8
Carbaryl	0.47	0.99	0.30	6	0.08	0.79	0.40	5
Endrin	-3.70	-2.26	0.90	6	-3.22	-1.91	0.76	5
Azinphos-methyl	-2.70	-0.48	1.27	6	-4.00	-1.59	1.44	5
Methoxychlor	-2.00	-1.17	0.43	5	-2.00	-1.38	0.50	5
Heptachlor	-2.39	-0.90	0.83	6	-2.40	-1.43	0.56	4
Atrazine	0.76	1.40	0.37	6	1.12	1.62	0.27	4
Diazinon	0.06	0.63	0.32	5	-1.40	0.07	0.84	5
Fenitrothion	0.19	0.55	0.20	4	-0.59	0.49	0.59	4

Table 2. The average potentially affected fraction PAFs (%) for native and non-native fish species in the River Rhine at Lobith per chemical stressor for the years 1992–2010. Average of annual maximum concentrations (log-transformed, mg/L) over the period 1992–2010 and SD for 9 stressors in the River Rhine at Lobith. Changes in annual maximum concentrations, tested using linear regression (p-values, significant at $p < 0.001$, slopes and intercepts are shown) for pesticides over the period 1992–2010 and for ammonium, phenol, and metals over the period 1978–2010. n.s. – not significant.

Chemical stressor	PAF (%) Non-native	PAF (%) Native	River water		p-value	Slope	Intercept
			Log max	SD			
Azinphos-metyl	0.12	2.62	-1.84	0.29	n.s.	1E-03	-1.21
Copper	2.45	2.58	-2.02	0.17	n.s.	0	0.95
Cadmium	1.4E-07	0.36	-3.74	0.24	<0.001	-5.4E-05	0.11
Zinc	1.03	0.01	-1.29	0.17	<0.001	-3.0E-03	6.01
Malathion	2.4E-12	1.4E-03	-4.85	0.31	n.s.	-2.5E-06	0.01
Pentachlorophenol	1.3E-04	4.4E-14	-4.62	0.35	n.s.	-2.6E-06	0.01
Fenitrothion	6.2E-20	4.5E-16	-1.92	0.38	n.s.	0	1.98
Atrazine	9.4E-48	1.7E-92	-4.23	0.45	<0.001	-9.5E-06	0.02

comparison among stressors. The average PAF over this period was used to rank the stressors according to the potential effect they have on each species group. The total PAF, or multi-substance msPAF, for pesticides and total msPAF for metals were calculated by response addition based on PAFs from individual stressor as:

$$msPAF = 1 - \prod_{i=1}^n (1 - PAF_i)$$

with n the number of stressors and PAF_i the PAF for each stressor individually (Traas et al. 2002).

The differences in PAFs between native and non-native species were compared by Wilcoxon signed rank test (at $p < 0.05$). The trends of PAFs and chemical concentrations in water over time were analyzed using linear regressions. All statistical tests were performed with SPSS 15.0 for Windows.

Results

Toxicity data on individual species

In total, 21 chemical stressors were included in the analysis, i.e., 3 metals, 16 pesticides, and 2 phenols. The amount of toxicity data available for native fish species (total 142 acute LC_{50} values) was larger than for non-native (total 129 acute LC_{50} values), with a maximum per compound of 13 for native fish species (for zinc) and of 9 for non-native species (for ammonium) (Table 1). Toxicity data were available for the same subset of fish species (Table 3).

Variation in tolerance to chemical stressors between native and non-native fish species

The HC_{50} values derived from SSDs for each chemical stressor did not differ significantly (t-test, all $p > 0.05$) between the native and non-native species (e.g., Figure 2 for copper). Yet, non-native species tended to be slightly more tolerant than native species for the majority of stressors studied (Table 1). The HC_{50} of non-native species to metals was consistently higher and HC_{50} to ammonium and phenols lower than for native species.

The standard deviations (SD) of 12 stressors out of 21 was larger for non-native than for native species while the SD for the remaining 9 stressors was smaller (Table 1), (all non-significant, Levene's test, $p > 0.05$). In general,

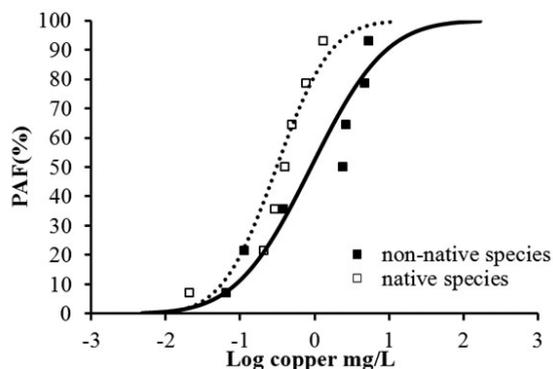


Figure 2. Species Sensitivity Distribution for copper used to derive Hazardous Concentrations for 5% and 50% (HC_5 and HC_{50} , respectively) of native and non-native fish species. PAF is Potentially Affected Fraction in percentage.

the differences between the most sensitive and the most tolerant fish species ranged from a factor of 2 to 800 for the non-native species, and from a factor of 4 to 1200 for the native species. In the non-native species group, *Oncorhynchus mykiss* (Walbaum, 1792) and *Micropterus salmoides* (Lacépède, 1802) were among the most sensitive species and *Carassius auratus* (Linnaeus, 1758) and *Cyprinus carpio* (Linnaeus, 1758) the most tolerant. In the native species group *Salmo trutta* (Linnaeus, 1758) was one of the most sensitive and *Gasterosteus aculeatus* (Linnaeus, 1758) and *Rutilus rutilus* (Linnaeus, 1758) were among the most tolerant. On average, the difference between the most sensitive species of the non-native and native species was a factor of 3, and between the most tolerant species of both groups a factor of 4.

Ranking stressors according to Potentially Affected Fractions

Based on the monitoring data, the average PAFs over the period 1992–2010 related to nine chemical stressors were calculated for native and non-native species (Table 2). The msPAF based on effects addition from all stressors was slightly higher for the native species (38.97%) than for the non-native species (31.86%). The differences between the PAFs of native and non-native species were not statistically significant (Wilcoxon signed rank tests, $Z = -0.27$, $p > 0.05$). Ammonium had the highest PAFs for both species groups (native 35.43%, non-native 29.34%). The ranking of the stressors slightly differed between species groups (Table 2). The fraction of the native species

Table 3. Fish species, native (0), non-native (1) recorded in main and side channels of the freshwater sections of River Rhine distributaries (Delta Rhine) in 1900–2010 (From Leuven et al. 2011), number of chemicals tested, and species presence (+) or absence (-) in two selected decades. Synonyms of species names are given in brackets. Climate in region of origin of species in brackets: (S) subtropics, (T) tropics.

Species	Native/non-native species	Number of chemicals tested per species	1980–1990	2000–2010
<i>Abramis brama</i>	0	4	+	+
<i>Acipenser baerii</i>	1	3	-	+ ^b
<i>Acipenser gueldenstaedtii</i>	1		-	+ ^b
<i>Acipenser ruthenus</i>	1	1	-	+ ^b
<i>Acipenser stellatus</i>	1		-	+ ^b
<i>Acipenser sturio</i>	0		-	-
<i>Alburnoides bipunctatus</i>	0		-	+
<i>Alburnus alburnus</i>	0	6	+	+
<i>Alosa alosa</i>	0		+	-
<i>Alosa fallax</i>	0		+	+
<i>Ameiurus melas (Ictalurus melas)</i>	1	10	-	-
<i>Ameiurus nebulosus (Ictalurus nebulosus)</i>	1	1	-	+ ^b
<i>Anguilla anguilla</i>	0	7	+	+
<i>Aspius aspius</i>	1		-	+
<i>Ballerus sapa (Abramis sapa)</i>	1		-	+
<i>Barbatula barbatulus (Noemacheilus barbatulus)</i>	0	3	+	+
<i>Barbus barbus</i>	0	1	+	+
<i>Blicca bjoerkna (Abramis bjoerkna)</i>	0		+	+
<i>Carassius carassius</i>	0	8	+	+
<i>Carassius gibelio (Carassius auratus)</i>	1	20	+	+
<i>Chondrostoma nasus</i>	0		+	+
<i>Cobitis taenia</i>	0		+	+
<i>Coregonus oxyrinchus</i>	0		-	+
<i>Cottus rhenanus / Cottus perifretum</i>	0		+	+
<i>Ctenopharyngodon idella (S)</i>	1	6	+ ^b	+ ^b
<i>Cyprinus carpio (S)</i>	1	21	+	+
<i>Esox lucius</i>	0	13	+	+
<i>Gasterosteus aculeatus</i>	0	16	+	+
<i>Gobio gobio</i>	0	3	+	+
<i>Gymnocephalus cernua</i>	0	2	+	+
<i>Hypophthalmichthys molitrix (Aristichthys molitrix) (S)</i>	1	6	+ ^b	+ ^b
<i>Lampetra fluviatilis</i>	0		+	+
<i>Leuciscus idus</i>	0	12	+	+
<i>Leuciscus leuciscus</i>	0	2	+	+
<i>Lota lota</i>	0		+	+
<i>Micropterus salmoides (S)</i>	1	17	-	-
<i>Misgurnus fossilis</i>	0		+	+
<i>Neogobius fluviatilis</i>	1		-	+
<i>Neogobius kessleri</i>	1		-	+
<i>Neogobius melanostomus</i>	1		-	+
<i>Oncorhynchus mykiss (S)</i>	1	20	+ ^b	+ ^b
<i>Osmerus eperlanus</i>	0		+	+
<i>Perca fluviatilis</i>	0	3	+	+
<i>Petromyzon marinus</i>	0		+	+
<i>Platichthys flesus</i>	0	5	+	+
<i>Poecilia reticulata (T)</i>	1	21	+ ^b	+ ^b
<i>Proterorhinus semilunaris</i>	1		-	+
<i>Pseudorasbora parva</i>	1	1	-	+
<i>Pungitius pungitius</i>	0	1	+	+
<i>Rhodeus amarus (Rhodeus sericeus)</i>	1 ^a	1	-	+
<i>Romanogobio belingi (Gobio albipinatus)</i>	1		-	+
<i>Rutilus rutilus</i>	0	10	+	+
<i>Salmo salar</i>	0	14	-	+
<i>Salmo trutta</i>	0	15	+	+
<i>Sander lucioperca (Stizostedion lucioperca)</i>	1	1	+	+
<i>Scardinius erythrophthalmus (Rutilus erythrophthalmus)</i>	0	5	+	+
<i>Silurus glanis</i>	0	3	+	+
<i>Squalius cephalus (Leuciscus cephalus)</i>	0	3	+	+
<i>Tinca tinca</i>	0	7	+	+
<i>Vimba vimba</i>	1		+	+

^a regarded as non-native species according to Van Damme et al. (2007); ^b incidentally recorded.

affected by the combined pesticides was higher than that of the non-natives (native 2.62%, non-native 0.12%). In contrast, the fraction of non-native species affected by metals was larger (native 2.94%, non-native 3.46%). Azinphos-methyl contributed most to the msPAF for pesticides, and copper dominated the msPAF for metals.

Temporal trends in Potentially Affected Fractions

Over the studied period, concentrations in Rhine water decreased for all chemical stressors, however, the decrease was significant for cadmium, zinc, ammonium, and atrazine only (Table 2). The msPAF for metals and the PAF for ammonium corresponding to the annual maximum water concentrations in the River Rhine at Lobith showed a significant linear decrease over the period 1978–2010 for both native and non-native species (Figure 3). For pesticides, there was no significant change in the PAF for the period 1992–2010 for either species group. Since we used the same species to determine the tolerance over time, these trends in PAFs were related to changes in concentrations of chemicals in water. Figure 3 shows that the drop in water concentrations of ammonium and metals in the River Rhine resulted in comparable values of the PAFs in the last decade for the native and non-native species, however, for ammonium it still remains high. The variability in the PAFs over the period 1978–1990 was rather low due to low variability in ammonium concentration over this period (e.g., between 1.2 and 2.0 mg/l), relatively low steepness of the species sensitivity curves and the PAF-scale used in the graph.

Discussion

Uncertainties

In general, the lack of available toxicity data can limit the number and diversity of species for SSD development (Raimondo et al. 2008). This limited availability of toxicity data for native and non-native species could introduce an uncertainty into the derived SSDs and HC_{50} . Usually, this uncertainty decreases with increasing number of species included in the HC_{50} calculations (Posthuma and Suter II 2011; Golsteijn et al. 2012). To deal with such data uncertainty, sample sizes can be enhanced by increasing the number of laboratory experiments. However, this is expensive, unfeasible for endangered/protected species and

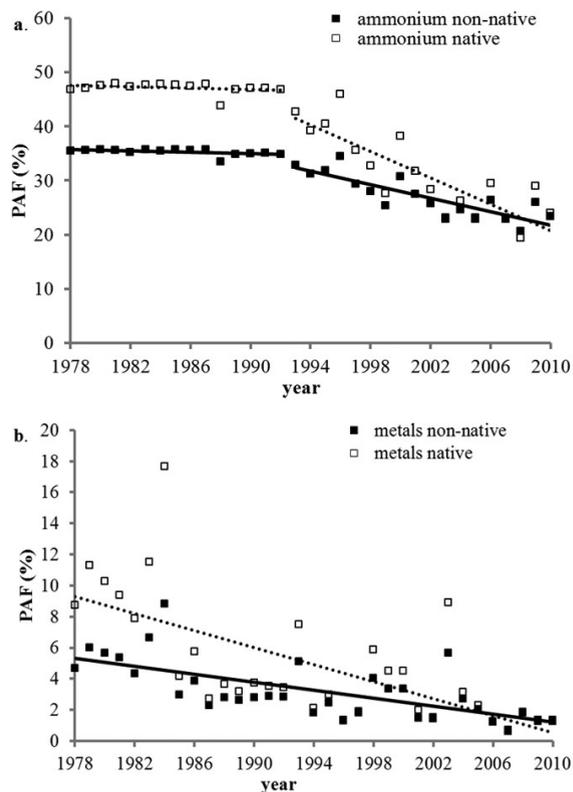


Figure 3. Potentially Affected Fraction (PAF) for ammonium (a) and total metals (b) for native and non-native fish species calculated using the maximum annual concentrations in the River Rhine at Lobith. Statistical specifications of linear regressions $PAF = a + b \cdot year$: Ammonium only since 1993 native $b = -1.21$, $a = 2459$, $R^2 = 0.70$, $p < 0.001$; non-native $b = -0.63$, $a = 1281$, $R^2 = 0.70$, $p < 0.001$. Metals native $b = -0.27$, $a = 550$, $R^2 = 0.45$, $p < 0.001$; non-native $b = -0.13$, $a = 259$, $R^2 = 0.43$, $p = 0.001$.

ethically controversial. Additional toxicity data for native and non-native fish species could be generated for chemical stressors of concern by using e.g., quantitative structure-activity relationships between chemicals (Devillers and Devillers 2009) or interspecies correlation estimation models (Dyer et al. 2008; Henning-de Jong et al. 2009; Golsteijn et al. 2012).

Tolerance and comparison with other studies

Previous studies on tolerance of native and non-native species have shown different results indicating that tolerance to abiotic stressors may vary from case to case. Studies on invertebrates have demonstrated different tolerances between native and non-native species to various stressors

(Piola and Johnston 2009; Verbrugge et al. 2012; Weir and Salice 2012). Contrasting results in tolerance of fish species have also been obtained, either with no differences in tolerance between native and non-native species to an organic compound (Jin et al. 2011) or a native fish species more tolerant to polycyclic aromatic hydrocarbons than a non-native species (Gevertz et al. 2012). Until now, however, no study has compared the tolerance of a whole fish community consisting of native and non-native fish species to a range of chemical stressors, including several metals, pesticides, and ammonium. The current study was based on the most comprehensive data available and showed no significant difference in tolerance to multiple chemical stressors between native and non-native fish species in the Delta Rhine. This lack of difference in tolerance to chemical stressors between native and non-native fish species may be related to introduction pathways, since many of the fish species in the River Rhine were deliberately introduced (Leuven et al. 2009). Species that survive harsh environmental conditions during dispersal routes (e.g., in ballast water or migration through canals between river basins) to become invasive elsewhere may, however, be more tolerant than native ones (Piscart et al. 2011).

In the last two decades, the chemical pollution in the Delta Rhine has decreased, whereas the number of non-native fish species has increased. The appearance of non-native fish species and disappearance of some native species in the Delta Rhine since 1980 cannot be explained by their tolerance to chemical stressors. However, the lack of toxicity data for many non-native species that have invaded the Delta Rhine only in the last two decades, underpins the uncertainty in terms of tolerance to chemicals for this species group. Possible reasons for the increase in the number of non-native species may be the increase in water temperature, known to affect non-native species less than native species (Leuven et al. 2011). Additionally, the opening of the Main-Danube canal in 1992 connecting the River Rhine with the River Danube may have accelerated the distribution of non-native species into the Delta Rhine (Leuven et al. 2009).

Overall, tolerance to chemical stress was not a trait that could explain the invasiveness of fish species. Other traits such as tolerance to high water temperature, trophic status, maximum adult size and prior invasion success may play a more important role (Marchetti et al. 2004; Leuven

et al. 2011). It should be noted, however, that comparative studies on traits between native and non-native species should account for the phylogenetic differences because closely related species may share a similar suite of traits through common ancestry (Jennings et al. 1999; Alcaraz et al. 2005). However, the amount of toxicity data available did not allow additional subdivision of fish species into phylogenetic groups in the current study.

Tendency in different tolerance between native and non-native fish species

Although differences in tolerance between native and non-native fish species were not statistically significant, a certain tendency was observed indicating possible difference in tolerance to different types of stressors. This might relate to the fact that most non-native species used in toxicity tests were from subtropical or tropical climate zones (Leuven et al. 2011; Table 3). Tropical species have been shown to be less tolerant to ammonium and phenol and more tolerant to metals than species from temperate climate (Brix et al. 2001; Kwok et al. 2007). These different responses of fish species toward stressors might be related to temperature-induced differences in toxicokinetics and toxicodynamics of the stressors among fish species (Smit et al. 2001; Kwok et al. 2007).

Combined effects of chemical and physical stressors

Even though the results of the current study suggest that there is no significant difference in tolerance between native and non-native fish species to certain chemical stressors, they may be important in combination with physical, morphological and ecological features of the habitats for the adaptation of non-native fish species to a new environment (Den Hartog et al. 1992). For example, the physical degradation of the River Rhine and the rise in water temperature has enabled a large number of new stagnant water fish and thermophilous species to establish themselves in this river (Den Hartog et al. 1992). Water temperature may have less impact on non-native fish species than on natives because non-native fish species of the Delta Rhine were found earlier to be more tolerant to high water temperatures than the native species (Leuven et al. 2011). Combined high water temperature and chemical contamination may have a different

effect on native and non-native fish species because an increase in temperature may enhance the toxicity of several groups of chemical compounds (Noyes et al. 2009). All these environmental factors may be interrelated, and tolerance of native and non-native species should be considered for multiple types of stressors together.

Effects of chemical stressors

The ecological condition of the River Rhine has improved since the 1970s due to river rehabilitation programs managed by the International Commission for the Protection of the Rhine and Dutch water authorities (Den Hartog et al. 1992; Nienhuis et al. 2002). With decreasing concentrations of chemical stressors in the water column, the PAF of fish species has been decreasing too. Current concentrations of metals in the Delta Rhine are lower in comparison to those in the period 1978–1985, yet, this yielded similar affected fractions for native and non-native species. The higher values of PAF for native species at higher metal concentrations indicate that native species might be less tolerant to high concentration of metals than non-native species. Figure 2 may underline this assumption indicating that at low metal concentrations the PAFs for both species groups are close but divergent at higher concentrations.

Although the concentration of ammonium in the River Rhine at Lobith has decreased from 1.4 mg/L to 0.2 mg/L over the period 1970–2010, it is currently still an important stressor causing the highest PAF of both native and non-native fish species. Considering the fact that un-ionized ammonia is more toxic than ammonium, the impact of other nitrogenous compounds may be severe (Camargo and Alonso 2006). High concentrations of ammonium and other nitrogenous compound in the river water are mainly caused by diffuse agricultural sources that are difficult to regulate (Erisman et al. 2002). The relative importance of other stressors studied varied between native and non-native fish species. In the native species group azinphos-methyl and copper contributed significantly to the overall PAF, whereas in the non-native species group copper and zinc were more important.

In the current study the location-specific SSD approach was applied to quantify and compare tolerance of native and non-native fish species to chemical stressors occurring in the River Rhine. Deriving the PAF for each species group was used to rank stressors and can be a useful tool for identifying stressors with the relatively highest

impact on the species of concern. This information can also be applied to water pollution control. The retrospective analysis of the trends of the PAF showed that at higher water concentrations of some chemical stressors there might be different responses between native and non-native species (Figure 2).

No significant difference was observed in the tolerance between native and non-native fish species to a range of chemical stressors. Recently, Elshout et al. (2013) described that there were no significant differences between sensitivity of native and non-native species to low oxygen content of water; however, their data on non-native species was limited. For the management of freshwater ecosystems potentially affected by introduced species, attention should therefore be given to other environmental variables such as temperature, hydrological regimes, habitat quality (Moyle and Light 1996; Holway et al. 2002; Leuven et al. 2011; Früh et al. 2012; Verbrugge et al. 2012) and to combined chemical and physical stressors.

Where sufficient data are available, additional analyses of difference in tolerance to chemical stressors between different life stages as well as between non-native fish that spawn and that do not spawn in the target location are recommended.

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