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Research Article

Spatio-temporal co-occurrence of alien and native molluscs: a modelling approach using physical-chemical predictors

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Abstract

The invasion of alien species can have serious economic and ecological impacts. Ecologically, invasions often lead to an increased rate of native species replacement and decreased biodiversity. A critical step in the dominance of alien species is their successful co-occurrence with native species. In this study, we assessed the occurrence of alien molluscs and their co-occurrence with native molluscs and identified the determining physical-chemical variables. We expected that a combination of some key variables of water quality could provide suitable conditions promoting alien molluscs to occur and to co-occur with native molluscs. The analyses were based on 20-year data, collected from river systems across Flanders (Belgium). Classification Trees (CTs) were used to perform the analyses and to develop the predictive models. Based on CT models, the co-occurrence of alien and native molluscs could be reliably predicted based on physical-chemical variables. However, there was insufficient data to determine the environmental conditions in which alien taxa dominate. From the past to the present, spatial co-occurrence significantly increased. Sinuosity, ammonium and nitrate concentrations, chemical oxygen demand, pH and conductivity were the key determining variables. Our findings suggest that the co-occurrence of alien and native molluscs mainly occurs in straight rivers with good chemical water quality. These results provide insights into the ecology and behaviour of alien species which could support management practices and priority setting for conservation planning in surface waters of Flanders and Europe.

Key words: Invasion, habitat suitability, classification trees, species replacement, water quality, Flanders

Introduction

Invasive species have become a major concern for the global economy and environment (Sala et al. 2000). A large proportion of the economy has been spent on the management of agriculture, grassland and various natural ecosystems to mitigate the effects of alien invasive species (Williams et al. 2010; Hulme 2012). Moreover, their spread threatens native species of the same taxonomic groups and surrounding biotic communities via e.g. species replacement, food web reorganization and community composition simplification (Gurevitch and Padilla 2004; Bernauer and Jansen 2006; Didham et al. 2007). Once invasive species have successfully colonized new habitats and co-exist with native species, eradication is rarely possible (Regan et al. 2006). Consequently, the rate of replacement of native species by invasive species increases, which can thus lead to an overall decrease of native species (Olden et al. 2004).

Invasion success depends on traits of the invaders and the suitability of invaded environments (Kolar and Lodge 2001). Some taxa, e.g. *Corbicula* spp., are highly successful invaders due to their rapid spreading ability and their capability to withstand a wide range of environmental conditions (Werner and Rothhaupt 2007; Pigneur et al. 2014). Habitat modifications, resulting in changed physical and chemical conditions, often promote the local abundance and regional distribution of alien species (Didham et al. 2007). Increased trade (shipping) and improved chemical water quality may also promote the number and abundance of alien species (IKSR 2002; Boets et al. 2016). Therefore, identifying the environmental conditions in which alien species solely exist or co-exist with native species and determining those locations that could be invaded in the future will provide essential knowledge to support environmental management and conservation planning.

River systems in Europe have been exposed to the introduction of alien macroinvertebrate species. In the river Rhine, for example, alien species contribute 11.3% of the total macroinvertebrate species richness (Leuven et al. 2009). Among the macroinvertebrate invaders, molluscs constitute a large proportion (Leuven et al. 2009; Nunes et al. 2015). However, for most river systems in Europe, e.g. river systems in Flanders, the environmental conditions in which only alien species occur or the conditions preferred by both alien and native species (co-occurrence) are poorly studied. Recently, an inventory and habitat suitability model of alien macrocrustaceans in Flanders was conducted (Boets et al. 2013; Boets et al. 2016). Moreover, Boets et al. (2016) reported that alien mollusc species, e.g. the New Zealand mud snail (*Potamopyrgus antipodarum* J.E.Gray, 1843) and the acute bladder snail (*Physella acuta* (Draparnaud, 1805)), are highly abundant in the river systems of Flanders. As such, there is an urgent need to gain insight into the environmental conditions preferred by alien molluscs and to determine the conditions that allow alien molluscs to co-occur with native molluscs, as a basis for invasion control (e.g. locations and type of actions which deserve priority).

The aim of our study is to 1) provide an analysis of the spatio-temporal occurrence of alien molluscs and their co-occurrence with native molluscs in the river systems of Flanders over the past two decades (1991–2010), and 2) identify key determining physical-chemical variables associated with the sole occurrence of alien molluscs and their co-occurrence with native molluscs. We expected that a combination of some key variables of water quality could provide suitable conditions promoting alien molluscs to occur and to co-occur with native molluscs.

Material and methods

Data collection and treatment

The Flemish Environment Agency (VMM) has collected biological and environmental data in Flanders since 1989. The samples have been collected at more than 2500 sites spread over different water bodies. In this monitoring program, a standard handnet was used to collect macroinvertebrates following the method described by Gabriels et al. (2010). At sampling sites where the kick sampling method was not possible, artificial substrates were used. Seven alien and 27 native mollusc genera were identified. Electrical conductivity (EC), pH and dissolved oxygen (DO) were measured in the field with a hand-held probe (Cond 315i, oxi 330, wtw, Germany and 826 pH mobile, Metrohm, Switzerland). All additional chemical variables, i.e. ammonium (NH_4^+), chemical oxygen demand (COD), biological oxygen demand (BOD), total phosphorus (Pt), nitrate (NO_3^-), nitrite (NO_2^-), Kjeldahl nitrogen (KjN), and orthophosphate (oPO4), were retrieved from the monitoring dataset compiled by the VMM and which is accessible online (www.vmm.be). Nutrient analysis was performed spectrophotometrically in accordance with ISO 17025. GIS software (version 9.3.1) applied to the Flemish Hydrographic Atlas was used to determine the slope and the sinuosity of a watercourse using the difference in height in between two points 1000 m apart, and on a stretch of 100 m, respectively. For further detailed information on the determination of physical-chemical variables, we refer to Boets et al. (2016).

Data from 1991 to 2010 were used for the analyses. The data were divided into 4 periods, each encompassing 5 years of sampling effort (i.e. 1991 to 1995, 1996 to 2000, 2001 to 2005 and 2006 to 2010). This division provided more samples with which to model the preferred environmental conditions, and can provide useful information on changes in the occurrence of alien molluscs and their co-occurrence with native molluscs for each period. Due to limited frequency of occurrence for most alien mollusc genera (Table 1, 2), we decided to merge all alien genera to form one categorical variable. This provided us with a higher number of instances for our predictive models and thus a better and more robust development of the model. Moreover, we were not aiming to make predictions for individual taxa but rather to reveal common environmental conditions that most of the alien molluscs prefer. In the same way, all native genera were also merged to form one categorical variable. Environmental preferences of each genus of alien and

Table 1. The occurrence instances of each alien mollusc genus and of all genera that are merged together, compared to all collected samples (7695 samples) within the studied period (1991–2010). The sum of occurrences of different alien genera is 2522 instances, and the overlapping occurrences of alien genera are 494 instances.

Genus	*Corbicula*	*Dreissena*	*Ferrisia*	*Lithoglyphus*	*Menetus*	*Physella*	*Viviparus*	Merging all genera
Instances	130	745	381	30	4	1138	94	2028
Instances (%)	1.4	8.8	4.5	0.4	<0.1	13.9	1.1	26.4

Table 2. List of alien and native molluscs, their occurrences and abundance recorded for each period. The total number of samples for each period is indicated in brackets.

	Occurrence				Abundance			
Taxa	1991-1995 (935)	1996-2000 (2021)	2001-2005 (2889)	2006-2010 (1850)	1991-1995 (935)	1996-2000 (2021)	2001-2005 (2889)	2006-2010 (1850)
Alien								
Corbicula	0	13	47	51	0	58	304	1092
Dreissena	59	132	257	227	683	1357	4980	29414
Ferrisia	10	17	199	120	125	40	1163	1133
Lithoglyphus	8	7	9	4	61	33	53	9
Menetus	0	0	0	4	0	0	0	11
Physella	2	1	236	831	13	2	15410	39361
Viviparus	15	27	32	12	142	162	148	126
Native								
Acroloxus	46	99	281	234	153	255	1760	1552
Ancylus	43	121	215	142	116	698	1097	1239
Anisus	152	318	472	235	633	2009	5493	2190
Anodonta	8	10	26	18	22	16	140	93
Aplexa	5	7	21	3	7	28	197	4
Armiger	25	63	260	148	63	150	1613	884
Bathyomphalus	84	189	237	159	447	915	1550	1864
Bithynia	225	498	808	514	2650	6639	27744	24220
Bythinella	3	4	0	0	33	16	0	0
Gyraulus	246	489	675	486	1598	2873	6471	11090
Hippeutis	27	50	206	131	121	131	1225	576
Lymnaea	512	1252	1677	918	6075	10458	23785	15381
Margaritifera	1	0	0	0	2	0	0	0
Marstoniopsis	1	0	0	0	1	0	0	0
Myxas	2	0	0	0	4	0	0	0
Physa	543	1103	1214	220	9358	13265	13918	4358
Pisidium	342	845	1272	799	3429	11237	36421	47265
Planorbarius	73	183	205	141	405	863	1297	948
Planorbis	113	230	280	195	786	1616	1841	2484
Potamopyrgus	165	306	529	365	3861	5424	28602	112402
Pseudamnicola	2	7	2	363	12	73	3	4088
Pseudanodonta	2	1	1	56	2	2	1	203
Segmentina	21	36	89	57	55	130	796	423
Sphaerium	224	451	873	526	2974	3386	7766	10878
Theodoxus	13	14	4	2	128	61	16	33
Unio	2	3	7	5	4	4	39	32
Valvata	242	445	734	458	3982	8193	25805	42883

native molluscs are provided in the Supplementary material Appendix 1. Each sampling site for each period was then categorized as: a "native" site (i.e. a site having only native molluscs present), an "alien" site (i.e. a site having only alien molluscs present) and a "co-occurrence" site (i.e. a site having both alien and native molluscs present), and this status was used as the response variable in analyses. Physical and chemical water quality variables that had missing values for more than 5% of the total samples were removed from the analyses. Each period thus consisted of one response categorical variable (native/alien/co-occurrence) and 13 predictor variables. To visualize the occurrence of alien molluscs and their co-occurrence with native molluscs, we produced an occurrence map using GIS-software (ArcGIS version 9.3.1). The summary of the physical and chemical water quality variables and of the response variable is shown in Table 3.

Table 3. Mean (and standard deviation) for environmental predictors and occurrence instances of each class of the response variable. NH_4^+: Ammonium, COD: Chemical Oxygen Demand, Pt: Total Phosphorus, EC: Electrical Conductivity, NO_3^-: Nitrate, NO_2^-: Nitrite, oPO_4: Orthophosphate, DO: Dissolved Oxygen. The number of sampled sites for each period is shown in square brackets.

Variable	Unit	Period			
		1991-1995 [509]	1996-2000 [991]	2001-2005 [1524]	2006-2010 [1250]
NH_4^+	mg/L	2.6 (4.8)	1.9 (3.4)	1.6 (2.8)	2.3 (5.1)
COD	mg/L	55 (43)	39 (42)	34 (34)	36 (41)
Pt	mg/L	1.0 (1.4)	0.9 (1.4)	0.9 (2.3)	0.8 (1.0)
EC	µS/cm	1320 (2618)	987 (1149)	998 (1509)	921 (949)
NO_3^-	mg/L	3.3 (4.1)	4.0 (4.7)	3.6 (3.7)	3.0 (3)
NO_2^-	mg/L	0.2 (0.3)	0.2 (0.3)	0.2 (0.2)	0.2 (0.2)
oPO_4	mg/L	0.6 (0.9)	0.5 (1.1)	0.4 (0.7)	0.5 (0.8)
pH		7.5 (0.6)	7.6 (0.5)	7.7 (0.4)	7.6 (0.4)
DO	mg/L	7.5 (3.5)	6.8 (3)	6.9 (3.1)	6.6 (2.9)
Sinuosity		1.1 (0.1)	1.1 (0.1)	1.1 (0.1)	1.1 (0.1)
Slope	m/1000m	1.0 (1.4)	1.6 (2.8)	2.0 (3.6)	1.7 (2.8)
Response	class				
Co-occurrence		*66*	*146*	*567*	*875*
Alien		*6*	*15*	*63*	*133*
Native		*863*	*1860*	*2259*	*842*
Total instances		935	2021	2889	1850

Modelling

A Classification Tree (CT) model was used to predict the occurrence of native and alien molluscs and their co-occurrence, and to identify the determining physical-chemical variables. The CT was chosen among other machine learning approaches according to its performance for both predictive power and the importance of input variables (Chen et al. 2015). Moreover, this decision tree model is relatively simple to implement, easy to interpret, and it tolerates missing values during both the training and testing phases (Therneau and Atkinson 1997; De'ath and Fabricius 2000).

A CT model is based on growing and pruning to select an optimal tree. In the growing phase, the decision trees were fitted using a recursive partitioning algorithm. In each growing phase, the root of a tree (the initial node) is built from the most informative input variables. From the root, the data is split into left and right branches based on the splitting rules defined by the values of selected input variables. The growing process continues up to the terminal node until all the data in that node are of the same class or until some other stopping criterion is reached. The terminal nodes are called leaves and are labelled with the corresponding class (Quinlan 1986). In the pruning phase, the tree was pruned by setting a complex parameter at cp=0.05. Where the tree had only a root, we decreased the cp to a level (e.g. cp = 0.04, 0.03, 0.02, 0.01) that at least two terminal nodes were produced. In practice, the first few splits mostly provide a very informative division of the data (Therneau and Atkinson 1997). These criteria were set in order to make the trees easily interpretable and comparable in terms of the number of variables and complexity.

For each period, a three-fold cross-validation was used to train and validate the models. To build reliable models and to avoid misidentifying the key variables determining each class of the response variable, we made 3 replications of the three-fold cross validation. For each 3-fold cross-validation, the data was shuffled and randomly split into three subsets; two subsets were used for training and one subset for validation. For the second and third replication, we re-shuffled the data and randomly split it into new training and validation sets following the same procedures. From each training and validation set, a model was built and in this way, a performance value and the importance of each variable (as a percentage) of nine different models (3 models of each three-fold cross validation × 3 replications) were calculated. A mean performance value, obtained from the nine models, was used as a final criterion for model evaluation. Cohen's Kappa Statistic (K) and Correctly Classified Instances (CCI) were used to evaluate the model performance. The higher the value of K (ranging from 0 to 1) and of CCI (ranging from 0 to 100), the better the model predicts the response variable. The importance of each variable determining the preferred environmental conditions of each class (native/alien/co-occurrence) was averaged across the nine models. To identify which

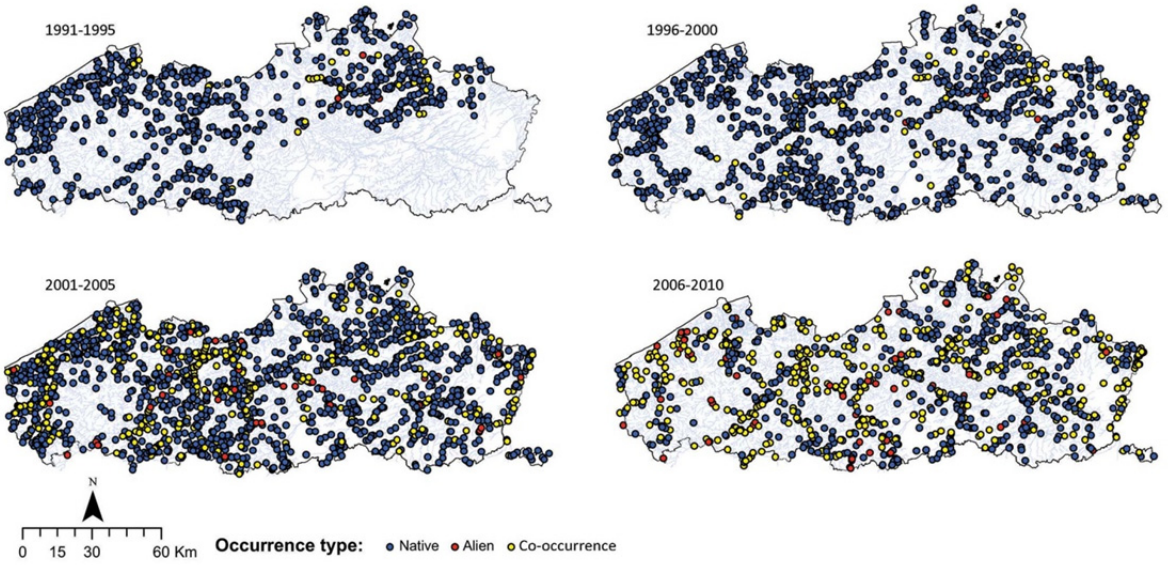

Figure 1. Sampling locations indicating the occurrence of native and alien molluscs and their co-occurrence for each period.

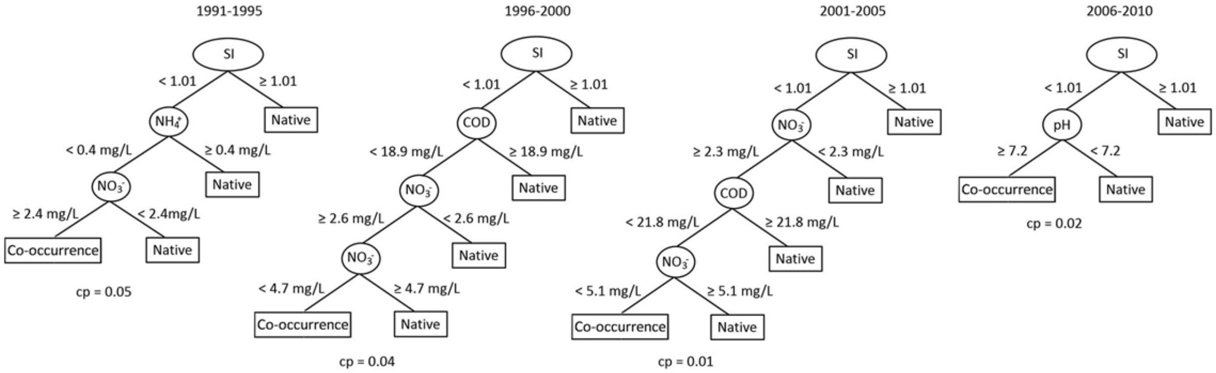

Figure 2. Classification trees predicting the "co-occurrence" and the "native" sites for each period. SI: Sinuosity, NH_4^+: Ammonium, NO_3^-: Nitrate, COD: Chemical Oxygen Demand, cp: pruning complex parameter.

variables significantly determine the preferred conditions of each class, the importance of each variable was compared based on the standard error. The same procedures and criteria were applied for modelling the data for each period across the whole studied period.

To develop and validate the models, and to construct the classification trees (for visualization), the package "rpart" in R (Breiman et al. 1984) was used. As we had many models which produced many trees for each period, we chose to construct the tree based on all data points of each period to be the representative one. All statistical analyses and calculations (K and CCI) were performed in R (R Core Team 2013).

Results

Occurrence of alien molluscs and their co-occurrence with natives

Overall, the occurrence of alien molluscs spatially and temporally increased in fluvial systems in Flanders (Figure 1). The "alien" sites accounted for 0.6%, 0.7%, 2.3% and 7.2% of the total samples for the period 1991–1995, 1996–2000, 2001–2005 and 2006–2010, respectively. The alien taxa which showed a notable increase in occurrences include *Corbicula, Dreissena, Ferrisia* and *Physella.* In the last period, a new alien genus (*Menetus*) was also recorded.

Detailed information on the occurrences and abundance of each alien mollusc genus is provided in Table 2. There was also a spectacular increase in the "co-occurrence" sites; they respectively accounted for 7.1% (66/935 samples), 7.2% (146/2020 samples), 21.1% (567/2689 samples) and 47.3% (875/1850 samples) for the corresponding periods (Table 3).

Key determining variables

For each period, the CT models were only able to reliably predict the "co-occurrence" and the "native" sites. The representative trees of the CT models for each period are shown in Figure 2. Sinuosity was always one of the most important variables for the models of each period and this, together with chemical water quality variables (e.g. NH_4^+, NO_3^-, COD, pH, Figure 2) and EC, were the key factors determining the predictive models (Figure 2, 3). When sinuosity was lower than 1.01, co-occurrence between alien and native molluscs was evident where NH_4^+ <0.4 mg/L and NO_3^- ≥2.4 mg/L for the period 1991–1995; COD <18.9 mg/L and NO_3^- in between 2.6–4.7 mg/L for 1996–2000; COD <21.8 mg/L and NO_3^- in between 2.3–5.1 mg/L for 2001–2005; and where pH ≥7.2 for 2006–2010 (Figure 2). Sites where only alien molluscs occurred could not be reliably predicted based on the available physical-chemical data (Figure 4).

Model performance

The mean Cohen's Kappa Statistic (K) and the mean overall Correctly Classified Instances (Overall-CCI) of the models decreased from the past to the most recent period (Figure 5A–B). For the four periods (1991–1995, 1996–2000, 2001–2005 and 2006–2010), the corresponding mean K was 0.34, 0.32, 0.22 and 0.16, and the corresponding mean Overall-CCI was 93%, 92%, 79% and 54%. The mean CCI of models predicting the "co-occurrence" sites (CCI-co-occurrence) was lower for the first 3 periods (28%, 25% and 23%), while it was higher for the most recent period (52%, Figure 5C). On the contrary, the mean CCI of models predicting the "native" sites (CCI-Native) for the past 3 periods (99%, 98%, and 96%) was substantially higher compared to that of the most recent period (66%, Figure 5D). The models predicting the "alien" sites for the 4 periods did not yield any reliable prediction. Only one model that was based on the data from the latest period correctly predicted one instance of "alien" sites. The confusion matrices obtained from the models and which were used to calculate the model performance measures (K and CCI) are provided in Appendix 2.

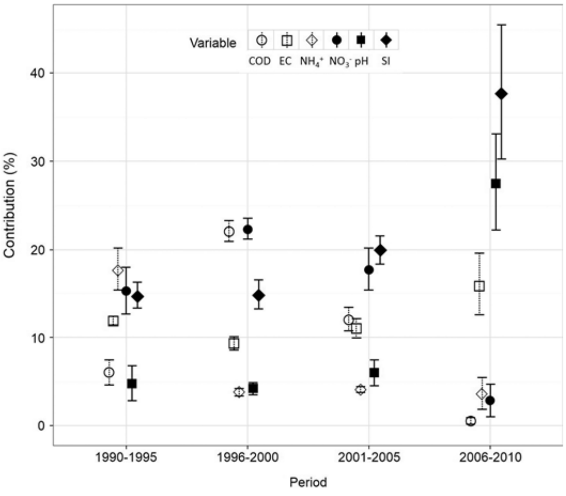

Figure 3. Mean and standard error bar showing the importance of the variables contributing most to the predictive models for each period. COD: Chemical Oxygen Demand, EC: Electrical Conductivity, NH_4^+: Ammonium, NO_3^-: Nitrate, SI: Sinuosity. Variables that contributed less to the predictive models are not shown.

Discussion

Occurrence of alien molluscs and their co-occurrence with the natives

Alien molluscs have spread spectacularly in several European river systems during the last few decades, e.g. the rivers Rhine and Meuse (Bernauer and Jansen 2006; Collas et al. 2014; Pigneur et al. 2014). This phenomenon is similarly observed in our study in the rivers in Flanders. The remarkable increase in the occurrence of alien molluscs over the past two decades may reveal their outbreak or invasion success across the fluvial systems in Flanders, which resulted in an increased incidence of co-occurrence of alien and native molluscs.

Key determining variables

CT models indicated that co-occurrence is mainly determined by sinuosity and by a set of chemical water quality variables (i.e. NH_4^+, NO_3^-, COD, pH and EC). Sinuosity was always one of the most important factors determining co-occurrence as it formed the main root in all models. Sites having a low sinuosity (<1.01), which corresponds to mainly straight rivers, may be subjected to a high number of passing ships, which is considered one of the main pathways of invasions (Bij de Vaate et al. 2002; Nunes et al. 2015). Straight rivers shorten travelling distances, resulting in more frequent transportation,

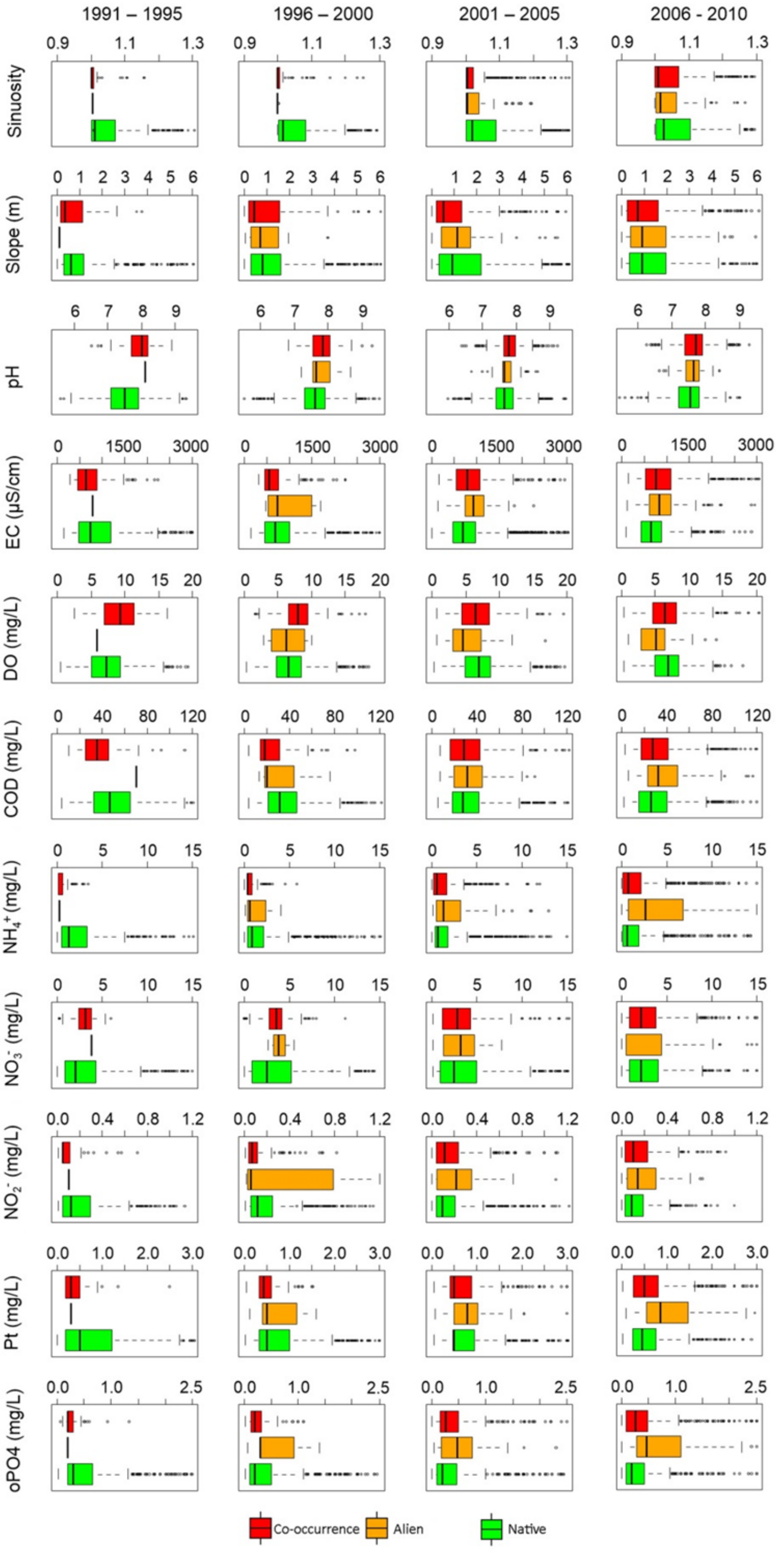

Figure 4. Box and whisker plots of physical-chemical variables in which each occurrence type occurred. Rectangles show first and third quartiles, dark bars are the medians, the lower and upper bars are the minimum and maximum values, and the circles are outliers. EC: Electrical Conductivity, DO: Dissolved Oxygen, COD: Chemical Oxygen Demand, NH_4^+: Ammonium, NO_3^-: Nitrate, NO_2^-: Nitrite, Pt: Total Phosphorus, oPO4: Orthophosphate.

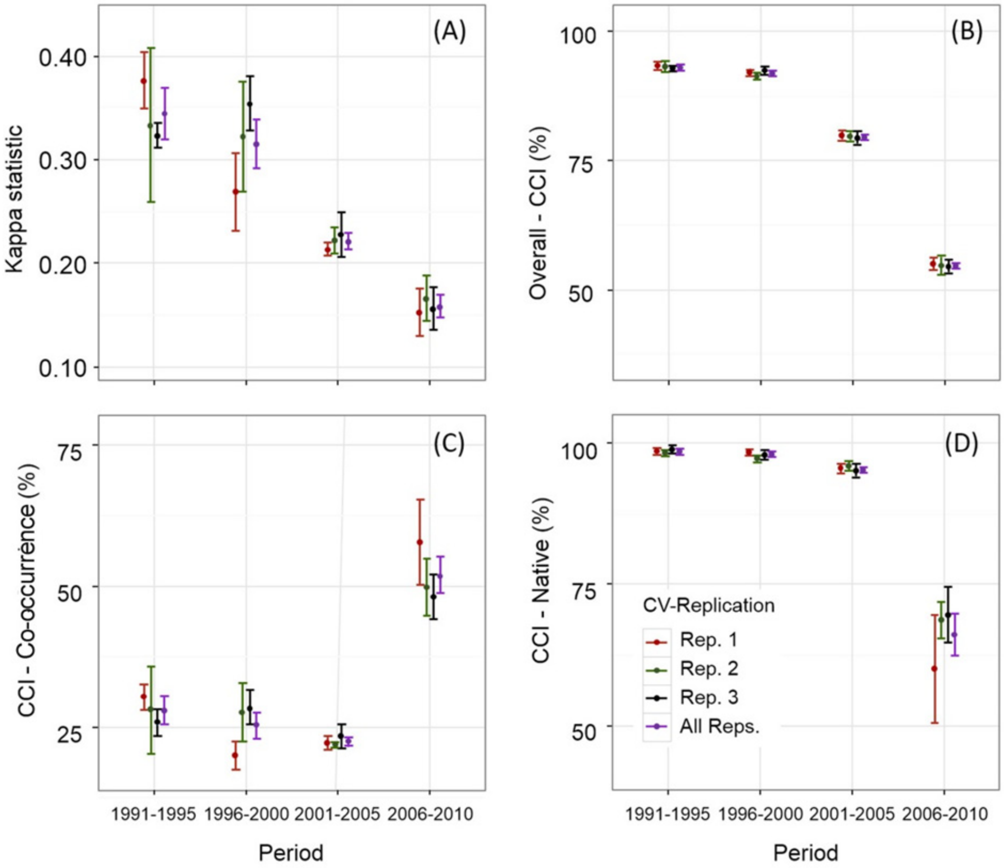

Figure 5. Mean and standard error bar indicating the overall model performance based on Cohen's Kappa statistic (A) and Correctly Classified Instances (CCI: B), and the predictive power of the predicted class "co-occurrence" (C) and the class "native" (D). CV: cross-validation, Reps: Replications.

thus allowing a large and frequent amount of ballast water to be released. Consequently, with a higher number of introductions, the survival rate of alien molluscs increases (Gollasch 2006). We found that the hotspots of mollusc invasion (the alien and the co-occurrence sites) were mainly situated in brackish polder watercourses and in large rivers that have a shorter distance to the ports in the Rhine delta, to the coast of the North Sea and to other large rivers (e.g. Meuse River). This observation is supported by evidence in Boets et al. (2016) and in Grabowski et al. (2009) who found that alien fauna mostly inhabited large rivers where intensive navigation takes place.

Besides river morphology, water quality is one of the major factors influencing the distribution and diversity of freshwater fauna (Leuven et al. 2009; Wang et al. 2012). Key variables used to evaluate water quality are NH_4^+, NO_3^+, COD, pH, DO, Chloride, and total phosphorus (US-EPA 1986; SEQ-Eau 2003; WWF 2007; Chea et al. 2016). Good water quality supports a high diversity of invertebrates (Leuven et al. 2009). In our study, where sampling sites had a low sinuosity (<1.01), co-occurrence was mainly dependent on chemical water quality status.

River systems in Europe as well as in Flanders have suffered from severe water quality degradation in previous decades. During these periods, some native species were reported to disappear (Bernauer and Jansen 2006) and only species that were able to withstand this water quality degradation remained. In the early 1990s, the invasion of alien molluscs seemed to be at an initial stage as reflected by the presence of only five alien taxa with limited spatial occurrence and low abundance (Figure 1, Table 2). Although water quality was degraded during these periods, we found that a low NH_4^+ concentration (<0.4 mg/L) and a nitrate concentration higher than 2.4 mg/L were preferred by both the alien and remaining native molluscs. This could be because higher NH_4^+ concentrations negatively affect their living conditions (Friberg et al. 2010). Moreover, an enrichment of nutrient content (e.g. NO_3^- >2.4 mg/L) could be advantageous for alien molluscs at the beginning of the invasion. Nutrient enrichment provides resources that can be used by alien molluscs and thus may enhance their proliferation (Hall et al. 2003; Strayer 2010). However, a very high nutrient concentration (e.g. NO_3^- >25 mg/L) is also an indication of a high level of water pollution (SEQ-Eau 2003), which can negatively affect both alien and native species (Boets et al. 2013). Nonetheless, a low NH_4^+ concentration and an optimum nutrient content (i.e. mean NO_3^- of 3.3 mg/L) facilitated alien and native molluscs co-existence during the early 1990s.

The late 1990s and early 2000s appeared to be an expansion phase for some alien molluscs (e.g. *Ferrisia, Physella, Dreissena* and *Corbicula*) since we found a large increase in their occurrences and abundance (Table 2). This increase may result in a substantial effect on water quality in river systems. A high abundance of filter-feeders (e.g. *Dreissena* and *Corbicula*) and *Physella,* which also feeds on phytoplankton, can lead to increased nutrient concentration. This is because the filter feeders consume phytoplankton, thus limiting the abundance of nutrient utilizing phytoplankton communities, allowing nutrient inputs (e.g. nitrate and phosphate) from surrounding areas to increase in river systems (Lavrentyev et al. 2000; Pigneur et al. 2014). However, this might not have led to a severe impact on water quality because during these periods there was a successful rehabilitation program for improving water quality, restoring riverine ecosystems and improving habitat connectivity in Europe (Leuven et al. 2009). A decreasing trend of nitrate and other water quality variables (e.g. COD) was observed in the river systems in Flanders (UN 2004). Therefore, it can be inferred that improved water quality and the rehabilitation programs not only helped to recover the diversity of native species but also promoted the occurrence and abundance of alien species. Indeed, Leuven et al. (2009) indicated that when hydromorphological conditions remain unchanged, improvement in water quality promotes alien species.

In the late 2000s, a high percentage of surface water bodies (43%) in Europe were considered to be at a "good status". The number of waterbodies increased to 53% in 2015, and Flanders was one of the regions that well implemented the policy of the Water Framework Directive (EU 2015). The improvement in water quality was associated with a great increase in the spatial occurrence and abundance of alien molluscs. The two filter feeders (i.e. *Corbicula* and *Dreissena*) always expanded their range and frequency in the late 2000s even though the number of sampling sites and total sample size were lower than the early 2000s. Moreover, a new alien genus, *Menetus,* also emerged in the late 2000s. This suggests that the late 2000s can be considered as the expansion phase of the existing alien molluscs and the beginning of the expansion phase of the recently introduced alien genus, *Menetus*. These results are therefore unlikely to be an effect of the sampling strategy, but rather reflect the suitable physical-chemical conditions for alien molluscs to spread and proliferate. However, we found that the pH value, which was the second most important variable in determining the co-occurrence of alien and native molluscs (Figure 2, 3) during this period, was relatively high (pH: 6.5–8.5). This is probably related to certain specific environmental conditions linked to geographic regions. For example, a high pH value could be mainly recorded from brackish polder watercourses and from the main harbour watercourses where a high level of seawater intrusion occurs and a high intensity of human-related activities takes place. The alkaline watercourses may have a higher concentration of calcium and magnesium compared to inland watercourses, thus may be preferred by some alien molluscs (e.g. *Ferrisia* and *Physella)* since they require a large amount of calcium and magnesium to form their shells (Brodersen and Madsen 2003; Gallardo and Aldridge 2013). Moreover, molluscs in the family Physidae, including the alien *Physella* and the native *Physa*, are pollution tolerant and can occur in areas with high pH values (Rodrigues Capítulo et al. 2001; De Troyer et al. 2016). Boets et al. (2013) also found an increase of alien species abundance with increasing pH.

Across the models of the four periods, other than NH_4^+, NO_3^-, COD and pH, EC was also selected as one of the key determining variables. Many studies have shown that high conductivity mostly favours alien species. For example, alien amphipods in the Vistula and Oder rivers of Poland, alien macro-crustaceans in fluvial systems in Flanders and alien gastropods in isolated ponds in Poland all benefited from high conductivity (Grabowski et al. 2009; Boets et al. 2013; Gallardo and Aldridge 2013; Spyra and Strzelec 2014). It is likely that alien mollusc species are well adapted to withstand high values of EC, while only a few native species (e.g. *Lymnaea stagnalis* (Linnaeus, 1758)), which occur in a wide range of environmental conditions (Brown et al. 2011), are able to co-exist in these areas. Our findings suggest that increased spatial co-occurrence results from the introduction or migration of alien species to the connected environments where native species are present or to new environments where conditions fit them best, e.g. sites having a high conductivity. Colonizing new niches where most of the native species are not able to thrive is one of the main strategies found among invaders (Verbrugge et al. 2012). This might further imply that, after success in co-existing with the native species, the aliens may overrun some native species and further spread to new areas. Clear evidence can be seen from the drastically increased occurrences and abundance of most alien molluscs from the past to the present, from the decrease in occurrences and abundance of the native *Physa, Aplexa* and *Theodoxus*, and from the disappearance of the native *Bythinella, Margaritifera, Marstoniopsis* and *Myxas* (Table 2).

Although not directly taken into account in our models, previous studies have demonstrated that dispersal vectors are important in making predictions on future locations that may be invaded by alien species. Indeed, recent research on the dispersal of alien macrocrustaceans in Flanders (Boets et al. 2013) showed that increased shipping and the connection between waterways promote the dispersal of alien species. Moreover, habitat conditions (bank structures and substrates) and hydrological variables (e.g. distance to ports/coast, flow regime and connectivity) can also influence the occurrence of alien species (Josens et al. 2005; Messiaen et al. 2010). However, further research suggested that although habitat and hydrological variables can improve model reliability when predicting the spreading rate of alien species, these variables are often not the limiting factor when making predictions on the scale of Flanders (Boets et al. 2014). Nevertheless, these variables should be taken into account for future research that aims to analyse and predict particular preferred conditions of alien molluscs at a larger scale.

Model performance

Predictive models are widely applied to assess the environments or areas that alien species have invaded or would invade (Pitt et al. 2009; Boets et al. 2013; Chen et al. 2015). In many cases, the performance of these predictive models ranges from fair to moderate (Gabriels et al. 2007; Boets et al. 2013). In our study, the overall performance of the models was moderate to good. When using data from the past period, the performance was higher than when using data from the most recent period (Figure 5A–B). This could be attributed to less complex biotic interactions and to the limited frequency of occurrences of alien species in the past. In this context, alien species may invade those sites where competition is low and where few native species occur. As alien species start to spread, competition with native species increases, and thus some native species which had a small environmental range may disappear (i.e. *Bythinella, Margaritifera, Marstoniopsis, Myxas*) or decrease their occurrence and abundance (e.g. *Aplexa, Theodoxus*). This is epitomized by alien and native molluscs in the family Physidae. In the past, the alien *Physella* occurred at only few sites with a few individuals, while the native *Physa* abundantly and widely occurred. A contrasting relationship between the two taxa was observed for the last period, in part due to a declining trend in the abundance and spatial occurrence of the native *Physa*. This could be a result of competition for food and niche with the alien *Physella*. Biotic interactions are important in predicting species distribution (Araújo and Luoto 2007; Meier et al. 2010), and when included will generally increase the predictive performance of a model. The exclusion of biotic interactions in our study may explain the overall lower performance of the models based on the most recent period. Moreover, during the late 2000s the range of environmental conditions where alien species occur increased (Figure 4) while many native species recovered their range and density (Table 2), due to improved water quality. This higher co-occurrence might also explain the lower performance of the models.

Similarly, higher co-occurrence may have influenced the higher reliability in the prediction of the “co-occurrence” of alien and native molluscs for the recent period compared to the first three periods. Likewise, models predicting the “native” sites yielded a high performance when the prediction was based on the periods (i.e. the first three periods) that have a large sample size of the “native” sites. This is quite logical as for predictive models the more input samples provided the better the models learn, and as a result, a higher predictive performance can be obtained (Stockwell and Peterson 2002; Hernandez et al. 2006). On the other hand, the models were not able to predict the “alien” sites (Appendix 2), due to a low number of instances of this particular class. Although the number of samples of the “alien” sites increased in the most recent period, it was still not sufficient for the models to learn and make a correct prediction. Small sample sizes, together with the opportunistic and generalistic characteristics of the alien species (Nehring 2006), are therefore considered the main reasons for the models to yield a very low performance. Additional observations or a particular optimization approach is thus recommended to better predict the “alien” sites and evaluate the predictive power of the models.

Conclusion

From the past to the most recent situation, there is an increasing trend in the spatial co-occurrence of alien and native molluscs in Flanders. Co-occurrence was predicted to mainly occur in rivers having low sinuosity and good chemical water quality. In addition, our most recent data indicated that alien molluscs have reached a relatively high number of sites where natives were not present, indicating either that alien molluscs have invaded more new sites or replaced native species at sites where they previously occurred. Given that our models were not able to make reliable predictions for environmental conditions preferred by alien molluscs, additional predictors and observations or perhaps a particular optimization approach is needed to predict the habitat conditions where alien molluscs are able to dominate the community. These results provide important information regarding the past and current co-existence of alien and native molluscs in Flanders. Our findings may be used to support management and conservation planning.

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References

Araújo MB, Luoto M (2007) The importance of biotic interactions for modelling species distributions under climate change. *Global Ecology and Biogeography* 16: 743–753, https://doi.org/10.1111/j.1466-8238.2007.00359.x

Bernauer D, Jansen W (2006) Recent invasions of alien macro-invertebrates and loss of native species in the upper Rhine River, Germany. *Aquatic Invasions* 1: 55–71, https://doi.org/10.3391/ai.2006.1.2.2

Bij de Vaate A, Jazdzewski K, Ketelaars H, Gollasch S, Van der Velde G (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. Canadian *Journal of Fisheries and Aquatic Sciences* 59: 1159–1174, https://doi.org/10.1139/f02-098

Boets P, Lock K, Goethals PLM (2013) Modelling habitat preference, abundance and species richness of alien macrocrustaceans in surface waters in Flanders (Belgium) using decision trees. *Ecological Informatics* 17: 73–81, https://doi.org/10.1016/j.ecoinf.2012.06.001

Boets P, Pauwels IS, Lock K, Goethals PLM (2014) Using an integrated modelling approach for risk assessment of the 'killer shrimp' *Dikerogammarus villosus*. *River Research and Applications* 30: 403–412, https://doi.org/10.1002/rra.2658

Boets P, Brosens D, Lock K, Adriaens T, Aelterman B, Mertens J, Goethals PLM (2016) Alien macroinvertebrates in Flanders (Belgium). *Aquatic Invasions* 11: 131–144, https://doi.org/10.3391/ai.2016.11.2.03

Breiman L, Friedman J, Olshen R, Stone C (1984) Classification and regression trees. Wadsworth International Group, Betmont, California, 368 pp

Brodersen J, Madsen H (2003) The effect of calcium concentration on the crushing resistance, weight and size of *Biomphalaria sudanica* (Gastropoda: Planorbidae). *Hydrobiologia* 490: 181–186, https://doi.org/10.1023/A:1023495326473

Brown R, Soldánová M, Barrett J, Kostadinova A (2011) Small-scale to large-scale and back: larval trematodes in *Lymnaea stagnalis* and *Planorbarius corneus* in Central Europe. *Parasitology Research* 108: 137–150, https://doi.org/10.1007/s00436-010-2047-z

Chea R, Grenouillet G, Lek S (2016) Evidence of water quality degradation in Lower Mekong Basin revealed by Self Organizing Map. *PLoS ONE* 11: e0145527, https://doi.org/10.1371/journal.pone.0145527

Chen L, Peng S, Yang B (2015) Predicting alien herb invasion with machine learning models: biogeographical and life-history traits both matter. *Biological Invasions* 17: 2187–2198, https://doi.org/10.1007/s10530-015-0870-y

Collas FPL, Koopman KR, Hendriks AJ, van der Velde G, Verbrugge LNH, Leuven RSEW (2014) Effects of desiccation on native and non-native molluscs in rivers. *Freshwater Biology* 59: 41–55, https://doi.org/10.1111/fwb.12244

De'ath G, Fabricius KE (2000) Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* 81: 3178–3192, https://doi.org/10.1890/0012-9658(2000)081[3178:CARTAP]2.0.CO;2

De Troyer N, Mereta S, Goethals P, Boets P (2016) Water quality assessment of streams and wetlands in a fast growing East African City. *Water* 8: 123, https://doi.org/10.3390/w8040123

Didham RK, Tylianakis JM, Gemmell NJ, Rand T, Ewers RM (2007) Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology and Evolution* 22: 489–96, https://doi.org/10.1016/j.tree.2007.07.001

EU (2015) 4th European Water Conference Conference report. Brussels, 45 pp

Friberg N, Skriver J, Larsen SE, Pedersen ML, Buffagni A (2010) Stream macroinvertebrate occurrence along gradients in organic pollution and eutrophication. *Freshwater Biology* 55: 1405–1419, https://doi.org/10.1111/j.1365-2427.2008.02164.x

Gabriels W, Goethals PLM, Dedecker AP, Lek S, De Pauw N (2007) Analysis of macrobenthic communities in Flanders, Belgium, using a stepwise input variable selection procedure with artificial neural networks. *Aquatic Ecology* 41: 427–441, https://doi.org/10.1007/s10452-007-9081-7

Gabriels W, Lock K, De Pauw N, Goethals PLM (2010) Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnologica* 40: 199–207, https://doi.org/10.1016/j.limno.2009.10.001

Gallardo B, Aldridge DC (2013) Priority setting for invasive species management: risk assessment of Ponto-Caspian invasive species into Great Britain. *Ecological Applications* 23: 352–364, https://doi.org/10.1890/12-1018.1

Gollasch S (2006) Overview on introduced aquatic species in European navigational and adjacent waters. *Helgoland Marine Research* 60: 84–89, https://doi.org/10.1007/s10152-006-0022-y

Grabowski M, Bacela K, Konopacka A, Jazdzewski K (2009) Salinity-related distribution of alien amphipods in rivers provides refugia for native species. *Biological Invasions* 11: 2107–2117, https://doi.org/10.1007/s10530-009-9502-8

Gurevitch J, Padilla DK (2004) Are invasive species a major cause of extinctions? *Trends in Ecology and Evolution* 19: 470–474, https://doi.org/10.1016/j.tree.2004.07.005

Hall RO, Tank JL, Dybdahl MF (2003) Exotic snails dominate nitrogen and carbon cycling in a highly productive system. *Frontiers in Ecology and the Environment* 1: 407–411, https://doi.org/10.1890/1540-9295(2003)001[0407:ESDNAC]2.0.CO;2

Hernandez PA, Graham CH, Master LL, Albert DL (2006) The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography* 29: 773–785, https://doi.org/10.1111/j.0906-7590.2006.04700.x

Hulme PE (2012) Weed risk assessment: a way forward or a waste of time? *Journal of Applied Ecology* 49: 10–19, https://doi.org/10.1111/j.1365-2664.2011.02069.x

IKSR (2002) Das Makrozoobenthos des Rheins 2000. Koblenz, 46 pp

Josens G, De Vaate AB, Usseglio-Polatera P, Cammaerts R, Chérot F, Grisez F, Verboonen P, Bossche JPV (2005) Native and exotic Amphipoda and other Peracarida in the River Meuse: new assemblages emerge from a fast changing fauna. *Hydrobiologia* 542: 203–220, https://doi.org/10.1007/s10750-004-8930-9

Kolar CS, Lodge DM (2001) Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* 16: 199–204, https://doi.org/10.1016/S0169-5347(01)02101-2

Lavrentyev PJ, Gardner WS, Yang LY (2000) Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment-water interface. *Aquatic Microbial Ecology* 21: 187–194, https://doi.org/10.3354/ame021187

Leuven RSEW, van der Velde G, Baijens I, Snijders J, van der Zwart C, Lenders HJR, bij de Vaate A (2009) The river Rhine: a global highway for dispersal of aquatic invasive species. *Biological Invasions* 11: 1989–2008, https://doi.org/10.1007/s10530-009-9491-7

Meier ES, Kienast F, Pearman PB, Svenning JC, Thuiller W, Araújo MB, Guisan A, Zimmermann NE (2010) Biotic and abiotic variables show little redundancy in explaining tree species distributions. *Ecography* 33: 1038–1048, https://doi.org/10.1111/j.1600-0587.2010.06229.x

Messiaen M, Lock K, Gabriels W, Vercauteren T, Wouters K, Boets P, Goethals PLM (2010) Alien macrocrustaceans in freshwater ecosystems in the eastern part of Flanders (Belgium). *Belgian Journal of Zoology* 140: 30–39

Nehring S (2006) Four arguments why so many alien species settle into estuaries, with special reference to the German river Elbe. *Helgoland Marine Research* 60: 127–134, https://doi.org/10.1007/s10152-006-0031-x

Nunes AL, Tricarico E, Panov VE, Cardoso AC, Katsanevakis S (2015) Pathways and gateways of freshwater invasions in Europe. *Aquatic Invasions* 10: 359–370, https://doi.org/10.3391/ai.2015.10.4.01

Olden JD, Poff NL, Douglas MR, Douglas ME, Fausch KD (2004) Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology and Evolution* 19: 18–24, https://doi.org/10.1016/j.tree.2003.09.010

Pigneur L-M, Falisse E, Roland K, Everbecq E, Deliège JF, Smitz, JS, Van Doninck K, Descy JP (2014) Impact of invasive Asian clams, *Corbicula* spp., on a large river ecosystem. *Freshwater Biology* 59: 573–583, https://doi.org/10.1111/fwb.12286

Pitt J, Worner S, Suarez AV (2009) Predicting Argentine ant spread over the heterogeneous landscape using a spatially explicit stochastic model. *Ecological Applications* 19: 1176–1186, https://doi.org/10.1890/08-1777.1

Quinlan JR (1986) Induction of Decision Trees. *Machine Learning* 1: 81–106, https://doi.org/10.1007/BF00116251

R Core Team (2013) R: a language and environment for statistical computing. http://www.r-project.org

Regan TJ, McCarthy MA, Baxter PWJ, Dane Panetta F, Possingham HP (2006) Optimal eradication: when to stop looking for an invasive plant. *Ecology Letters* 9: 759–766, https://doi.org/10.1111/j.1461-0248.2006.00920.x

Rodrigues Capítulo A, Tangorra M, Ocón C (2001) Use of benthic macroinvertebrates to assess the biological status of Pampean streams in Argentina. *Aquatic Ecology* 35: 109–119, https://doi.org/10.1023/A:1011456916792

Sala OE, Chapin III FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Skykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* 287: 1770–1774, https://doi.org/10.1126/science.287.5459.1770

SEQ-Eau (2003) Système d'évaluation de la qualité de l'eau des cours d'eau. Agences de l'eau-Minist ère de l'écologie et du développement durable, 40 pp

Spyra A, Strzelec M (2014) Identifying factors linked to the occurrence of alien gastropods in isolated woodland water bodies. *Naturwissenschaften* 101: 229–239, https://doi.org/10.1007/s00114-014-1153-7

Stockwell DR, Peterson AT (2002) Effects of sample size on accuracy of species distribution models. *Ecological Modelling* 148: 1–13, https://doi.org/10.1016/S0304-3800(01)00388-X

Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55: 152–174, https://doi.org/10.1111/j.1365-2427.2009.02380.x

Therneau TM, Atkinson EJ (1997) An introduction to recursive partitioning using the rpart routines. Technical report no. 61. Rochester, Minnesota, 67 pp

UN (2004) Freshwater Country Profile-Belgium, 29 pp, http://www.google.be/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&ved=0ahUKEwj2haLAmqDOAhVHGsAKHSqrAHUQFggcMAA&url=http://www.un.org/esa/agenda21/natlinfo/countr/belgium/belgiumwater04f.pdf&usg=AFQjCNFyyRBxfJJxe5tFoXKJJjLM-Xiq4w&bvm=bv.128617741,d.ZGg (accessed on 1 August 2016)

US-EPA (1986) Quality criteria for water. Office of Water Regulations and Standards. Washington D.C., 450 pp

Verbrugge LNH, Schipper AM, Huijbregts MAJ, van der Velde G, Leuven RSEW (2012) Sensitivity of native and non-native mollusc species to changing river water temperature and salinity. *Biological Invasions* 14: 1187–1199, https://doi.org/10.1007/s10530-011-0148-y

Wang B, Liu D, Liu S, Zhang Y, Lu D, Wang L (2012) Impacts of urbanization on stream habitats and macroinvertebrate communities in the tributaries of Qiangtang River, China. *Hydrobiologia* 680: 39–51, https://doi.org/10.1007/s10750-011-0899-6

Werner S, Rothhaupt KO (2007) Effects of the invasive bivalve *Corbicula fluminea* on settling juveniles and other benthic taxa. *Journal of the North American Benthological Society* 26: 673–680, https://doi.org/10.1899/07-017R.1

Williams F, Eschen R, Harris A, Djeddour D, Pratt C, Shaw R, Varia S, Lamontagne-Godwin J, Thomas S, Murphy S (2010) The economic cost of invasive non-native species on Great Britain. Wallingford, 199 pp

WWF (2007) National surface water classification criteria & irrigation water quality guidelines for Pakistan. Lahore, Pakistan. WWF-Pakistan, 33 pp

Supplementary material

The following supplementary material is available for this article:

Appendix 1. Mean as well as minimum and maximum values of each physical-chemical variable measured at sites where the presence of each genus of alien and native molluscs was recorded.

Appendix 2. Confusion matrices showing the observed and predicted classes obtained from Classification Tree models of the four periods.

This material is available as part of online article from:
http://www.aquaticinvasions.net/2017/Supplements/AI_2017_Sor_etal_Supplement.pdf