Impact assessment of alien macroinvertebrates in Flanders (Belgium)

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List of abbreviations

BOD	Biological Oxygen Demand
CCI	Correctly Classified Instances
COD	Chemical Oxygen Demand
HSM	Habitat Suitability Model
IAC	Invasive Alien Crustaceans
IAS	Invasive Alien Species
K	Cohens Kappa statistic
MMIF	Multimetric Macroinvertebrate Index Flanders
RBINS	Royal Belgian Institute for Natural Sciences
SBCI	Site-specific Biocontamination Index
WFD	Water Framework Directive

Box 1. *Checklist for definitions: questions that should be answered when defining alien or invasive species (Heger et al., in press).*

Alien species

1. Is human-mediated transport a necessary criterion?
   If yes:
   - How are unrecorded, unintentional introductions distinguished from natural dispersal?
   - Does human mediation include indirect effects such as facilitation of dispersal via construction of waterways?
2. Are species that continuously expand their range regarded as aliens?
   If not:
   - Which criterion is used to distinguish continuous from non-continuous spread (e.g. distance between populations, existence of a barrier to dispersal)?
3. Are species regarded as aliens if they evolved in the region, became extinct and were reintroduced?
4. Are species regarded as aliens even if they have been present in the new region for quite a long time?
   If not:
   - After which time period do we consider them to be indigenous?

Invasive species

5. Can indigenous species also be invasive or are invasive species a subset of alien species?
6. Is impact a necessary criterion?
   If yes:
   - How is impact defined?
7. Is success a necessary criterion?
   If yes:
   - How is success defined? Indicators may be large distribution, high local abundance or fast spread.
8. Is occurrence in natural habitats a necessary criterion?

1.2 Impact of alien species

Alien species are believed to have ecological, economic as well as genetic effects on invaded communities and are considered the second-greatest threat to the survival of threatened or endangered species, only preceded by habitat destruction (Pimentel et al., 2005; Rahel, 2000; Wilcove et al., 1998). However, we should be careful with these generalisations about alien species since many of the claims, driving the perception that introduced species pose an apocalyptic threat to biodiversity, are not supported by data (Davis et al., 2011). Indigenous species are often threatened by multiple stressors and it is not always clear if alien species are the major cause of extinction or if they are coincidental to the disturbance caused (Gurevitch and Padilla, 2004). The positive correlation between indigenous species decline and invasive alien species dominance does not necessarily means that invasive alien species are the drivers of the observed change. Many alien species take opportunistic advantage of other forms of ecosystem change, such as habitat disturbance, rather than being the drivers of change themselves (Gurevitch and Padilla, 2004).

Nevertheless, there is substantial evidence that invasive alien species can cause a serious threat to biodiversity and enhance global environmental change (Vitousek et al., 1996; Clavero and García-Berthou, 2005; Vilá et al., 2010). From an ecological viewpoint, invasive alien species can cause a destabilisation of the ecosystem because there is often a decrease in abundance and diversity of indigenous species observed in invaded aquatic ecosystems (Bernauer and Jansen, 2006; Jazdzewski et al., 2004; van Riel et al., 2006a). The introduction of alien species can have an effect at different trophic levels and directly or indirectly change the population biology of other species (Vander Zanden et al. 1999). A long term effect of these invasions is an increased homogenisation of aquatic ecosystems (Rahel, 2002). Species are naturally isolated from each other via geographic barriers. The disappearance of these natural barriers as a consequence of globalisation puts natural species pools under stress. Homogenisation can change the functional diversity (variety of biological processes, functions or characteristics) of an ecosystem. Most successful invasive alien species are characterised by several traits that enable them to survive the introduction phase, establish a new population and dominate the community. This implies that the introduction of these species can alter functional diversity, which can on the long run lead to the loss of unique and often complex ecosystems that are replaced by single-species dominated ecosystems (McKinney and Lockwood, 1999).

Alien species are often characterised by a reduced genetic diversity as a result of bottlenecks, which has two main consequences: (1) a reduction of the chance that a population can sustain; (2) a limitation of evolutionary capacities (Sakai et al., 2001). However, in the aquatic environment, there is mounting evidence that reduced genetic diversity in alien populations is not as commonplace as expected (Roman and Darling, 2007). Recent studies indicate that high propagule vectors, such as ballast water and shellfish transplantations and multiple introductions contribute to the elimination of founder effects in the majority of successful aquatic invasions (Hänfling et al., 2011). In particular, multiple introductions can promote range expansion of introduced populations through both genetic and demographic mechanisms. Furthermore, high propagule pressure may contribute to maintain high levels of dispersal) only 10% of the species are successful (only one out of 1000 reaches the final stage). The hypothesis is mainly applicable for terrestrial species (birds, plants, insects), but is less suitable for aquatic species. Well documented exceptions to the tens rule are those species which are purposely introduced into their new environment and in this way having a higher chance to become successful. According to Jeschke et al. (2012) major differences exist among taxa and habitats. They show that empirical data from plants more frequently support the tens rule than data from animals, yet levels of support for this hypothesis do not exceed 50% across taxonomic groups and habitats.

1.4 Alien macroinvertebrates: the 'underdog' of invasive species?

Historically, most attention in invasion biology has been attributed to plants, mammals and fish, while only a limited group of researchers have been focussing on macroinvertebrates. Nevertheless, invasive alien macroinvertebrates constitute an important group of species with high economic and ecological impacts (Hänfling et al., 2011). Typical examples of well know invasive macroinvertebrates are molluscs, such as *Dreissena polymorpha*, crayfish, such as *Procambarus clarkii* (Girard, 1852) and amphipods, such as the killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894). Currently, more attention is attributed to some highly invasive key macroinvertebrate species by scientist as well as the general public. This has resulted in an environmental impact assessment not only for large mammals and plants, but also for macroinvertebrate species (Zaiko et al., 2011). In this doctoral thesis, I mainly focused on alien macrocrustaceans since these are widespread and represent, together with molluscs, the most important share of alien macroinvertebrates in many large rivers across Europe (Bernauer and Jansen, 2006; Nehring, 2006; Messiaen et al., 2010; Boets et al., 2011a). The impacts of invasive alien crustaceans (IAC) are often substantial due to the complex trophic role of most of these species leading to cascading effects throughout the invaded ecosystems. IAC also have the potential to cause a shift in the 'keystone' ecosystem functions, changing energy flux and nutrient cycles, which together affect critical ecosystem services, such as biodiversity, fisheries yield and water quality (Hänfling et al., 2011; MacNeil et al., 2011). Invasive alien macrocrustaceans are often very successful in their new habitat. Their intrinsic characteristics, such as a short generation time, rapid growth with early sexual maturity, high fecundity and their euryhaline and omnivorous character make them extremely suitable for rapid expansion and establishment in freshwater ecosystems (Bij de Vaate et al., 2002). Although IAC are widespread (Vilá et al., 2010), there appear to be some geographic hotspots for their invasion. For example, Crustacea have played a major role in the mass invasions recently experienced in the Baltic Sea, California Bay and the Laurentian Great Lakes (Cohen and Carlton, 1998; Olenin and Leppakoski, 1999; Ricciardi and MacIsaac, 2000). Conversely, the Ponto-Caspian region represents one of the most important sources of IAC to Europe and North-America (Ricciardi and Rasmussen, 1998). *Dikerogammarus villosus* is such a highly invasive crustacean originating from the Ponto-Caspian area. The species invaded the east of Flanders in 1997 and has since then spread throughout Flanders mainly via large waterways and canals.

1.5 Introduction prevention and control of invasive alien species

Ecologists as well as economists agree on the need for preventive steps to reduce the further introduction and spread of invasive alien species, given that control and eradication of already established populations is more difficult and costly (Pimentel et al., 2005; Hulme et al., 2009). Prevention of alien species introductions would be more effective if the set of environmental conditions that allow a net positive population increase could be estimated, since the geographic projection of those conditions provides a preliminary, but reliable, indication of sites suitable for invasion (Peterson, 2003). In this respect, modelling techniques are a powerful tool to develop risk assessment maps of invasive alien species. Recently, habitat suitability models (HSMs) have been used in risk assessment to predict the future distribution of invasive alien species (Ficetola et al., 2007; Ba et al., 2010; Boets et al., 2010a; Jiménez-Valverde et al., 2011 Gallardo et al., 2012).

For management purposes, invasive alien species with a high level impact should be listed on an 'alert list' in a future early warning system in order to prevent further spread (Zaiko et al., 2011). Prevention could for example be obtained by better regulations regarding the trade and deliberate introduction of these invasive alien species. Currently, there are preliminary guidelines for environmental impact assessment and classification of alien species in some European countries (Verbrugge et al., 2012) and in Belgium (Branquart, 2011). In Belgium, this classification is based on a simplified environmental impact assessment protocol and the geographic distribution of alien species. Such a categorisation provides a scientific background to prioritise actions to prevent introduction and mitigate the impact of invasive alien species, including the improvement of the legislative framework. One example of an effective law to control the introductions of alien species is the International Convention for the Control and Management of Ships' Ballast Water and Sediments (http://www.imo.org). However, what remains an essential prerequisite of any attempt to prevent the introduction of invasive alien species and to mitigate their impact, is the thorough understanding of their ecology.

1.6 Problem formulation and knowledge gaps

Although extensive research has been carried out on invasive alien macroinvertebrate species, there seems to be a lot of inconsistency on conclusions regarding the key factors determining the impact and spread of alien macroinvertebrates. Some researchers attribute the success of invasive alien species to specific traits enabling them to compete with indigenous species (Grabowski et al., 2007; Statzner et al., 2008). Others are convinced that the ruling environmental conditions are determining whether a species can establish or not and thus win the competition from other species (Vermonden et al., 2010, Früh et al., 2012). This inconsistency could attribute to uncertainties on the environmental conditions under which species are thriving well and can have a substantial impact on the macroinvertebrate community. Large or small scale changes of environmental and habitat conditions can have an effect on the establishment success of alien species (Leuven et al., 2009). Formerly degraded ecosystems are often seen as vulnerable to invasions (Den Hartog et al., 1992). In this respect, it is thought that invasive alien species are taking advantage of these changes rather than changing the ecosystem themselves (MacDougall et al., 2005). The improving water quality in Flanders could not only be beneficial for indigenous species, but also alien species could directly benefit from this improvement. Since the environment is under constant change due to human impacts and natural phenomena, it is important to include these changes when making predictions about the future impact and dispersal of these aquatic alien species.

Another problem related to invasive alien species is their direct and indirect impact on water quality monitoring. The Water Framework Directive (WFD) (European Parliament & Council, 2000) aims to improve water quality throughout Europe and in many countries, WFD ecological status assessment of freshwaters rely on generating biotic indices derived from benthic macroinvertebrate assemblages in rivers and lakes. Unfortunately, invasive alien species can have a major impact on resident macroinvertebrate assemblage structure and diversity, thus having a confounding effect on interpreting assemblage structure as a bioassessment tool for WFD purposes (Arbačiauskas et al., 2008; Cardoso and Free, 2008; Arndt et al., 2009). Biotic indices, such as the Multimetric Macroinvertebrate Index Flanders (MMIF) and similar genus- and family-level derived indices, do not distinguish between natives and invaders, when invaders can be more tolerant to organic pollution or more predatory than the indigenous species they replace (MacNeil et al., 2000; MacNeil and Briffa, 2009; MacNeil et al., 2010b). Directly, alien species could influence the outcome of the MMIF via their higher tolerance score. Indirectly, they can alter the macroinvertebrate community leading to a lower biotic index. To this end, we investigated how alien macroinvertebrate species influence macroinvertebrate communities and the outcome of biotic indices.

Most studies investigate a single species or a specific ecosystem. In this dissertation, we aimed for an integrated (modelling) approach. A combination of field data analysis, laboratory studies and data-driven modelling techniques were used to get insight in (1) the ecology of alien macroinvertebrates, (2) the key factors determining their success and (3) their current and future impact and spread. A problem with aquatic alien species is that once they are established, it is very difficult (or even impossible) and especially costly to eradicate them. Performing environmental impact assessment of some key species and monitoring those sites which can be seen as hot spot for biodiversity, are considered efficient to prevent the further introduction and spread of alien species. We are convinced that the final models constructed in this dissertation could be used by policy makers in order to set up an effective management that reduces the impact and spread of invasive alien macroinvertebrates not only in Flanders, but also in other parts of the world.

1.7 'Field guide' to the different chapters of this dissertation

In this chapter, we gave a general introduction about the definition of alien and invasive species, their impacts and the importance of alien macroinvertebrates as a group of species with possible high economic and ecological impacts. Furthermore, the problems related to invasive alien species and the knowledge gaps are discussed.

Chapter 2 gives an overview of the different alien macroinvertebrates encountered in Flanders anno 2012. Mainly fresh and brackish waters were investigated. Three new species for Flanders were discovered and reported during this study. An update was made on the geographic distribution of the different macrocrustaceans encountered in Flanders. This chapter shows that alien macroinvertebrates make up a substantial part of our aquatic ecosystems and especially artificial watercourses (e.g. canals).

In chapter 3, the biocontamination by alien macroinvertebrates was assessed in order to disentangle the general key elements determining their establishment and spread. It was investigated to which extent the establishment and spread of alien macroinvertebrates was determined by habitat, water quality, biotic interactions and shipping. To this end, over 1800 macroinvertebrate samples collected by the Flemish Environment Agency during 1999, 2004 and 2009 were analysed. Based on the modelled results of the improvements in water quality, predictions were made on the future prevalence of alien macroinvertebrates in surface waters in Flanders.

In the next three chapters, based on three different case studies, the changes in macroinvertebrate composition are highlighted due to the introduction of alien macroinvertebrates and changes in environmental and habitat conditions. In these studies, we focussed mainly on macro-Crustacea, the most prominent group of alien species present in the macroinvertebrate community. Due to the differences in environmental and habitat conditions, all three case studies provided useful insight in the ecology of alien macroinvertebrates and the interrelationship with environmental and habitat conditions.

Chapter 4 is based on a case study on the harbour of Ghent, a former heavily polluted ecosystem with a north-south salinity gradient. We investigated the changes in macroinvertebrate composition using long-term biological and chemical monitoring data collected by the Flemish Environment Agency over the last two decades. The relationship between the changes in water quality and the establishment of alien macroinvertebrates was examined in order to gain insight in the drivers that caused changes in macroinvertebrate species composition.

In chapter 5, we investigated the changes in the gammarid composition in polder waters, an ecosystem that is characterised by brackish water conditions and low natural species diversity. Originally, only two typical brackish water gammarids occurred in these waters. Due the introduction of a new gammarid species, *Gammarus tigrinus* Sexton, 1939, we expected changes in the prevalence of the indigenous species. Biological and physical-chemical samples of the last 20 years were investigated via multivariate analysis in order to explain the observed changes.

Besides a formerly polluted system and the brackish water polders, we also investigated the macroinvertebrate community of the Belgian coastal harbours and their adjacent watercourses, which are discussed in chapter 6. In this case study, we investigated the presence of alien and indigenous macro-Crustacea in the main four Belgian coastal harbours

(Nieuwpoort, Oostende, Zeebrugge and Blankenberge). We mapped the degree of biocontamination to reveal the difference in presence and abundance of alien macroinvertebrates along a salinity gradient. The importance of several abiotic factors was linked to the occurrence of these macroinvertebrates. Since shipping is seen as an important vector for alien species introductions, ship movements of yachts and container ships were analysed.

After acquiring the necessary knowledge and insight in the ecology of alien macroinvertebrates based on the different case studies, we used data-driven modelling techniques to determine the preferred habitat of alien macro-Crustacea, which is discussed in Chapter 7. Different techniques, such as regression and classification trees in combination with several optimisation methods (e.g. pruning) were used to construct the models. Afterwards, we made predictions on their future distribution in Flanders taking into account the possible changes in water quality in the near future based on an integrated modelling approach. For this, we selected the river basin of the 'Gentse kanalen', since we gained already some knowledge on this area from previous research (see chapter 4).

In chapter 8, we made spatio-temporal predictions on the future distribution of *Dikerogammarus villosus*, an invasive alien macroinvertebrate species. Based on the coupling of a habitat suitability model with a water quality model and a spatial model, we performed a risk assessment for this species in Flanders. With this approach, we tried to provide a practical method that can be applied by decision makers in order to take preventive measures against the further spread of invasive alien macroinvertebrates.

In the last chapter of this dissertation, the general conclusions and opportunities for further research are discussed.

Chapter 2: Distribution of alien macroinvertebrates in Flanders

Based on:

Boets P., Lock K., Cammaerts R., Plu D., Goethals P.L.M. (2009). Occurrence of the invasive crayfish *Procambarus clarkii* (Girard, 1852) in Belgium (Crustacea: Cambaridae). Belgian Journal of Zoology 139(2): 173-175.

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Chapter 2: Distribution of alien macroinvertebrates in Flanders

Abstract

During the present study an inventory was made of the alien macroinvertebrates occuring in Flanders. To this end large collections of biological samples were investigated and supplemented with own sampling campaigns. Mainly fresh and brackish surface waters were investigated. Three new macroinvertebrate species for Flanders were discovered. In total 41 alien macroinvertebrates were encountered in fresh and slightly brackish surface waters in Flanders. Additionally, 24 alien macroinvertebrate species have been reported for the Belgian part of the North Sea and its adjacent estuaries. Most alien macroinvertebrates belonged to the crustaceans and molluscs. Over 2,500 samples containing macrocrustaceans were identified to species level, which allowed us to accurately map their distribution in Flanders. Alien species found in the fresh and brackish water environment, mainly originated from the Ponto-Caspian area and North-America followed by Asia and South- and East-Europe. This overview shows that alien macroinvertebrates are widespread and abundantly present in many watercourses in Flanders. Based on observations in neighbouring countries, several additional species are expected to arrive in the near future. A follow-up of the invasive alien species together with a monitoring scheme to detect new incoming species is valuable to estimate the size of the problem and to be able to closely follow their ecological and economic impact.

2.1 Introduction

During the last century, an increasing number of alien species has been observed in watercourses worldwide. Although migration of species can be considered as a natural process, anthropogenic influences have altered the geographical scale, speed and dispersal mode of invaders (Elton, 1958). In Europe, different groups of exotic macroinvertebrates are found in fresh- and brackish water systems, of which the majority belongs to the crustaceans and the molluscs originating from the Ponto-Caspian basin (Bij de Vaate et al., 2002).

Because of the growing number of invaders and the ecological and economic consequences, the invasion issue has received special attention. As a result, the number of publications, workshops, congresses and journals about invasive alien species increased substantially (Pysek et al., 2006). In contrast to other countries and even Wallonia, the southern region of Belgium (Vanden Bossche et al., 2001; Vanden Bossche, 2002; Josens et al., 2005), the presence and spread of alien macroinvertebrates has hardly been examined in Flanders. Wouters (2002) gave an overview of the alien macrocrustaceans in the whole of Belgium. He stated that at least 13 macrocrustaceans have invaded Belgium. For marine and brackish waters in Flanders, an overview of all alien taxa has been presented by Kerckhof et al. (2007). More recently, the Flanders Marine Institute published a list of all alien species of the Belgian part of the North Sea and its adjacent estuaries (Vandepitte et al., 2012). They listed 40 alien macroinvertebrate species including crustaceans, molluscs and worms. The proximity of the sea, the interconnection between different waterways, the high degree of canalisation, intensive boat transport and the presence of harbours make Flemish watercourses susceptible for aquatic invasions. In this study, we mainly focused on alien macrocrustaceans since these are widespread and represent, together with molluscs, the most important share of alien macroinvertebrates in many rivers across Europe (Bernauer and Jansen, 2006; Nehring, 2006; Messiaen et al., 2010). The present study aims to make an inventory of the occurence of alien macroinvertebrate species in Flemish watercourses based on samples of the Flemish Environment Agency and available literature, supplemented with own sampling campaigns.

2.2 Materials en Methods

2.2.1 Study area

Our study was conducted in Flanders (northern part of Belgium), which is situated in West-Europe and is bordered in the northwest by the North Sea (Fig. 2.1).

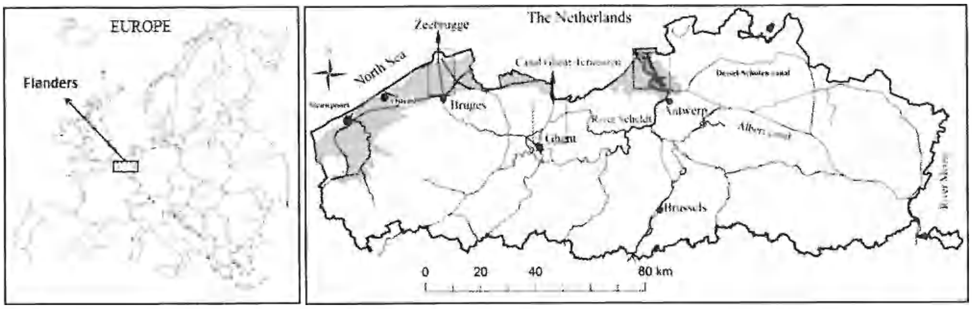

Figure 2.1 *Overview of the study area (Flanders, Belgium) with indication of the most important watercourses and geographical locations, an indication of the polder area (grey) and the three main harbours which are indicated by rectangles.*

Flanders comprises a total area of 14,000 km^2, is highly urbanised and is characterized by a high population density (on average 460 inhabitants km^{-2}). Flanders is classified as lowland and has a dense network of small and large watercourses including a large network of navigable canals (over 1000 km). The dominant land uses are agriculture, industry and residential area (Gobin et al., 2009). Different aquatic habitats were monitored including all types of watercourses (see Jochems et al., 2002), lakes and (coastal) harbours.

2.2.2 Data collection

Data from different sources (e.g. VMM, own sampling campaigns, historical data) were collected for the different studies performed during this PhD. Each study required its own sampling method and approach, which is discussed in detail in the different chapters. However, we briefly discuss the different datasets and methods that were used to be able to give an overview of alien macroinvertebrates present in Flanders. Only benthic macroinvertebrates were considered in this study and consequently the following groups: Cnidaria, Ctenophora, Bryozoa, Coelenterata and Porifera were not included in the analysis

Different sources of data available:

(1) Data collected by the Flemish Environment Agency (VMM) of over 4,600 sampling locations situated in inland waters (fresh and brackish water). Biological samples were taken yearly and each sampling location was sampled on average every three years resulting in a large dataset of more than 11,000 biological samples collected between 1989 and 2012. The samples are stored at the Royal Belgian Institute of Natural Sciences (RBINS). Since samples of the VMM are identified to genus or family level, information about alien macrocrustacean species, such as *Dikerogammarus villosus* (Sowinsky, 1894) or *Gammarus tigrinus* Sexton, 1939 was not available since both species belong to the same family Gammaridae as the indigenous species *G. pulex* (Linnaeus, 1758). Although we try to give a complete overview of all alien macroinvertebrates encountered in Flanders, we mainly focused on aquatic macrocrustaceans in Flanders. Consequently, we identified all crustaceans of over 2,500 samples to species level.

(2) Data collected during the four years of this study (2008-2012) at different sampling locations where alien macroinvertebrate species were expected based on historical records, information retrieved from databases or observations made by colleague zoologists. In addition, data were collected in the scope of a study that was performed in the Belgian coastal harbours to assess the diversity and abundance of alien macro-Crustacea (Malacostraca).

(3) Data retrieved from the collections of the RBINS and from literature reporting on the occurrence of alien macroinvertebrates.

The standard method used for biological monitoring of aquatic macroinvertebrates is the one used by the VMM and described by Gabriels et al. (2010). Depending on the depth of the watercourses, macroinvertebrates were sampled by means of a standard handnet or artificial substrates (Gabriels et al., 2010). The handnet consists of a metal frame of approximately 0.2 m by 0.3 m to which a conical net is attached with a mesh size of 300 µm. With the handnet, a stretch of approximately 10-20 m was sampled during three minutes for watercourses less than 2 m wide or five minutes for larger rivers. Sampling effort was proportionally distributed over all accessible aquatic habitats. In addition to the handnet sampling, macroinvertebrates were manually picked from stones, leaves, or branches along the same stretch (Gabriels et al. 2010). Artificial substrates were used for deep waters like canals where handnet sampling was not possible (Gabriels et al., 2010). Three replicates of artificial substrates, which consist of polypropylene bags (5L) filled with bricks of different sizes, were left in the water for a period of at least three weeks before they were retrieved. In this way, species had the time to colonise the substrates. Both sampling methods are standardised semi-quantitative methods (Gabriels et al., 2010).

When investigating the macroinvertebrate fauna of the Belgian coastal harbours we used, besides the abovementioned sampling techniques, a trawl net with a circular diameter of 100 cm, a length of 3 m and a mesh size of 200 µm. This sampling method was used for qualitative analysis only and the samples were used to assess the species present in the water column between 0.2 m and 1.2 m above the bottom. This sampling technique was used to catch mobile species, such as Mysida, since these are often missed when using a handnet or artificial substrates. The trawl net was with a long rope attached to a zodiac and left into the water to the appropriate depth for sampling. Samples were taken by sailing within a radius of 100 m from a predetermined fixed sampling location (GPS determined) within the harbour for 10 minutes at an average ship speed (4 km $hour^{-1}$ relative to the bottom). All samples were taken during day time when hyperbenthic organisms are known to be concentrated near the bottom.

In order to be able to give a representative overview of the distribution of crayfish in Flanders we did not only use the standard sampling techniques as described by Gabriels et al. (2010), but we also used fyke nets (0.25 m diameter and a length of 0.50 m) specifically designed to catch crayfish and bait (cat food). Historical records as well as recent observations of crayfish were checked from October 2010 to May 2011.

2.3 Results

2.3.1 Macroinvertebrates

Based on all investigated samples (from aal different case stdueis) a total of 65 alien macroinvertebrates have been encountered in Flanders of which 40 are regularly encountered in fresh and slightly brackish waters. The remaining 25 species are restricted to the marine environment. An overview of the alien macroinvertebrates that have been detected in Flanders is given in Table 2.1.

Most alien macroinvertebrate species belonged to the crustaceans (56%), followed by Mollusca (32%), Annelida (9%) and Platyhelminthes (3%) (Fig. 2.2). Most alien macroinvertebrates originated from North-America (37%) or the Ponto-Caspian region (19%) (Fig. 2.3).

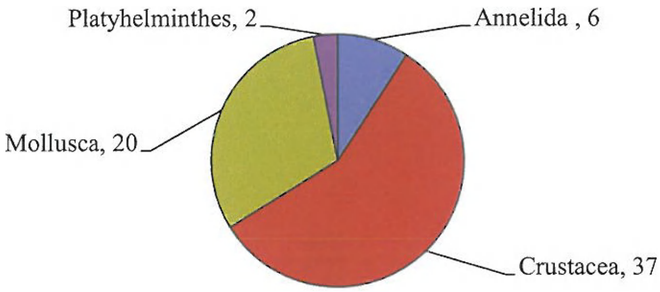

Figure 2.2 *Pie chart showing the distribution of alien aquatic macroinvertebrates among the different phyla.*

When analysing the number of alien macroinvertebrate species established in function of time a serious increase is observed in the number of alien species established during the last two decades. Before 1970, the number of alien species encountered was quite low whereas after the sevenies many alien macroinvertebrates were found in our Flemish watercourses. Since 2000, 15 new alien species were recorded for the first time in Flanders (Fig. 2.4). More detailed results on the occurrence and distribution of macrocrustaceans are given in section 2.3.2.

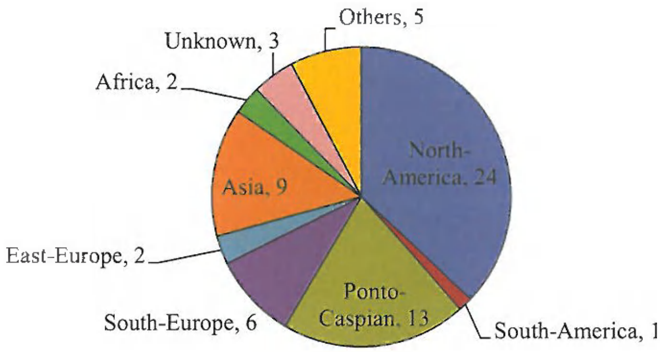

Figure 2.3 *Pie chart presenting the origin of the different alien aquatic macroinvertebrate species encountered in Flanders.*

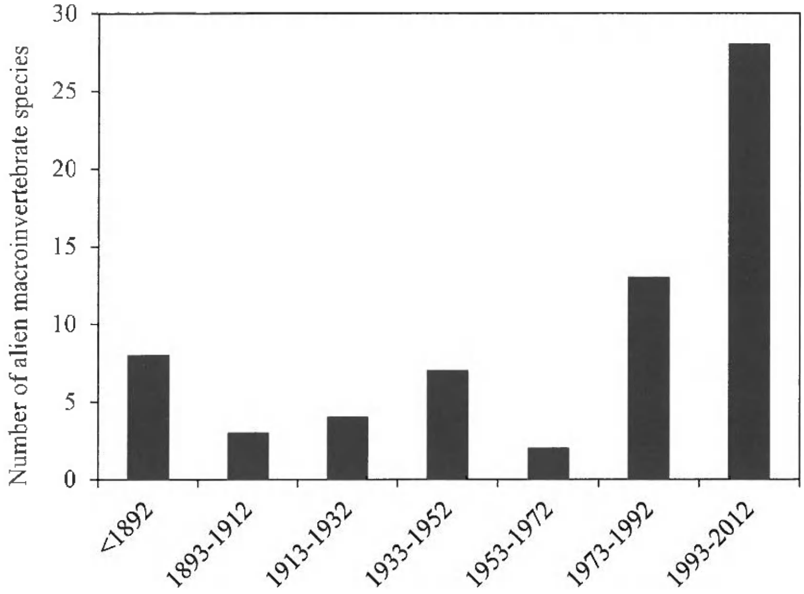

Figure 2.4 *Number of established alien aquatic macroinvertebrate species in Flanders in function of time intervals.*

Table 2.1 *Overview of alien macroinvertebrates observed in surface waters in Flanders with the first year of occurrence, the origin and salinity zone. Only species in bold were encountered during the study. RBINS=Royal Belgian Institute for Natural Sciences, VLIZ=Flanders Marine Institute.*

Phylum/Order	Family	Species	Origin	First occurrence in Flanders	Salinity zone	Reference
ANNELIDA						
Haplotaxida	Tubificidae	*Tubificoides heterochaetus*	North-America	1952	B	Konietzko 1953
	Tubificidae	*Quistadrilus multisetosus*	North-America	1996	F	Seys et al. 1999
	Tubificidae	*Branchiura sowerbyi*	East-Asia	1931	F/B	Damas 1938
Sabellida	Serpulidae	***Ficopomatus enigmaticus***	Asia	1950	F/B	Leloup and Lefevere 1952
Spionida	Spionidae	*Marenzelleria neglecta*	North-America	1996	B	Ysebaert et al. 1997
Terebellida	Ampharetidae	***Hypania invalida***	Ponto-Caspian	2000	F	Vercauteren et al. 2003
CRUSTACEA						
Amphipoda	Caprellidae	***Caprella mutica***	Northeast-Asia	1998	M	Cook et al. 2007
	Corophidae	***Chelicorophium curvispinum***	Ponto-Caspian	1990	F/B	Vercauteren et al. 2003
		Monocorophium sextonae	New Zealand	1993	B/M	Dewicke 2002
	Crangonictidae	***Crangonyx pseudogracilis***	North-America	1992	F	Wouters 2002
		Synurella ambulans	Ponto-Caspian	2003	F	Boets et al. 2010b
	Gammaridae	***Dikerogammarus villosus***	Ponto-Caspian	1997	F/B	Messiaen et al. 2010
		Echinogammarus berilloni	South-Europe	1925	F	Wouters 2002
		Echinogammarus trichiatus	Ponto-Caspian	2009	F	Boets et al. 2012b
		Gammarus roeseli	South-Europe	1937	F	Collection RBINS
		Gammarus tigrinus	North-America	1991	F/B	Messiaen et al. 2010
	Melitidae	***Melita nitida***	North-America	1996	M	Faasse and Van Moorsel 2003
	Pleustidae	***Incisocalliope aestuarius***	North-America	1996	B/M	Faasse and Van Moorsel 2003
	Talitridae	***Orchestia cavimana***	Ponto-Caspian	1927	F/B	Wouters 2002
Decapoda	Astacidae	***Astacus leptodactylus***	East-Europe	1986	F	Gérard 1986

		Pacifastacus leniusculus	North-America	1986	F	Gérard 1986
	Atyidae	***Athyaephyra desmaresti***	South-Europe	1895	F	Wouters 2002
	Cambaridae	***Procambarus clarkii***	North-America	2008	F	Boets et al. 2009
		Orconectes limosus	North-America	1977	F	Wouters 2002
	Panopeidae	***Rhithropanopeus harrisii***	North-America	1991	F/B	Van Damme et al. 1992
	Palaemonidae	***Palaemon macrodactylus***	Southeast-Asia	1999	F/B	Boets et al. 2011a
	Portunidae	*Callinectes sapidus*	North-America	1993	B/M	Van Damme and Maes 1993
	Varunidae	***Eriocheir sinensis***	Southeast-Asia	1933	F/B	Wouters 2002
		Hemigrapsus takanoi	North-America	2003	M	Dumoulin 2004
		Hemigrapsus sanguineus	North-America	2006	M	Nuyttens et al. 2006; d'Udekem d'Acoz 2006
Isopoda	Asellidae	***Proasellus coxalis***	South-Europe	1998	F/B	Boets et al. unpub data
		Proasellus meridianus	South-Europe	1945	F/B	Collection RBINS
	Idoteidae	***Synidotea laticauda***	North-America	2005	B	Soors et al. 2010
	Janiridae	***Jaera istri***	Ponto-Caspian	2000	F	Messiaen et al. 2010
Mysida	Mysidae	***Hemimysis anomala***	Ponto-Caspian	1999	F/B	Verslycke et al. 2000
		Limnomysis benedeni	Ponto-Caspian	2005	F	Lock et al. 2007
Sessilia	Austrobalanidae	*Elminius modestus*	Australia-Asia	1950	M	Leloup and Lefevere 1952
	Balanidae	*Amphibalanus amphitrite*	South-Europe	1952	M	Kerckhof and Catrijsse 2001
		Amphibalanus improvisus	West-Atlantic	before 1700	M	Kerckhof and Catrijsse 2001
		Megabalanus tintinnabulum	West-Africa, Indio-Pacific	1998	M	Kerckhof and Catrijsse 2001
		Megabalanus coccopoma	South-America	1997	M	Kerckhof and Catrijsse 2001
		Amphibalanus reticulatus	Tropical and warm seas	1997	M	Kerckhof and Catrijsse 2001
Tanaidaea	Tanaidae	***Sinelobus stanfordi***	Unknown	2007	B	Van Haaren and Soors 2009
MOLLUSCA						
Architaenioglossa	Viviparidae	***Viviparus viviparus***	East-Europe	<1874	F	Adam 1947
Euheterodonta	Pharidae	*Ensis directus*	North-America	1987	M	Kerckhof and Dumoulin 1987
Littorinimorpha	Calyptraeidae	*Crepidula fornicata*	North-America	1911	M	Adam and Leloup 1934
Myoida	Myidae	*Mya arenaria*	North-America	< 1700	M	kerckhof et al. 2007

	Teredinidae	*Teredo navalis*	Unknown	1730-1732	M	Sellius 1733
		Psiloteredo megotara	Unknown	<1600	M	Redeke 1912
Neotaenioglossa	Hydrobiidae	***Lithoglyphus naticoides***	Ponto-Caspian	1924	F	Adam 1947
		Potamopyrgus antipodarum	New Zealand	1927	F/B	Adam 1947
Ostreoida	Ostreidae	***Crassostrea gigas***	Southeast-Asia	1969	B/M	Leloup 1971
Pulmonata	Planorbidae	*Menetus dilatatus*	North-America	1998	F	Sablon et al. 2010a
		Ferrissia fragilis	North-Africa	1937	F	Van Goethem and Sablon 1986
	Physidae	***Physella acuta***	North-America	1869	F	Adam 1947
Unionoida	Unionidae	*Sinanodonta woodiana*	Asia	1999	F	Keppens and Mienis 2004
Veneroidea	Corbiculidae	***Corbicula fluminalis***	Asia	1992	F	Swinnen et al. 1998
		Corbicula fluminea	Asia	1992	F	Swinnen et al. 1998
	Dreissenidae	***Dreissena polymorpha***	Ponto-Caspian	1834	F	Adam 1947
		Dreissena rostriformis bugensis	Ponto-Caspian	2009	F	Sablon et al. 2010b
		Mytilopsis leucophaeta	North-America	1835	F/B	Nyst 1835; Adam 1947
	Mactridae	***Rangia cuneata***	North-America	2004	F/B	Verween et al. 2006
	Veneridae	*Petricolaria pholadiformis*	North-America	1899	M	Loppens 1902
PLATYHELMINTHES						
Seriata	Dugesiidae	***Dugesia tigrina***	North-America	2004	F	Vercauteren et al. 2005
	Dendrocoelidae	***Dendrocoelum romanodanubiale***	Ponto-Caspian	2001	F	Vercauteren et al. 2003

2.3.2 Macro-Crustacea

In the following section an overview is presented of all alien macro-Crustacea encountered in surface waters of Flanders. We did not investigate the Sessilia (barnacles) during our study, because we mainly focussed on benthic macroinvertebrates.

2.3.2.1 Caprellidae

Caprella mutica Schurin 1935 was recorded for the first time in Belgium in 1998 on a buoy that was situated at the entrance of the harbour of Zeebrugge (Cook et al., 2007). The species can regularly be found on pontoons in the harbour of Zeebrugge. Although we found *C. mutica* only in the harbour of Zeebrugge the species has been reported from the harbour of Ostend and at other locations along the Belgian coast (Cook et al., 2007).

2.3.2.2 Corophidae

The Ponto-Caspian invader *Chelicorophium curvispinum* (Sars, 1895) has been observed in the canals in the eastern part of Flanders since 1990 (Vercauteren et al., 2003). The current study revealed that the species also occurred in the Kleine Nete in 2005. This species was also found in the 'Prinsenpark' in Retie (Vercauteren et al., 2006) and the gravel pits Kessenich and Heerenlaak along the Border Meuse (Lock et al., 2007). The other species belonging to this family, *Monocorophium sextonae* Crawford 1937, is restricted to the marine environment. The species was recorded for the first time in 1993 in samples that were taken along the North Sea coast (Dewicke, 2002). Besides the ship wrecks in the North Sea where the species is abundantly present (Mallefet et al., 2008), the species was also recorded in 2009 in the harbour of Zeebrugge.

2.3.2.3 Crangonictidae

Crangonyx pseudogracilis Bousfield, 1958 was first reported for Flanders in 1998 in a ditch near Puurs, probably introduced with *Ludwigia grandiflora*. However, analysis of historical samples in a nearby ditch indicated its presence since 1992 (Vercauteren and Wouters, 1999). A few specimens were found in the Dessel-Schoten canal in 1997 and 2005 and in the Bocholt-Herentals canal in 1996 and 1998. This species was also found in the Old Meuse in Stokkem, in two brooks in Genk and Herk-de-Stad (Vercauteren, pers. comm.) and in a lake in Harelbeke (Ghyselbrecht, pers. comm.). Since 2002, a growing number of localities in the western part of Flanders have been invaded by this species (Ghyselbrecht, pers. comm.).

Currently, the species is widely distributed in Flanders and occurs in all types off habitats, but is mainly found in stagnant or slow flowing rivers.

Synurella ambulans (Müller, 1846) was recorded for the first time in Belgium in 2003 in the samples taken by the VMM, at two localities in Snellegem in the West of Flanders. Both sampling locations were situated in a small brook (Jabeekse beek) in the nature reserve Vloethemveld. Due to the isolated occurrence it is still unclear how *S. ambulans* reached this new location. One of the possible vectors is anthropogenic distribution via introduction of fish.

2.3.2.4 Gammaridae

Various gammarid species were found in the analysed samples. Although the earliest report of the alien gammarid *Dikerogammarus villosus* in Flanders dated from 2000 (Vercauteren et al., 2006), analysis of the VMM samples showed that this species was already present in 1997 in the East of Flanders, at least in the Albert canal and the Dessel-Kwaadmechelen canal. The species has been spreading gradually and since 2002, some occurrences in the central and western part of Flanders have been reported. The species is currently also present in the canal Ghent-Terneuzen, the canal Kortrijk-Bossuit and the canal Ghent-Ostend.

Gammarus tigrinus Sexton, 1939 was reported for the first time in Flanders in 1996 (Vercauteren and Wouters, 1999), but *G. tigrinus* has been present in the VMM samples since 1991. The species has now invaded watercourses all over Flanders and can reach very high densities in canals and the brackish polder waters.

Both *Echinogammarus berilloni* (Catta, 1878) and *Gammarus roeseli* Gervais, 1835 can be considered as naturalized, as they have already been present in Flanders for a few decades. Both species have a restricted occurrence in Flanders and are only sporadically encountered in the samples. They are associated with fast flowing rivers with a relatively good water quality.

The most recently discovered gammarid is *Echinogammarus trichiatus* (Marynov, 1932). The species was sampled for the first time in June 2009 in an artificial lake in the East of Flanders. The lake resulted from water intrusion into an old sand pit, where excavation took place in previous decades. The pond is situated close to the Bocholt-Herentals canal and is connected with the Dessel-Kwaadmechelen canal, which could indicate that the species was most likely introduced via human-mediated transport.

2001 and in the Dessel-Kwaadmechelen canal in 2004. Analysis of the samples of the VMM showed that one individual was found in 2004 in Mechelen in a tributary of the river Dijle. The occurrence of the species is mainly restricted to the rivers and canals in the eastern part of Flanders.

2.3.2.19 Mysidae

Hemimysis anomala (Sars, 1907) was observed for the first time in Flanders in 1999 in a brackish pond 'Galgenweel' near Antwerp (Verslycke et al., 2000). This species mainly occurs in lentic environments and can withstand a wide range of salinities up to 19 ‰ (Bij de Vaate et al., 2002). A few years later the species was also found in the cooling installation of BASF in Antwerp and in a ditch near Ostend. Nevertheless the distribution of this species remains limited. *Limnomysis benedeni* Czerniavsky, 1882 mainly occurs in freshwater and was first recorded in Flanders in 2005 in the Kessenich and Herenlaak (Lock et al., 2007). The species has a scattered distribution and can be found also in the Donkmeer, the river Kleine Nete, Schulensmeer and in some gravel pits near Mol.

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2.3.2.5 Melitidae

Melita nitida Smith 1873 was recorded for the first time in 1996 near Doel (Faasse and Van Moorsel, 2003). The species has been found several times at the same location in 2003 en 2004 and is regularly reported in the river Scheldt. *M. nitida* has been sampled in the Belgian coastal harbours in 2009, but at very low densities, indicating that the species has a limited occurrence in these harbours.

2.3.2.6 Pleustidae

The first record for *Incisocalliope aestuarius* (Watling and Maurer, 1973) took place in the river Scheldt near the Dutch border in 1996 (Faasse and Van Moorsel, 2003). The species occurrence is very rare and can mainly be found under the low tide line.

2.3.2.7 Talitridae

Orchestia cavimana Heller, 1865 was observed for the first time in Flanders in 1927 and was found in the Zuid-Willemsvaart and Bocholt-Herentals canal in 1980, in the Dessel-Schoten canal in 1981 and in other rivers, such as the river Leie, Scheldt and Dender (Wouters, 2002). More recently, the species was also encountered in the river Yser, in the Stenensluisvaart canal in Woumen and the Zuidervaartje canal in Bruges (Ghyselbrecht, pers. comm.). During sampling in the harbour of Ghent, the species was also found near the banks of the canal Ghent-Terneuzen.

2.3.2.8 Tanaidae

In Flanders, the first individual of *Sinelobus stanfordi* (Richardson, 1901) was found in 2007 in the Verrebroekdok in the harbour of Antwerp on artificial substrates that are used to monitor glass eel. The species can reach high abundances and was also recorded more inland and in the canal Ghent-Terneuzen. In the Verrebroekdok, the species was accompanied by a community dominated by the introduced amphipod *Gammarus tigrinus*, small numbers of the alien crab *Rhithropanopeus harrisii* (Gould, 1841) and the invasive alien snail *Potamopyrgus antipodarum* (Gray, 1843) (van Haaren and Soors, 2009).

2.3.2.9 Astacidae

Astacus leptodactylus Eschscholtz, 1823, originating from East-Europe was introduced for the first time in Belgium in the 1970s and was first recorded in Flanders in 1986 (Gérard, 1986). *A. leptodactylus* was originally introduced to replace stocks of crayfish but also seemed to be vulnerable for the crayfish plague and consequently did not fulfil the expected yield (Pérez et al., 1997). Currently, the species occurs at six scattered locations in Flanders: three ponds, one small river and two canals. The species has similar habitat preferences compared to *Astacus astacus* (Linnaeus, 1758) (Gérard, 1986), but has a competitive advantage over the indigenous species (Stucki and Romer, 2001) and is thought to outcompete the remaining populations of *A. astacus* in the southern parts of Belgium.

Pacifastacus leniusculus (Dana, 1852) was introduced for the first time in Flanders in 1979 (Edsman et al., 2010) and was recorded at three locations before the 1990s in Flanders. During recent sampling, the species was only found in one pond near Hasselt. Although this species is known to be successful and widespread throughout Europe (Holdich et al., 2009), the species seems to have a restricted distribution in Flanders.

3.2.10 Atyidae

The arrival of *Atyaephyra desmaresti* (Millet, 1831) in Flanders is estimated around 1895 (Wouters, 2002). The species was regularly found in the canals and rivers in the eastern part of Flanders. *A. desmaresti* has been found in the river Scheldt, the Old Meuse, the river Warnp and the Roeselare-Leie canal. In the western part of Flanders, the species has also been recorded in the canal Kortrijk-Bossuit.

2.3.2.11 Cambaridae

Orconectes limosus (Rafinesque, 1817) originating from North-America was found for the first time in Flanders in 1977 (Wouters, 2002). This very successful species is widely distributed, occurs in all types of aquatic systems (canals, rivers, brooks and ponds) and is the most common crayfish species in Flanders. This species started its colonisation in the eastern part of Flanders were it rapidly invaded large watercourses. Since the 1990s *O. limosus* has been spreading to the West of Flanders with an average speed of 10 km per year.

The most recently introduced crayfish species is *Procambarus clarkii* (Girard, 1852), which was discovered in a pond near Zammel in 2008. Currently, the species is reported at four other locations: in a pond near Laakdaal not far from its first observation, in a pond near Mechelen and in several canals with slow running water near Bruges. This indicates that the species is in full expansion, which is eased by the fact that this species can spread over land and is thus not restricted to the aquatic environment for its dispersal.

2.3.2.12 Panopeidae

Rhithropanopeus harrisii was recorded for the first time in Flanders in 1991 in the cooling water system of the nuclear power plant of Doel (Van Damme et al., 1992). The species is now commonly found in the Scheldt estuary and in the harbour of Antwerp. *R. harrisii* has a well-established population in the canal Ghent-Terneuzen and was in 2009 also recorded in a small river near the harbour of Nieuwpoort.

2.3.2.13 Palaemonidae

Palaemon macrodactylus Rathbun 1902, originating from Southeast Asia, was recorded for the first time in Belgium in 2003 in the harbour of Antwerp (Soors et al., 2010) and in 2004 in the harbour of Zeebrugge (d'Udekem d'Acoz et al., 2005). However, a study of the samples of the canal Ghent-Terneuzen showed that the species was already present in 1998 in the samples taken closest to the Dutch border. Although the first European occurrence of *P. macrodactylus* was recorded in 1999 (Spain; Cuesta et al., 2004), the species was already present in Flanders one year earlier. d'Udekem d'Acoz et al. (2005) stated that *P. macrodactylus* is most likely to occur in the mesohaline parts of estuaries, albeit this species was also recorded in the upstream part of the canal Ghent-Terneuzen in water with a low salinity (1.9‰), where it reached high abundances (> 100 individuals per sample) in 2006.

2.3.2.14 Portunidae

The first record of a live specimen of *Callinectes sapidus* Rathbun 1896 dates back from 1993 near Doel (Van Damme and Maes, 1993). The species has also been reported along the Belgian coast in Oostduinkerke and Knokke-Heist. Living ovigorous females were found in 2004 by fishermen in the Belgian part of the North Sea. Although we did not encounter *C. sapidus* during our sampling campaigns, the frequent reports suggests that the species has established populations in some Belgian harbours and estuaries.

2.3.2.15 Varunidae

Eriocheir sinensis H Milne Edwards 1853 was observed for the first time in 1933 and is now widely distributed throughout Flanders (Wouters, 2002). Based upon the review made by Wouters (2002), this species occurs both in natural habitats as well as in canalised rivers. However, this species was not frequently found in the dataset of the VMM, probably due to the used sampling method.

Hemigrapsus penicillatus Asakura and Watanabe *2005*, which is present in Belgium since 2003, has rapidly spread and colonised several habitats. *H. penicillatus* can be found under boulders and rocks or between oysters, mainly in estuaries and harbours (Dumoulin, 2004). *H. penicillatus* predominantly occurs along the Belgian coast, but was also recorded in the harbour of Antwerp (Soors et al., 2010).

Hemigrapsus sanguineus (De Haan 1835) was far less recorded during our sampling campaign compared to *H. penicillatus*, although Kerckhof et al. (2007) stated that this species is abundantly present along the Belgian coast. The first specimen was recorded in Knokke-Heist in 2006. Anno 2008, the species spread over the entire coast and was recorded in Zeebrugge, Oostende and Nieuwpoort. The species has even been recorded near the banks of the river Scheldt (VLIZ, 2011).

2.3.2.16 Asellidae

In the studied area, the family Asellidae consisted of two alien species: *Proasellus meridianus* (Racovitza, 1919) and *Proasellus coxalis* (Dolfus, 1892). Although Josens et al. (2005) consider *P. meridianus* as an alien species originating from southern Europe, its alien status can be questioned. According to different authors, this species originates from western Europe, namely Germany and the British isles (Grüner, 1965; Moon and Harding, 1981). *P. meridianus* has a scattered distribution in Flanders and can be frequently found in the samples of the VMM. The first record of *P. coxalis* dates from 1998. Although the species is mainly found in fresh water, it has also been encountered in the brackish polder waters and in the canal Ghent-Terneuzen.

2.3.2.17 Idoteidae

Synidotea laticauda Benedict 1897 was recorded for the first time in 2005 in a sample taken in the river Scheldt near Doel (Soors et al., 2010). This species has a very limited distribution and most of the individuals can be found on the filters of the cooling water installation of the nuclear power plant in Doel.

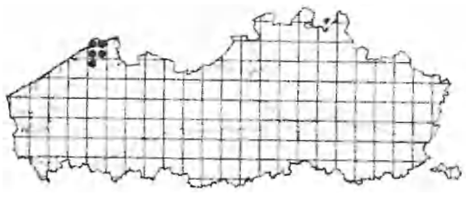
2.3.2.18 Janiridae

Jaera istri Veuille, 1979, an isopod originating from the Ponto-Caspian basin, has been present in watercourses in the east of Flanders since 2000. This species occurs in the Albert canal since 2001 (Vercauteren et al., 2003). It was observed in the Bocholt-Herentals canal in

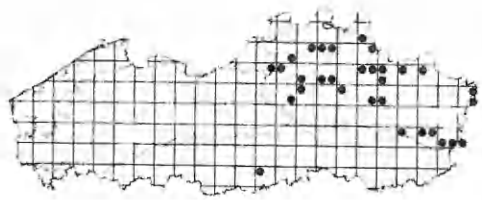
2001 and in the Dessel-Kwaadmechelen canal in 2004. Analysis of the samples of the VMM showed that one individual was found in 2004 in Mechelen in a tributary of the river Dijle. The occurrence of the species is mainly restricted to the rivers and canals in the eastern part of Flanders.

2.3.2.19 Mysidae

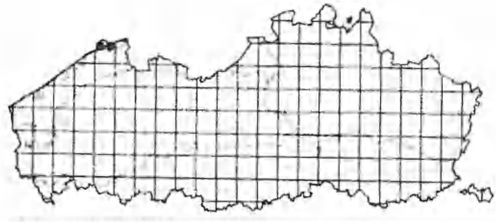
Hemimysis anomala (Sars, 1907) was observed for the first time in Flanders in 1999 in a brackish pond 'Galgenweel' near Antwerp (Verslycke et al., 2000). This species mainly occurs in lentic environments and can withstand a wide range of salinities up to 19 ‰ (Bij de Vaate et al., 2002). A few years later the species was also found in the cooling installation of BASF in Antwerp and in a ditch near Ostend. Nevertheless the distribution of this species remains limited. *Limnomysis benedeni* Czerniavsky, 1882 mainly occurs in freshwater and was first recorded in Flanders in 2005 in the Kessenich and Herenlaak (Lock et al., 2007). The species has a scattered distribution and can be found also in the Donkmeer, the river Kleine Nete, Schulensmeer and in some gravel pits near Mol.



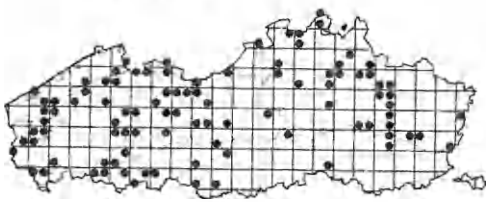
Caprella mutica



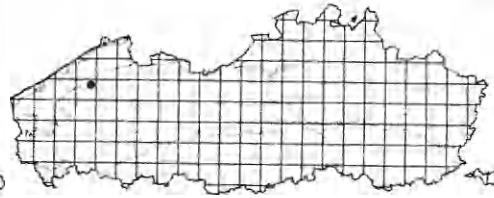
Chelicorophium curvispinum



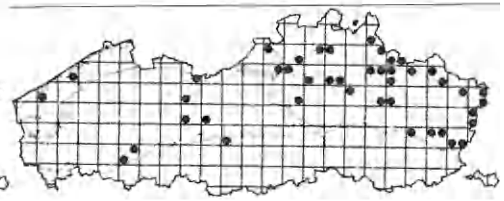
Monocorophium sextonae



Crangonyx pseudogracilis

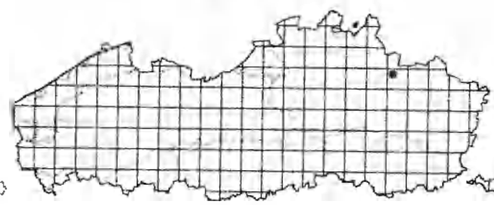


Synurella ambulans

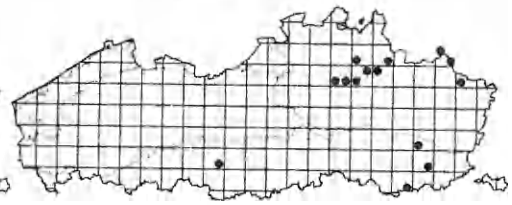


Dikerogammarus villosus

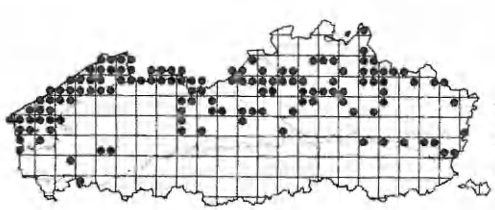
Echinogammarus berilloni



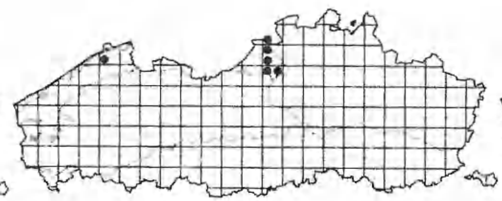
Echinorgammarus trichiatus



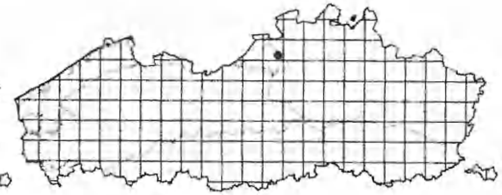
Gammarus roeseli



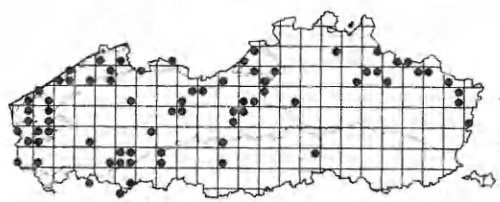
Gammarus tigrinus



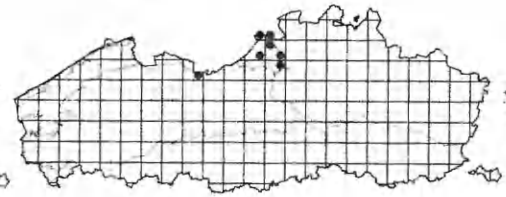
Melita nitida



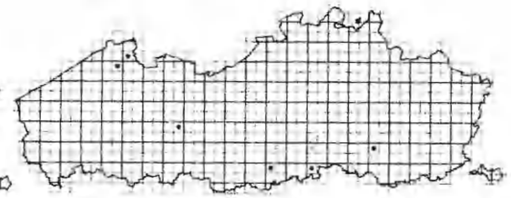
Incisocalliope aestuarius



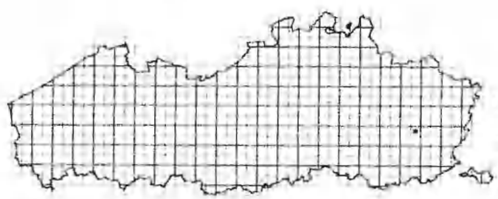
Orchestia cavimana



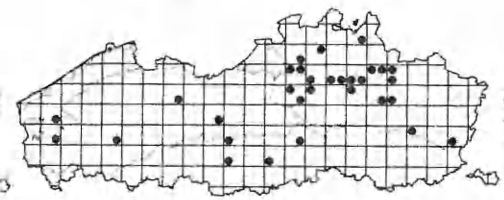
Sinelobus stanfordi



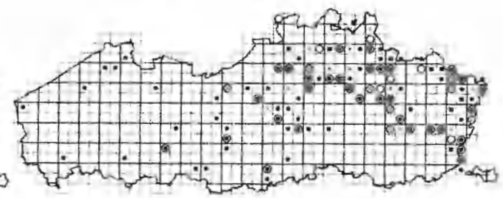
Astacus leptodactylus



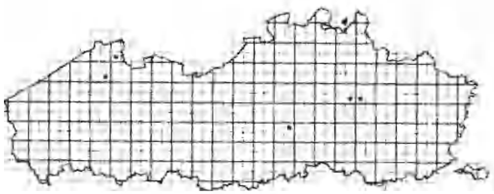
Pacifastacus leniusculus



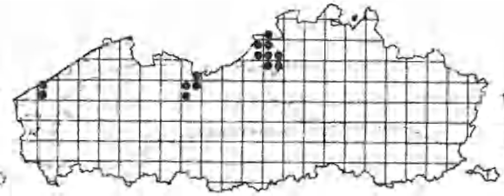
Athyaephyra desmaresti



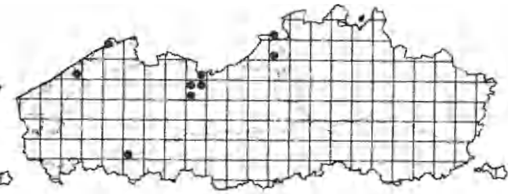
Orconectes limosus



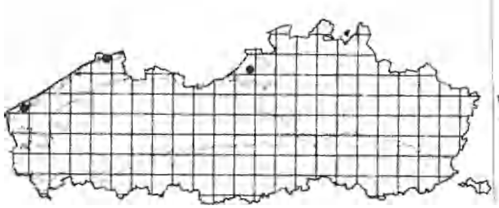
Procambarus clarkii



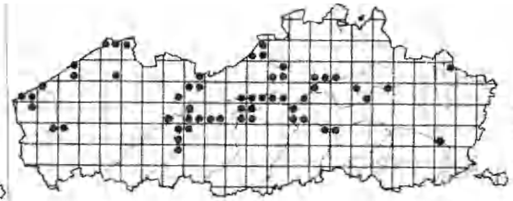
Rhithropanopeus harrisii



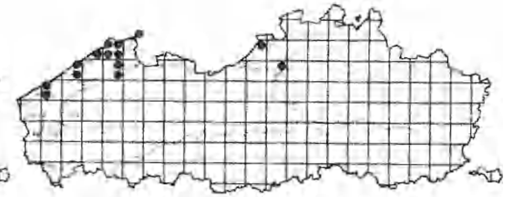
Palaemon macrodactylus



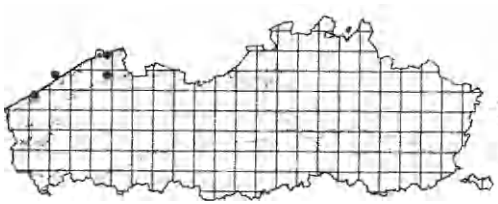
Callinectes sapidus



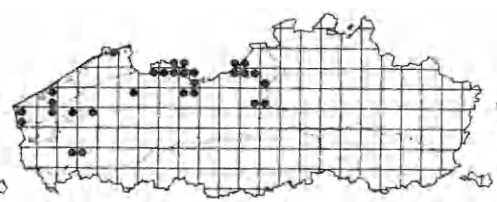
Eriocheir sinensis



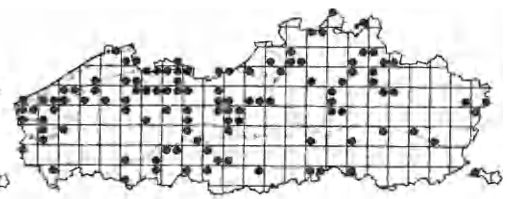
Hemigrapsus penicillatus



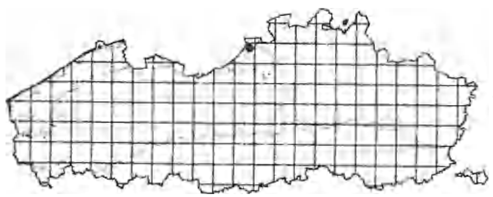
Hemigrapsus sanguineus



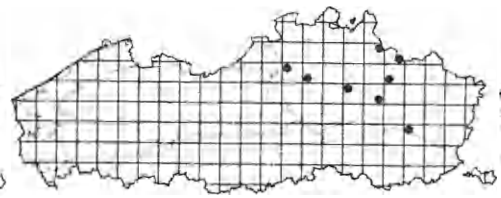
Proasellus coxalis



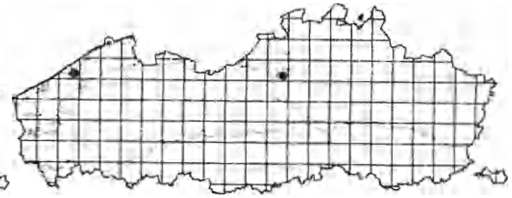
Proasellus meridianus



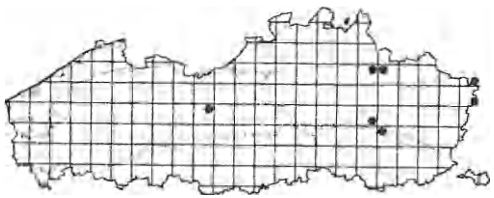
Synidotea laticauda



Jaera istri



Hemimysis anomala



Lymnomysis benedeni

Figure 2.5 *Distribution maps of all alien Crustacea encountered in Flanders (except Sessilia) on a 5X5 UTM grid. Black dots represent the occurrence of the different species. For crayfish, a different legend is applicable: before 1990 (cross), from 1990 to 1999 (circle) and since 2000 (black dot).*

2.4 Discussion

Apart from occasional observations, a large study regarding the occurrence of alien macro-invertebrates in Flanders was not carried out yet. The present study reveals that there are more than 60 alien macroinvertebrate species occuring in Flemish watercourses and that many species were introduced during the last few decades probably as a result of increased trade in the world, the disappearance of natural migration barriers and the degradation of habitats in former decades (Hulme et al., 2009). The present study on the detailed distribution of macrocrustaceans found in surface waters in Flanders, revealed that at least 30 different alien species are present. Especially in fresh water the number of alien crustaceans (21 species) is relatively high compared to the number of indigenous species (3 species). One species, the indigenous crayfish *Astacus astacus*, is extinct in Flanders and highly threatened in the southern parts of Belgium and even Europe due to the crayfish plague, habitat deterioration and a decrease in water quality. Four alien crayfish species replaced the indigenous one of which *Orconectes limosus* is the most abundant and widespread species encountered in Flanders. *O. limosus* appears not to be as sensitive to land use changes and human activities as the indigenous crayfish species (Schulz et al., 2002) and it can withstand habitats unfavourable to the indigenous species, such as soft substrates, turbid and muddy waters, polluted canals and organically enriched ponds and lakes (Pucky, 2009). *P. leniusculus* has similar habitat preferences compared to *A. astacus* (Westman et al., 2002). Despite the reported co-occurrence (Koutrakis et al., 2007), *P. leniusculus* is able to outcompete the indigenous species (Westman et al., 2002). *P. leniusculus* has a similar size, but grows faster, has an earlier sexual maturity, produces larger clutches and is resistant to the crayfish plague (Huner, 1994). It is expected that *P. clarkii* can become the next dominant crayfish species since it can outcompete several other crayfish species (Gherardi, 2007a). The species is known to contribute to biodiversity losses and habitat degradation in several freshwater systems of south central Europe (Gherardi, 2007a) and is therefore also expected to have negative impacts on aquatic communities in Flanders.

Besides crayfish also Amphipoda are represented by several alien species. The best known invasive alien amphipod is *Dikerogammarus villosus*. This species, originating from the Ponto-Caspian area started its colonisation in Flanders in the Albert canal in 1997. Due to the hard bank structures and stony substrates present in canals and large watercourses in Flanders the species quickly dispersed. Currently, the species is reported from several locations, mainly in large watercourses in the central and eastern parts of Flanders. Although the range expansion of *D. villosus* may be influenced by a myriad of factors including hydrological regime, temperature, salinity, water quality and food availability (Devin et al., 2003; Josens et al., 2005; Boets et al., 2010a), habitat heterogeneity and substratum in particular may play a crucial role in its dispersal. Detailed research on this species based on data-driven models indicates that it mainly prefers large rivers and canals with a hard bank structure and a good chemical water quality (Boets et al., 2010a). Where the species arrived, it always replaced the indigenous *G. pulex* and in some cases the alien *G. tigrinus* as well (Messiaen et al., 2010). The effect of *D. villosus* on other amphipods could also be observed in neighbouring countries including the Netherlands (van der Velde et al., 2000; van Riel et al., 2006a; 2007) and France (Devin et al., 2005a). Many studies show that this 'killer shrimp' can prey upon a diverse array of macroinvertebrate species, including indigenous and other previously successful invasive alien amphipods, as well as eggs and larvae of fish (van Riel et al., 2006a; 2007). In order to minimise the impact on local macroinvertebrate communities, it is important to get insight in the ecology of this species. This could be achieved via a combination of field data analysis and experimental laboratory studies.

Although *G. tigrinus* was reported for the first time in Flanders in 1996 in a pond near Antwerp (Vercauteren and Wouters, 1999) the species was already present in the samples of the VMM in 1991, which indicates that it arrived earlier in Flanders, probably via the Scheldt-Rhine connection. Via this route, *G. tigrinus* probably invaded the Albert canal and the other canals. The Ponto-caspian invader *Jaera istri* has been found in the Netherlands since 1997, in the Rhine, the IJssel and the Waal (Kelleher et al., 2000). Soon after its arrival in 2001 in the Albert canal, it became locally abundant (Schöll, 2000). *J. istri* thrives well on places with stony substrates and in dammed canals (Kelleher et al., 2000; Vercauteren et al., 2006; van Riel et al., 2007). Many of these Ponto-Caspian invaders have a preference for these canals with artificial bank structures (reinforced by stones and concrete) and stony substrates (van Riel et al., 2007).

Analysis of the VMM samples indicated that canals in the East of Flanders are extremely prone to colonisation by invaders because of their high degree of connectivity and structural characteristics. Many alien macroinvertebrates were first recorded in the east of Flanders and gradually colonised the rest of Flanders.

In the marine environment, the number of alien macrocrustaceans was quite limited and their distribution remained mostly restricted to the main coastal harbours or the Scheldt estuary.

Most of these marine and brackish species were only recently discovered in Flanders or the Belgian part of the North Sea. *Synidotea laticauda* was recorded for the first time in 2005 in the harbour of Antwerp. Although the species was found only at low abundances, it is expected that the species is more common than the few observations suggest (Soors et al., 2010). With four species, Decapoda represented an important part of the alien fauna found in the brackish and marine environment. *Rhithropanopeus harrisii* is reported form marine (Nieuwpoort) as well as inland harbours (Ghent and Antwerp). Most larvae of the Decapoda were probably introduced via ballast water of ships, which allows them to easily disperse not only within Europe, but also in Flanders.

Although almost all alien macrocrustaceans known to occur in Flanders and even some newly established alien species were found during this study (Table 2.1), some species were less frequently caught than others. This has mainly two reasons. First because some species have a limited distribution with only a few established populations present in Flanders. Secondly, the sampling techniques used (mainly handnet or artificial substrates), are not always effective for catching certain mobile or bigger species, such as crabs, mysids and crayfish. The sampling method can have an impact on the assessment of the distribution of certain invaders, such as *E. sinensis*, *H. anomala*, *L. benedeni* and *O. cavimana*. Based on the review by Wouters (2002), *E. sinensis* is widely distributed in Flanders. The limited occurrence of this species in the dataset of the VMM is probably a consequence of the sampling method. The species is difficult to catch with artificial substrates since the organisms are highly mobile both on land and in the water. Therefore, other sampling techniques, e.g. fyke nets would give a more accurate picture of its actual distribution. Invaders like *H. anomala, L. benedeni* and *O. cavimana* are not often observed, which is probably also due to the sampling method (artificial substrates and handnet), since *H. anomala* and *L. benedeni* are fast swimmers, while *O. cavimana* lives along the river banks in organic debris of fresh and brackish waters (Josens et al., 2005). Additional sampling of the river banks and stone sampling might reveal that the latter species is more abundant than the used sampling techniques suggest, while a hyperbenthic sledge would be a good sampling device to sample mysid shrimp.

Crustacean invaders, such as *Dikerogammarus haemobaphes* (Eichwald, 1841), that has already invaded the Meuse (Josens et al., 2005) and *Echinogammarus ischnus* (Stebbing, 1899) (Wouters, 2002), *Orconectes immunis* (Hagen, 1870) and *Chelicorophium robustum* (Sars, 1895), which have already invaded the river Rhine (Bernauer and Jansen, 2006), can be expected to arrive soon in Flanders. Also the Northern crayfish *Orconectes virilis* (Hagen, 1870), the marbled crayfish *Procambarus* species and the white river crayfish *Procambarus acutus* (Girard, 1852) / *zonangulus* Hobs and Hobs, 1990, which have already been reported in the Netherlands (Koese, 2008), can be expected in the near future. However, their spread will largely depend on their interference with the species that are already present (Kley and Maier, 2006).

A continuous monitoring of new and formerly established invaders is necessary, in particular regarding their habitat preferences and their effect on indigenous species, to obtain a better understanding of their impact due to competition and predation. Moreover, migration models can be relevant to make better predictions regarding the migration speed of alien macroinvertebrates in rivers (Dedecker et al., 2006), whereas habitat suitability models can help to indicate those rivers that are suitable for alien macroinvertebrates to establish.

Alien species are not mentioned specifically in the European water framework directive (European Community, 2000). However, the precautionary principle in the broad sense could be applicable for the negative effects of invasive alien species because aquatic ecosystems have to be guarded from decline. Invasive alien species can modify the natural biological structure and ecological functioning of aquatic systems and the assessment of invasive alien species as biological pressure should therefore be considered as a part of a catchment management policy together with other pressures. However, effective control measures to eradicate invasive alien aquatic macroinvertebrates are not feasible and at best slow down their dispersal rate. Control measures usually cause more damage (i.e. application of pesticides) or other risks (introduction of natural predator of alien species). The Belgium Forum of Invasive Species (www. http://ias.biodiversity.be/) proposes a system of lists, which are based on the ability of a species to disperse and on its environmental impact. It is suggested that this list can be used to ban import, trade and introduction in the natural environment and for control measures or eradication. In such a system, it is necessary to demonstrate significant damage before a species is listed (currently no alien macrocrustaceans or molluscs are listed). However, by the time damage can be demonstrated, the considered species has usually already invaded the area and nothing can be done to stop its further spread. In our opinion, preventive measures, such as the obligatory treatment of ballast water and a controlled trade, are the only possible solutions to reduce the influx of alien aquatic macroinvertebrates.

Chapter 3: Which key factors favour the establishment success and spread of alien macroinvertebrates

Adapted from:

Boets P., Lock K., Goethals P.L.M. (submitted). Assessment of biocontamination in aquatic ecosystems: analysing key factors favouring the establishment and spread of alien macroinvertebrates. Biological invasions.

Chapter 3: Which key factors favour the establishment success and spread of alien macroinvertebrates in Flanders

Abstract

In this chapter, it has been investigated to which extent the establishment and spread of alien macroinvertebrates is determined by habitat, water quality, biotic interactions and shipping. For this, we analysed over 1800 macroinvertebrate samples from 1999, 2004 and 2009, which were scattered over different water types in Flanders (Belgium). In ten years time, the prevalence of alien species significantly increased. When mapping the site-specific biocontamination index (SBCI), which reflects the number of alien species and their abundance, it was found that navigable waterways, harbours and brackish waters were hotspots for the occurrence of alien macroinvertebrates. For water with a good chemical water quality, a negative correlation was found between the SBCI and the Multimetric Macroinvertebrate Index Flanders (MMIF), indicating that water bodies with a high SBCI usually had a relatively low ecological water quality. An integrated model was developed to predict the future prevalence of alien macroinvertebrates. Modelled changes of oxygen and nutrient concentrations due to the construction of planned waste water treatment plants in combination with a habitat suitability model predicting the presence or absence of alien species, indicated that a further increase in the prevalence of these alien species can be expected, especially in those water bodies evolving from a bad or poor to a moderate water quality status. However, waters with a high ecological or hydro-morphological water quality status were found to be less prone to invasions.

3.1 Introduction

In order to set up a cost-effective management that controls and reduces the further spread of invasive alien species, it is important to determine the factors, such as water quality, shipping and biotic interactions that favour the establishment and spread of invasive alien species. Such findings could be supplemented with modelling techniques predicting the future distribution of invasive alien species and in this way, disclosing vulnerable areas for invasions.

In this chapter, it was investigated to which extent the establishment and spread of alien macroinvertebrates is determined by habitat, water quality, shipping and biotic interactions. While there are several studies that investigate the effect of one single factor on individual alien species or narrow taxonomical groups for a specific system (e.g. van Riel et al., 2006b; Grabowski et al., 2007), there only a few studies addressing the importance of environmental, biotic and hydro-morphological variables on alien species from different taxonomic groups at a large geographic scale (e.g. Vermonden et al., 2010; Früh et al., 2012). The site-specific biocontamination index (SBCI, Arbačiauskas et al., 2008) was calculated for different water types in order to quantify the species richness of alien macroinvertebrates and their abundance in the aquatic ecosystem. In order to detect shifts in biocontamination, three different years with time intervals of five years were investigated. Based on biocontamination maps, hotspots of biocontamination were identified on a catchment district scale. In addition, the relationship between the ecological water quality (expressed as a multimetric macroinvertebrate index) and the SBCI was investigated. It was expected that alien species were numerous in waters with a poor ecological water quality and a low biotic resistance. Finally, the future prevalence of alien macroinvertebrates was predicted based on a combination of modelling techniques and the expected changes in chemical water quality due to the installation of planned wastewater treatment plants.

3.2 Materials and methods

3.2.1 Study area

The study area includes two important international catchments: the Scheldt catchment and the catchment of the Meuse (VMM, 2012). An overview of the study area with indication of the most important watercourses and geographic locations as well as an indication of the polder area (grey) can be found in Chapter 2 (Fig. 2.1).

3.2.2 Data collection

The presented data originates from the Flemish Environment Agency (VMM), which coordinates the integrated water management in Flanders. The VMM has been monitoring the water quality in Flanders since 1989 and collected biological, physical-chemical and hydro-morphological data of over 4,600 sampling locations situated in inland waters (fresh and brackish water). Physical-chemical analyses were conducted several times a year (usually on a monthly basis) at about half of these sampling locations, whereas for biological samples each sampling location was sampled on average every three years. Measured standard physical-chemical parameters include pH, oxygen concentration and conductivity. A large dataset of more than 11,000 biological samples and 250,000 physical-chemical samples is available. Macroinvertebrates were collected by standard hand net sampling or via artificial substrates if it was not possible to use the kick sampling method (Gabriels et al., 2010). The hand net consists of a metal frame of approximately 0.2 m by 0.3 m to which a conical net is attached with a mesh size of 300 µm. With the hand net, a stretch of approximately 10-20 m was sampled during three minutes for watercourses less than 2 m wide or five minutes for larger rivers. Sampling effort was proportionally distributed over all accessible aquatic habitats. In addition to the hand net sampling, macroinvertebrates were manually picked from stones, leaves, or branches along the same stretch (Gabriels et al., 2010). Artificial substrates were used for deep waters like canals, where hand net sampling was not possible (Gabriels et al., 2010). Three replicates of artificial substrates, which consist of polypropylene bags (5 l) filled with bricks of different sizes, were left in the water for a period of at least three weeks after which they were retrieved. In this way, species could colonize the substrates. Both sampling methods are standardised semi-quantitative methods (Gabriels et al., 2010).

In the VMM database, macroinvertebrates were identified to the level needed for the calculation of the Multimetric Macroinvertebrate Index Flanders (MMIF; Gabriels et al., 2010). Indigenous and alien species can belong to the same taxon and therefore, it was not always clear from the VMM database if alien macroinvertebrates occurred in the samples or not. Therefore, we only identified all taxa that possibly were represented by alien species (Annelida, Crustacea, Mollusca or Platyhelminthes) to species level. The samples of three years were analysed: 1999 (n=622), 2004 (n=793) and 2009 (n=408). In total, 1823 samples were investigated and data on abundance of indigenous and alien macroinvertebrates as well as related physical-chemical (ammonium (NH_4), biological oxygen demand (BOD_5), chemical oxygen demand (COD), conductivity (EC), total phosphorous (Pt), Kjeldahl nitrogen (KjN), nitrate (NO_3), nitrite (NO_2), orthophosphate (oPO_4), pH and dissolved oxygen) and hydro-morphological data (slope, water type) were analysed. Classification of all different water types in Flanders was based on Jochems et al. (2002) and Gabriels et al. (2010). For practical reasons, several types were clustered into more general types leading to six major water types (Table 3.1).

Table 3.1 *Overview of different water types in Flanders (Belgium), based on Jochems et al. (2002) and Gabriels et al. (2010).*

Water type	Abbreviation	Catchment area
Small stream	Bk	<50 km^2
Large stream	Bg	50-300 km^2
Small river	Rk	300-600 km^2
Large river	Rg	>600 km^2
Polder water	P	Not applicable
Lake	S	Not applicable

3.2.3 Data analysis

The site-specific biocontamination index (SBCI) can be derived from two metrics: abundance contamination index (ACI) and richness contamination index (RCI), both at ordinal rank (Arbačiauskas et al., 2008). These indices were calculated as: ACI=Na/Nt, where Na is the total number of alien individuals and Nt the total number of individuals in a sample, and RCI=Ta/Tt, where Ta is the total number of alien taxa and Tt is the total number of taxa per sample. With the ACI and RCI values, the site-specific biocontamination index (SBCI) can then be derived from the matrix in Table 3.2. SBCI classes rank from 0 (no biocontamination) to 4 (very high biocontamination). The threshold for the lowest quality limit ("bad" class) is based on the assumption that when alien species represent more than half the detected taxa or when their abundance exceeds 50% of the individuals, the community/assemblage has developed as a consequence of the invasion by alien species. The SBCI was mapped and visualized per year using a Geographic Information System (Arcmap version 9.3). A distinction was made between sampling sites where shipping was present (square) or absent (circle). Information on shipping was retrieved from Waterwegen en Zeekanaal nv and the River Information Services.

***Table 3.2** Assessment of site-specific biocontamination index (SBCI) based on abundance contamination index (ACI) and ordinal richness contamination index (RCI) (Arbačiauskas et al., 2008).*

RCI	ACI				
	none	0.01 – 0.10	0.11 – 0.20	0.21 – 0.50	>0.50
none	0				
0.01 – 0.10		1	2	3	4
0.11 – 0.20		2	2	3	[illegible]
0.21 – 0.50		3	3	3	4
>0.50		4	4	4	4

Next to the SBCI, the MMIF (Gabriels et al., 2010) was calculated for all three years and all water types. The MMIF is a type-specific multimetric index based on five equally weighted metrics: taxa richness, number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa, number of other sensitive taxa, the Shannon-Wiener diversity index and the mean tolerance score. The final index value is expressed as an Ecological Quality Ratio ranging from zero for bad ecological quality to one for very good ecological quality. Alien species were included in the calculation of the MMIF, since we followed the standard method as described in Gabriels et al. (2010), where no distinction is made between alien and indigenous taxa. The relationship between the SBCI and the MMIF was investigated based on scatter plots made in the free software package R (version 2.11.0), where the size (diameter) of the dots represent the number of samples. The basic Prati was calculated per sampling point based on measured values for COD, dissolved oxygen and NH_4 concentration (Prati et al., 1971). Based on this index, five different classes ranging from excellent to very bad water quality could be distinguished. Samples classified as excellent or acceptable were grouped under a good chemical water quality, whereas samples classified as slightly polluted, polluted and heavily polluted under a bad chemical water quality. This classification according to Prati et al. (1971) was used to investigate the relationship between the SBCI and the MMIF under good and bad chemical water quality conditions. The relationship between the SBCI and the MMIF as well as the relationship between the SBCI and the measured physical-chemical parameters was tested using Spearman's Rank Correlation Coefficient. A factorial ANOVA was used to test the difference in SBCI between the different years and different river types as well as the interaction effect. A Mann-Whitney U test was used to test whether there was a significant difference in measured nutrient concentrations between the year 1999 and 2009 for the 57 selected sites. In addition, Kruskal-Wallis ANOVA was used to test the difference in physical-chemical parameters between the different water types, where water type was the independent variable and the physical-chemical variables the dependent variables. The significance level for hypothesis testing was set at $p \leq 0.05$. All statistical analyses were performed using Statistica 7.0 (Statsoft Inc., 2004).

Besides the basic statistics, multivariate data analysis was performed using CANOCO for Windows 4.5 (ter Braak and Šmilauer, 2002) to investigate which environmental parameters were detrimental for the species composition. Only the most frequently encountered alien taxa as well as the related indigenous taxa were plotted. Direct gradient analysis was used, since environmental variables were explicitly incorporated in the analysis. To test whether a linear or unimodal method was needed, a Detrended Correspondence Analysis (DCA) was performed. If the Length of Gradient (LoG) > 4, a unimodal method is needed, whereas if the LoG < 3 a linear method is designated. In our case, a Canonical Correspondence Analysis (CCA) was used since the LoG = 5.1. Averages were used for missing data (229 out of 1823 samples were missing) and a log (x+1) transformation was performed prior to CCA to normalize the data. One of both of the following environmental variables were removed prior to analysis because they were highly correlated with each other: BOD and COD ($r = 0.58$, $p = 0.001$), Pt and oPO_4 ($r = 0.73$, $p < 0.001$) and KjN and NH_4 ($r = 0.78$, $p < 0.001$).

3.2.4 Integrated modelling approach

Predictions on the future prevalence of alien macroinvertebrates in water bodies in Flanders were made based on an integrated modelling approach, where a habitat suitability model (based on a data-driven classification tree) was combined with predictions on the improvement of the chemical water quality (PEGASE water quality model) due to the installation of planned wastewater treatment plants (Ronse and D'heygere, 2007; Deliège et al., 2009).

The PEGASE model is a detailed hydrodynamic, deterministic water quality model that consists of three submodels: a combined hydrological and hydrodynamic submodel, a thermal submodel and a biological submodel (Ronse and D'heygere, 2007). The flow (m^3 day^{-1}) is indispensable when determining the water quality because pollution loads are characterised by the flow and the concentration of pollutants in the water. The average flow per watercourse was calculated based on series of field measurements. Based on the measured flow, the natural flow could be determined by means of the mass balance. Besides flow, also the width of the watercourse (m), the slope (mm m^{-1}) and the coefficient of Manning (s $m^{-1/3}$) were incorporated. In addition, a digital elevation model and the land use were used to determine the natural flow. An important parameter of the thermal submodel was water temperature (°C), since this parameter influences the kinetics of processes in the water. Besides series of measurements of the water temperature, also measurements of solar radiation were used as input for the thermal model. Four types of processes were taken into account for the biological submodel: transportation and dilution, biochemical processes in the water column, interactions of the water column with the air near the water surface and interactions of the water column with the soil. Regarding the biochemical processes, the biomass of phytoplankton and –benthos, zooplankton and benthic filterers and heterotrophic bacteria were modelled. The integrated PEGASE model was calibrated and validated with measurements of BOD, COD, oxygen concentration, ammonium, nitrate, nitrite, Kjeldahl nitrogen, total nitrogen, orthophosphate and total phosphorous. The validation of the model with measured values generally resulted in accurate predictions of the different parameters (Ronse and D'heygere, 2007; Deliège et al., 2009; MIRA, 2009). With the PEGASE model, physical-chemical data were generated for three years: 2006 (reference data) and predictions for the year 2015 and 2027 according to the deadlines set by the European Water Framework Directive. In the first scenario (2015), the standard policy as well as the proposed measures in the first period of the district plans are implemented. In the second scenario (2027), all proposed measures are implemented and the receiving water of the neighbouring countries is expected to be of good quality (MIRA, 2009). To check the accuracy of the water quality model, the calculation of the presence of alien species in the samples of the year 2006 based on the output of the water quality model was compared with the results obtained when running the habitat suitability model on the measured physical-chemical data of the year 2006.

For our habitat suitability model, a data-driven classification tree model was constructed based on data of all three years (measured values) including only those physical-chemical parameters that were also available in the water quality model (NH_4, BOD, COD, KjN, NO_3, NO_2, Pt, oPO_4, oxygen concentration), the slope and the presence (n=873) or absence (n=950) of alien macroinvertebrates. Only a limited number of physical-chemical parameters were included in the water quality model and consequently, only these parameters were used to build the habitat suitability model predicting the prevalence of alien macroinvertebrates. Except for slope, hydro-morphological parameters were not consistently monitored in

Flanders and therefore, only one hydro-morphological parameter could be included in our analysis. Classification trees were grown with a recursive partitioning algorithm from a training set of records, which is known as 'Top-Down Induction of Decision Trees' (Quinlan, 1992). For each step, the most informative input variable is selected as the root of the sub-tree and the current training set is split into subsets according to the values of the selected input variable. In this way, rules are generated that relate the predictor (independent) variables (e.g. oxygen concentration) with the response (dependent) variables (prevalence of alien macroinvertebrates). For the construction of the classification tree, the J48 algorithm was applied (Hall et al., 2009), which is a re-implementation of the C4.5 algorithm. Standard settings and three-fold cross-validation were used to construct the classification tree. The dataset was, after reshuffling, randomly split in three subsets: two thirds were used for training and one third for validation. For each training and validation set, a model was build and in this way, a performance value for each of the three different models was obtained. Final model performance was based on the percentage Correctly Classified Instances (CCI) and Cohen's Kappa Statistic (K). The performance of the habitat suitability model was calculated for each year as well as for all years together (entire dataset). The model was built using WEKA software (version 3.0). We strived for an optimal model with a good balance between a satisfying performance on the one hand and a high ecological relevance and reduced complexity on the other hand.

In the final step, the habitat suitability model was applied on the data generated by the water quality model. The data of 2006 were used as a reference, whereas the data of 2015 and 2027 were used to make predictions on the future prevalence of alien macroinvertebrates in surface waters in Flanders. Based on the integrated model, the prevalence of alien macroinvertebrates was calculated.

3.3 Results

Based on the 1823 biological samples, a total of 29 alien macroinvertebrate species belonging to four different Phyla were identified (see Chapter 2 for detailed species list). Crustacea represented 59% of all alien species, followed by Mollusca (31%), Platyhelminthes (7%) and Annelida (3%). Most alien macroinvertebrates originated from the Ponto-Caspian region (35%) or North-America (24%), 14% from Asia, 21% from South Europe and only one species from New Zealand and one from Africa (both 3%).

3.3.1 Assessment of biocontamination

For the six different water types (Table 3.1), the SBCI was calculated (Fig. 3.1). The results show that in large and small streams, 60 to 70% of the samples were free of alien macroinvertebrates. In contrast, large and small rivers, polder waters and lakes were mostly classified as biocontaminated (Fig. 3.2). Most samples classified as severely biocontaminated (SBCI class 4) could be found in large rivers and polder waters. Alien macroinvertebrates were encountered in more than 70% of all samples in polder waters and lakes, which were highly biocontaminated. The highest number of alien macroinvertebrate species (10 species at one site) was encountered in a lake, whereas the highest density (>1000 individuals per three bags of artificial substrates) was found in a large canal.

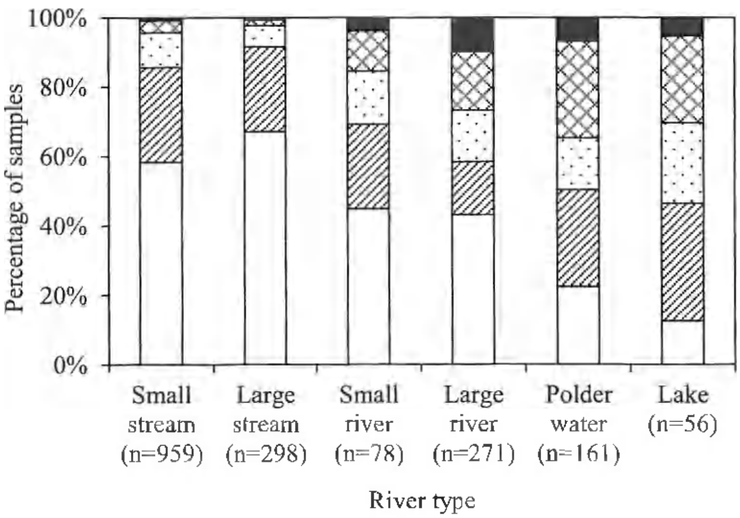


Figure 3.1 *Site-specific biocontamination index for the different water types (no biocontamination (white), low biocontamination (striped), moderate biocontamination (dotted), high biocontamination (crossed) and very high biocontamination (black)).*

Analysis of the physical-chemical characteristics of the different water types revealed that there were significant differences between the different water types for all physical-chemical parameters that were measured (Table 3.3a and 3.3b). On average, the highest nutrient levels were measured in the polder waters (Table 3.3a). The high conductivity measured in the polder waters could be attributed to the brackish water conditions. Large rivers contained relatively low nutrient levels, but sometimes had a high conductivity. Lakes scored best regarding chemical water quality parameters. The highest nutrient values could be found in small streams. There was a difference in the dominance of alien macroinvertebrates encountered in the different water types (Table 3.4). In terms of alien biota, small and large streams were mainly colonized by crustaceans: *Proasellus meridianus* (Racovitza, 1919) and *Crangonyx pseudogracilis* (Bousfield, 1958). Typical euryhaline species, like the amphipod *Gammarus tigrinus* Sexton, 1939 and the New Zealand mud snail *Potamopyrgus antipodarum* (JE Gray, 1843), were found in about half of the samples taken in polder waters. In large rivers, mainly alien gammarids as well as the Ponto-Caspian bivalve *Dreissena polymorpha* dominated the samples. Several alien macroinvertebrate species were encountered in lakes and especially the flatworm *Dugesia tigrina* (Girard) as well as the New Zealand mud snail (*Potamopyrgus antipodarum*) were frequently found.

Table 3.3a *Average and minimum and maximum values (over three years) of ammonium (NH_4), biochemical oxygen demand for 5 days (BOD_5), total phosphorous (Pt), Kjeldahl nitrogen (KjN), nitrate (NO_3), nitrite (NO_2), orthophosphate (oPO_4), pH and dissolved oxygen (O_2) for the different water types: small stream (Bk), large stream (Bg), small river (Rk), large river (Rk), polder water (P), lake (S).*

Type	NH_4 (mg l^{-1})	BOD_5 (mg O_2 l^{-1})	Pt (mg l^{-1})	EC (µS cm^{-1})	KjN (mg l^{-1})	NO_3 (mg l^{-1})	NO_2 (mg l^{-1})	oPO_4 (mg l^{-1})	pH	O_2 (mg l^{-1})
Bk	2.7 (0.0-73)	6.9 (1.0-354)	1.1 (0.05-100)	705 (92-18000)	5.4 (0.75-163)	3.5 (0.03-36)	0.1 (0.0-2.7)	0.5 (0.0-16)	7.5 (6.0-9.1)	6.5 (0.2-19)
Bg	2.0 (0.1-16)	5.2 (1.0-48)	0.7 (0.06-3.7)	760 (165-4940)	4.1 (0.75-28)	3.1 (0.06-14)	0.2 (0.0-1.1)	0.4 (0.0-3.0)	7.6 (6.0-8.8)	6.7 (0.2-15)
Rk	1.4 (0.1-14)	5.6 (1.1-31)	0.8 (0.1-9.0)	1427 (365-4860)	3.2 (0.9-16	3.0 (0.10-13)	0.2 (0.0-0.8)	0.3 (0.0-3.2)	7.7 (6.4-9.2)	6.5 (1.4-22)
Rg	1.9 (0.1-11)	4.6 (1.0-30)	0.8 (0.08-4.6)	1060 (315-9070)	3.7 (0.45-25)	3.7 (0.23-14)	0.3 (0.0-3.0)	0.5 (0.0-1.9)	7.8 (6.9-9.4)	6.2 (0.9-19)
P	2.5 (0.1-42)	6.7 (1.0-30)	1.3 (0.2-6.8)	3109 (310-45300)	4.6 (1.2-48)	3.1 (0.06-34)	0.2 (0.0-2.9)	0.9 (0.0-6.6)	8.0 (7.0-9.1)	7.0 (0.5-28)
S	0.6 (0.1-3)	5.4 (1.3-14)	0.6 (0.08-1.8)	554 (128-2570)	2.3 (0.75-7.7)	0.99 (0.06-5)	0.04 (0.0-0.3)	0.2 (0.0-1.2)	8.2 (6.4-9.4)	9.0 (2.9-17)

Table 3.3b *Results of Kruskal-Wallis ANOVA representing the significant differences found between the different water types for all physical-chemical parameters measured. For explanations of physical-chemical variables see Table 3.2a.*

Variable	Chi-square	df	*P*-value
NH_4 (mg l^{-1})	21.15	5	0.008
BOD_5 (mg O_2 l^{-1})	57.8	5	<0.001
Pt (mg l^{-1})	54.29	5	<0.001
EC (µS cm^{-1})	272.03	5	<0.001
KjN (mg l^{-1})	27.02	5	0.001
NO_3 (mg l^{-1})	113.78	5	<0.001
NO_2 (mg l^{-1})	153.48	5	<0.002
oPO_4 (mg l^{-1})	97.4	5	<0.003
pH	135.45	5	<0.004
O_2 (mg l^{-1})	35.5	5	<0.005

Table 3.4 *Prevalence (%) (calculated as the number of samples where the species was recorded relative to the total number of samples for that specific water type) of the most frequently encountered alien macroinvertebrates (present in more than 3% of all samples) in the different water types: small stream (Bk), large stream (Bg), small river (Rk), large river (Rk), polder water (P) and lake (S).*

Species	Bk	Bg	Rk	Rg	P	S
Dugesia tigrina	2.8	5.7	15.4	13.7	9.3	33.9
Chelicorophium curvispinum	0.1	-	-	12.5	-	10.7
Crangonyx pseudogracilis	8.2	3.7	6.4	1.5	2.5	14.3
Dikerogammarus villosus	0.1	-	-	15.1	0.6	14.3
Gammarus tigrinus	4.2	5.0	37.2	20.3	52.8	21.4
Proasellus coxalis	1.5	0.7	6.4	0.4	6.2	3.6
Proasellus meridianus	11.9	6.4	10.3	4.4	10.6	21.4
Orconectes limosus	1.0	2.3	1.3	6.6	-	12.5
Corbicula spec.	0.3	-	1.3	6.6	-	16.1
Dreissena polymorpha	0.5	0.3	3.8	35.1	2.5	26.8
Ferrissia clessiniana	1.6	1.7	7.7	10.0	1.2	8.9
Physella acuta	11.7	6.7	6.4	3.7	6.2	19.6
Potamopyrgus antipodarum	12.6	7.7	15.4	15.5	41.0	48.2

When analysing the CCA biplot of frequently encountered species and environmental variables, the first and the second axis had an Eigenvalue of 0.449 and 0.206, respectively. The first and second axis explained 50% and 23% of the variance in species composition, respectively. SBCI, conductivity and pH explained most of the variance in the species composition and were strongly correlated with the first axis $r = 0.85$, $r = 0.24$ and $r = 0.50$, respectively. Ammonium ($r = -0.33$), conductivity ($r = -0.40$) and COD ($r = -0.30$) were negetaively correlated with the second axis (Fig. 3.2a). Alien and indigenous species could be clearly separated from each other (Fig. 3.2a). The indigenous species *Gammarus pulex* mainly occurred in small streams (high slope) with a good ecological water quality (high MMIF), whereas the alien gammarid *G. tigrinus* mainly preferred rivers with a high conductivity. Most alien species that occurred frequently in large rivers (canals) clustered together (Fig. 3.2a). The biplot of the samples and environmental variables revealed that small (Bk) and large brooks (Bg) were mainly associated with a high slope and sinuosity, whereas small (Rk) and large rivers (Rg) were associated with the SBCI (Fig 3.2b).

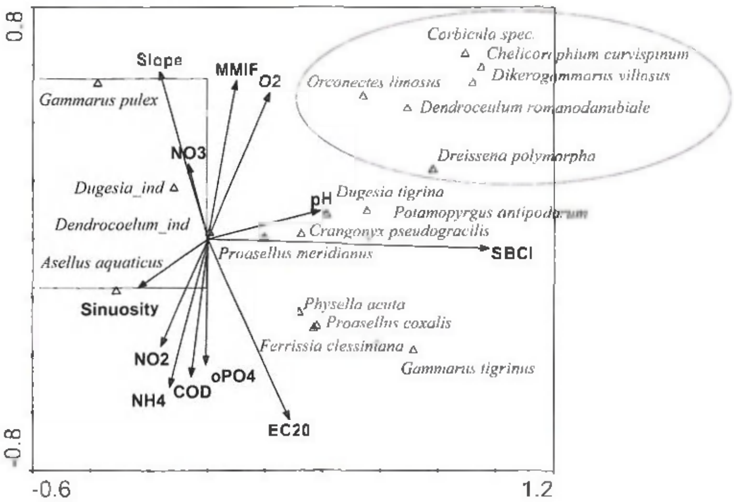


Figure 3.2a *Canonical Correspondence analysis (CCA) showing the relationship between the environmental variables (see Table 3.2a) and the most frequently encountered alien species (Table 3.3 and Dendrocoelum romanodanubiale) and indigenous (indicated by the square) species in Flanders. The circle clusters all alien species which commonly occur together in large watercourses in Flanders.*

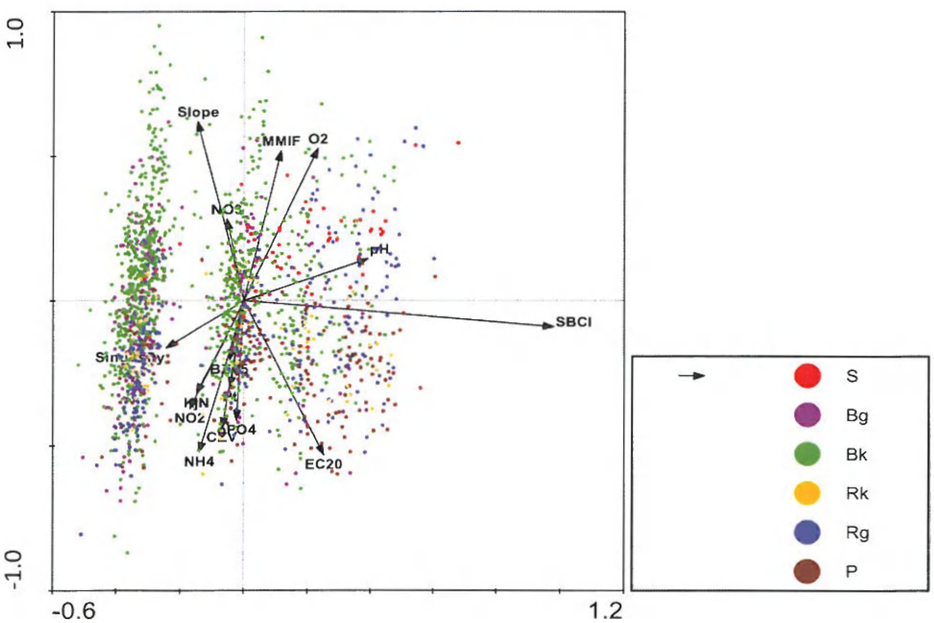


Figure 3.2b *Biplot of samples and environmental variables. For the used abbreviations of the river types see Table 3.1.*

Based on the ANOVA, a significant difference in the biocontamination could be observed between the three years (df = 2, F = 20.2, $p < 0.001$), between the differen river types (df = 5, F = 53.8, $p < 0.001$) as well as the interaction effect year * river type (df = 10, F = 3.95, $p < 0.00002$) (Fig. 3.3a). In 2009, more than 50% of the samples contained alien macroinvertebrates and there was an increase in the high and very high biocontamination classes compared to 2004 and 1999. In polder waters, the biocontamination level fluctuated and only 20 to 30% of the sites were free of alien macroinvertebrates (Fig. 3.3b). A serious increase in biocontamination was observed for large rivers and in 2009, up to 10% of the sites could be classified as severely biocontaminated (SBCI = 4), indicating that at these sites more than 50% of the taxa are alien and/or alien taxa make up more than 50% of the total abundance (Fig. 3.3c). The fraction biocontaminated small rivers remained more or less constant, but there was a shift towards higher biocontamination classes (Fig. 3.3d). Only a small increase in the number of alien macroinvertebrates was observed in small and large streams (Fig. 3.3e, 3.3f). Lakes are invaded by alien macroinvertebrates and in 2009, alien species were found in all sampled locations (Fig. 3.3g). In Fig. 3.3h, the 57 sites, (distributed over different water types) that were monitored in all three years, are compared. Similar to the graph based on all data (Fig. 3.3a), an increase in the number of sites comprising alien macroinvertebrates was observed, with mainly an increase in sites of moderate to high biocontamination.

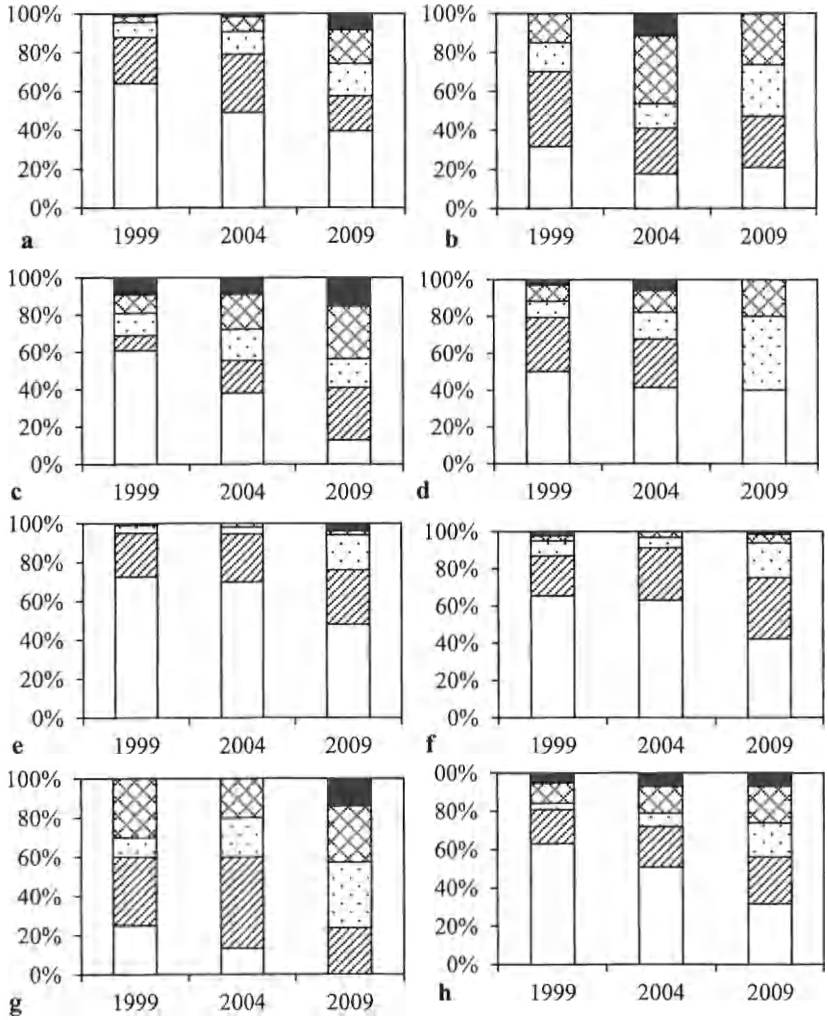


Figure 3.3 Site-specific biocontamination index for the three different years (1999, 2004 and 2009) for (a) all data, (b) polder waters, (c) large rivers, (d) small rivers, (e) large streams, (f) small streams, (g) lakes and (h) a selection of 57 sites that were monitored in each year (no biocontamination (white), low biocontamination (striped), moderate biocontamination (dotted), high biocontamination (crossed) and very high biocontamination (black)).

Based on the biocontamination maps (Fig. 3.4), an increase in the number of biocontaminated sites could be observed over the three years. In the year 1999, the hotspots for biocontamination were mainly situated in the large canals with intensive shipping in the eastern part of Flanders (Fig. 3.4a). Only five years later, the harbour of Ghent and the canal Ghent-Terneuzen became highly biocontaminated. The brackish polder waters also became a hotspot for alien macroinvertebrates since 2004 (Fig. 3.4b). Based on the biocontamination map, another general increase in the number of sites containing alien macroinvertebrates could be observed in 2009. Alien species dispersed and many previously uncontaminated large and small rivers were classified in 2009 as biocontaminated (Fig. 3.4c).

3.3.2 Relation between water quality and biocontamination

When plotting the site-specific biocontamination index (SBCI) in function of the multimetric macroinvertebrate index Flanders (MMIF), a decrease in the ecological water quality (MMIF) was observed with increasing contamination by alien species (SBCI), except for the lowest SBCI class zero (Fig. 3.5a). An SBCI of zero indicates that there were no alien species encountered, which can be the case in pristine waters that have not been colonized by aliens. However, it should be taken into account that such a low SBCI can also be caused by a water quality that is very poor and hardly supports any species (thus also no alien species). To overcome this ambiguity, data were separated into samples with a good or a bad chemical water quality based on the basic Prati index (Fig. 3.5b, 3.5c). Samples with a good chemical water quality had a high MMIF and often had low levels of biocontamination (Fig. 3.5b). In samples with a good chemical water quality, high SBCI values were usually related with low MMIF values, indicating a negative correlation between the SBCI and the MMIF ($r = 0.27$, $p = 0.002$). When considering only samples with a bad chemical water quality (Fig. 4C), there was no unambiguous relationship between the SBCI and the MMIF ($r = 0.12$, $p = 0.08$). There was a significant positive correlation between the SBCI and conductivity ($r = 0.12$, $p < 0.001$) and the SBCI and oxygen concentration ($r = 0.17$, $p < 0.001$). A negative correlation between the SBCI and environmental variables was found for: KjN ($r = 0.2$, $p < 0.001$), NH_4 ($r = 0.18$, $p < 0.001$) and Pt ($r = 0.14$, $p = 0.02$).

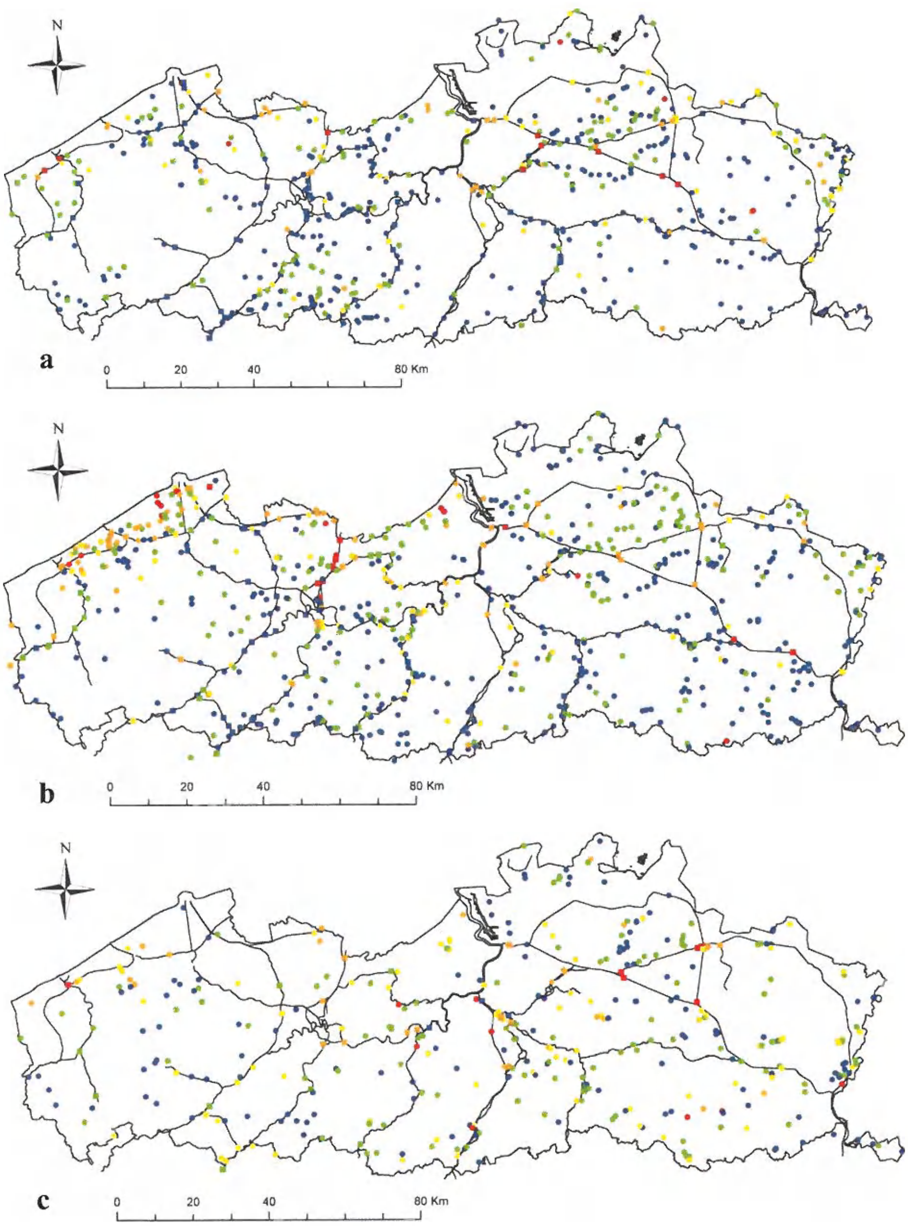


Figure 3.4 *Biocontamination map of Flanders for (a) 1999, (b) 2004 and (c) 2009 based on the site specific biocontamination index (SBCI, Arbačiauskas et al. 2008). Blue=no biocontamination, green=low biocontamination, yellow=moderate biocontamination, orange=high biocontamination and red=very high biocontamination. Circles represent sampling sites without shipping and squares indicate sampling sites with shipping.*

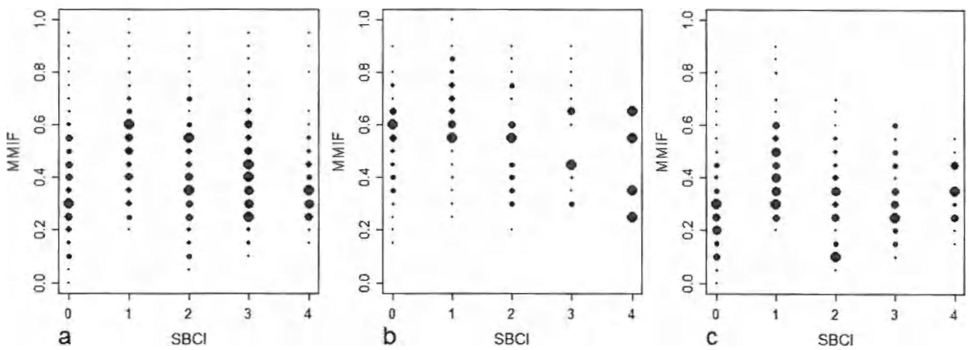


__Figure 3.5__ Site-specific biocontamination Index (SBCI) in function of the Multimetric Macroinvertebrate Index Flanders (MMIF) for (a) all data, (b) a good chemical water quality and (c) a bad chemical water quality.

An analysis of the physical-chemical parameters of the 57 selected sites, that were monitored in all three years, indicated a significant decrease between 1999 and 2009 for all measured nutrient levels NH_4 ($U = 3.372$, $p < 0.001$), BOD ($U = 4.665$, $p < 0.001$), Pt ($U = 2.794$, $p = 0.005$), KjN ($U = 6.239$, $p < 0.001$), NO_3 ($U = 2.645$, $p = 0.008$), NO_2 ($U = 2.579$, $p = 0.01$), oPO_4 ($U = 2.751$, $p = 0.03$) (Fig. 3.6), which indicates an improvement of the chemical water quality over the years.

3.3.3 Integrated model

Our habitat suitability model had, based on a three-fold cross-validation, a fair performance (CCI = 61% and $K = 0.23$) and contained two chemical variables (NH_4 and KjN) and one hydro-morphological variable (slope) (Fig. 3.7). The performance when calculated for 1999, 2004 and 2009 was also fair with CCI = 65%, $K = 0.21$; CCI = 59%, $K = 0.20$ and CCI = 61%, $K = 0.20$, respectively. When running our habitat suitability model on the measured physical-chemical field data of the VMM, there is an underestimation of 40% for 1999, an overestimation of 28% for 2004 and an underestimation of 13% for 2009 compared to the observed results (Fig. 3.7). The calculated number of samples containing alien taxa is comparable when running the habitat suitability model on the water quality data simulations of 2006 (62%) and the measured physical-chemical data of the VMM of 2006 (56%).

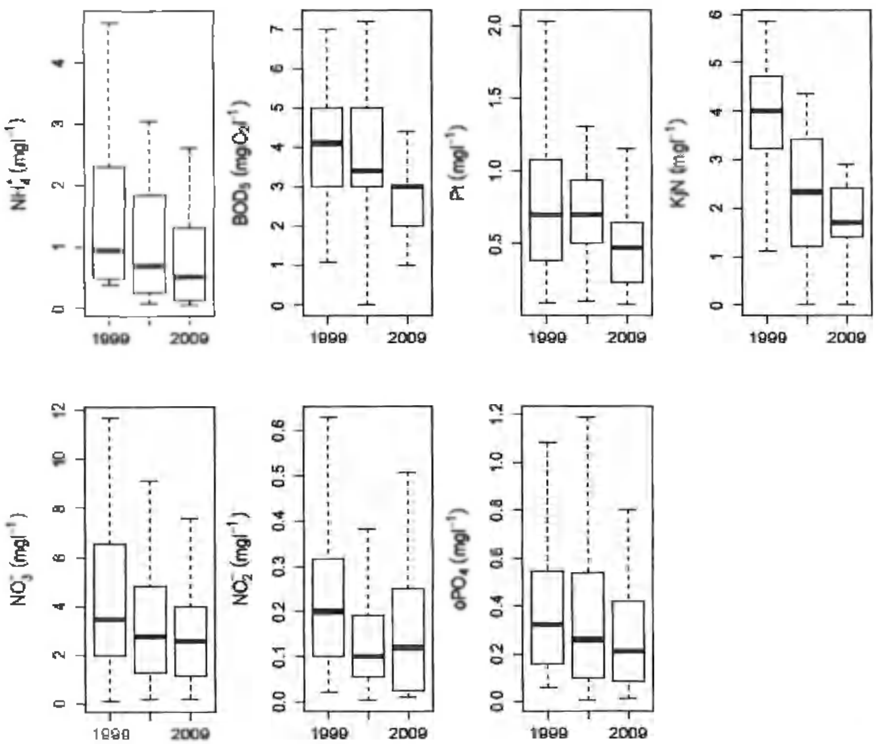


Figure 3.6 *Box- and Whisker-plots for the chemical water quality parameters of the 57 selected sites that were monitored in all three years (1999, 2004 and 2009). Ammonium (NH_4^+), biochemical oxygen demand for 5 days (BOD_5), total phosphorous (Pt), Kjeldahl nitrogen (KjN), nitrate (NO_3^-), nitrite (NO_2^-) and orthophosphate (oPO_4).*

The predictions made based on the integrated model show an increase in the number of samples containing alien macroinvertebrates in 2015 and 2027 (Fig. 3.8). Based on our integrated model, it is expected that by the year 2027, alien macroinvertebrates will be present in more than 75% of our water bodies. This could mainly be attributed to a reduction in the concentration of ammonium and Kjeldahl nitrogen, which were the only chemical parameters that could be included in the classification tree model. When running the habitat suitability model on the output of the water quality model simulations of 2006, the number of samples containing alien macroinvertebrates (62%) is in between the observed values for the year 2004 (47%) and the year 2009 (63%), indicating a reasonable prediction made by our integrated model.

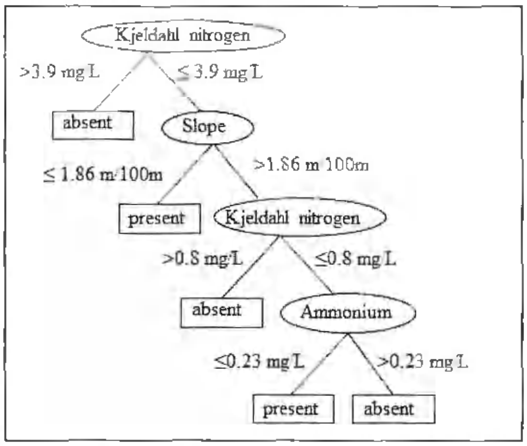


Figure 3.7 *Classification tree model based on all physical-chemical and hydro-morphological data (of the three years) indicating the habitat preference of alien macroinvertebrates.*

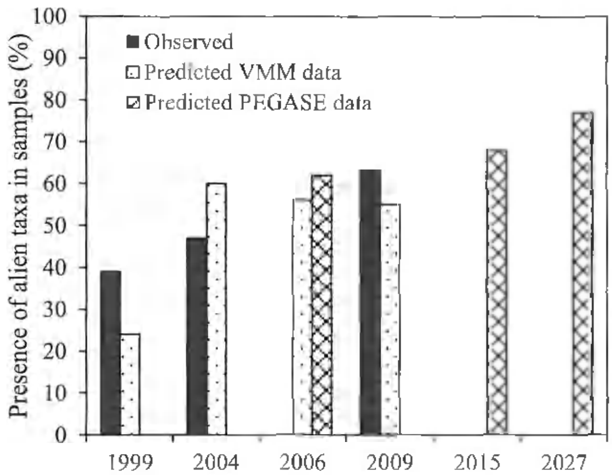


Figure 3.8 *Observed prevalence of alien macroinvertebrate taxa in the samples of 1999, 2004 and 2009 (black bars), predicted prevalence of alien macroinvertebrate taxa for 1999, 2004, 2006 and 2009, based on the developed habitat suitability model and the physical-chemical values measured by the Flemish environment Agency (VMM) (dotted) and predicted prevalence of alien macroinvertebrate taxa for 2006, 2015 and 2027, based on a the data generated by the water quality model (PEGASE) (crossed bars).*

3.4 Discussion

Most identified alien macroinvertebrates found in the samples belonged to the Crustacea (59%) and the Mollusca (31%), followed by Platyhelminthes (7%) and Annelida (3%), which is similar to recent studies conducted on the macroinvertebrate composition in large water bodies in Europe (Bernauer and Jansen, 2006; Leuven et al., 2009; Arbačiauskas et al., 2011; Labat et al., 2011; Zaiko et al., 2011). In Flanders, most crustaceans encountered in fresh and brackish water habitats are alien (Messiaen et al., 2010; Boets et al., 2011a; 2011b; 2012c). To date, 23 different alien macrocrustaceans are found in fresh- and brackish water in Flanders. More than one third of the alien macroinvertebrates encountered in Belgium originate from the Ponto-Caspian region, which is the primary donor region of alien species for many West-European countries (Bij de Vaate et al., 2002). Mainly since the opening of the Rhine-Main-Danube canal in 1992, many species, especially crustaceans and molluscs, invaded large rivers in Western Europe (van der Velde et al., 2002). Bij de Vaate et al. (2002) distinguished three different inland invasion corridors for Ponto-Caspian macroinvertebrates: a northern corridor connecting several inland lakes with the Baltic Sea, a central corridor connecting the rivers Dnieper, Vistula, Oder Elbe and Rhine and a southern corridor connecting the rivers Danube and Rhine. The interconnection of river basins has facilitated the distribution of alien species in Europe due to the disappearance of natural geographic barriers (Bij de Vaate et al., 2002). This resulted in invasion corridors for aquatic animals, that can migrate actively or passively (e.g. via ballast water and hull fouling of ships) from one geographic region to another. Based on our analysis, we found that mainly large rivers are colonised by alien species and therefore, we expect that the dense network of waterways and canals probably also facilitates the dispersal of alien macroinvertebrates in Flanders.

3.4.1 Biocontamination and environmental impact assessment

The site-specific biocontamination index (SBCI) revealed a significant increase in prevalence of alien macroinvertebrates in ten years time (from 1999 to 2009). The highest increase was observed in large rivers and in lakes (Fig. 3.3). The dominant species in these systems were mainly *Dikerogammarus villosus*, *Gammarus tigrinus*, *Dreissena polymorpha* and *Potamopyrgus antipodarum*. All these species are known to cause high ecological and/or economic impacts (e.g. Karatayev et al., 1997; Jazdzewski et al., 2004). For example the 'killer shrimp' (*D. villosus*), which arrived in 1997 in Flanders (Messiaen et al., 2010), is still spreading to the west of Europe and reached the British Isles in 2010 (MacNeil et al., 2010a).

This species shows a high feeding plasticity from detritus consumption and grazing to predation on free swimming macroinvertebrates and even fish larvae (Arbačiauskas et al., 2011). Given the fact that this species can reach high abundances, dominating the macroinvertebrate community (van Riel et al., 2006a), it is expected to have also a major impact in the British Isles on biodiversity and overall assemblage structure through competition and predation (MacNeil et al., 2010a; 2010b; 2012). Another species with a high economic as well as ecological impact is *Dreissena polymorpha*. This mussel functions as an ecosystem engineer by reducing the suspended organic matter (and thus affecting light penetration) and by providing a structurally complex habitat for other species and a refuge from predators (Ricciardi et al., 1997). This can have a positive effect on macroinvertebrate communities by enhancing the water clarity and providing habitat for species, but it is also known to cause the decline of other filter feeders or some burying organisms due to niche overlap (Ricciardi et al., 1997). In addition, this species can reach very high densities, clogging installations and pipes and in this way cost a lot of money for the removal of these dense layers of mussels (Pimentel et al., 2005).

According to Panov et al. (2009), all abovementioned species have been classified as high impact species, which were assigned as 'blacklist species' for European inland waters. Special attention should be given to these species since dispersal from Flanders to neighbouring countries is likely and in this way, Flanders can act as a donor region as well. For management purposes, species with a high level impact should be listed on an 'alert list' in a future early warning system in order to prevent further spread of alien species (Zaiko et al., 2011). Currently, there are preliminary guidelines for environmental impact assessment and classification of alien species in some European countries (Verbrugge et al., 2012) and in Belgium (Branquart, 2011). In Belgium, this classification is based on a simplified environmental impact assessment protocol and the geographic distribution of alien species. Such a categorisation provides a scientific background to prioritise actions to prevent introduction and mitigate the impact of alien species, including the improvement of the legislative framework. Until now, most attention has been given to vascular plants, fish and mammals and no alien macroinvertebrates are included. Urgent actions to include and assess the impact of alien macroinvertebrates are necessary, considering the impact that several of these invasive alien macroinvertebrates can cause. This is often time and money consuming and needs well-funded research in order to make reliable impact assessments. In this respect, not only ecologic impact assessment is important, but also the economic impact and dangers to human health should be incorporated in the final assessment.

3.4.2 Hotspots for alien macroinvertebrates

Based on the SBCI, several hotspots for alien macroinvertebrates could be identified. Large rivers and canals, harbours and brackish polder waters contained most alien species, often at high abundances, leading to a high biocontamination (Fig. 3.4). Rivers and canals are subjected to several anthropogenic pressures (e.g. intensive shipping, pollution), making them favourable habitats for alien macroinvertebrates to establish. Most European rivers are dominated by alien macroinvertebrates, which can make up to 75% of all macroinvertebrate species encountered (Bernauer and Jansen, 2006; Žganec et al., 2009; Arbačiauskas et al., 2011; Labat et al., 2011). Invasive alien species are very opportunistic, can easily cope with changing environmental conditions and often have low habitat requirements (Statzner et al., 2008). In former decades, many rivers in Europe suffered from environmental degradation and habitat deterioration. Due to stricter environmental regulations and treatment of wastewater, the water quality strongly improved, allowing macroinvertebrates to (re)colonize these niches (Den Hartog et al., 1992). Unfortunately, many of the established species are alien due to high propagule pressure caused by intensive shipping and the ability of alien species to establish easily in degraded habitats. A case study on the harbour of Ghent by Boets et al. (2011a) demonstrated that, although the chemical water quality improved significantly, mainly alien species colonised the previously degraded ecosystem. Large waterways are often suffering from environmental constraints like intensive navigation or irreversible habitat modification by hydraulic engineering (e.g. stony banks), which hamper a complete recovery. Many alien macroinvertebrates seem to have a preference for these modified habitats, which are typical for navigable waterways. Hard substrates often even promote the establishment success of alien macroinvertebrates (van Riel et al., 2006b). For example, a species that is very successful in these canals and quickly spreads in Flanders is *Dikerogammarus villosus*. This species prefers watercourses with a non-natural bank structure (e.g. concrete banks of canals) and a relatively good chemical water quality (high oxygen concentration and low nutrient levels) (Boets et al., 2010a).

Harbours, which are often situated in estuarine regions and are prone to high anthropogenic pressures and intensive international shipping, tend to be very susceptible for invasions as well (Gaonkar et al., 2010). Most invasive alien species are generalists (Statzner et al., 2008)

3.4.4 Future distribution of alien macroinvertebrates

The analysis of the chemical water quality parameters of the 57 sites, that were monitored in all three years, showed a significant decrease in all nutrient levels, together with an increase in alien macroinvertebrate presence and abundance. Alien species not only have a competitive advantage in habitats with a low ecological water quality, they also benefit from an improvement in the chemical water quality. Modelling is often used to predict the future distribution of alien species (Gallien et al., 2010). Based on our habitat suitability model that was combined with the water quality model, it is expected that by the year 2027, more than 75% of our water bodies could be colonized by alien macroinvertebrates. The main underlying reason for this is the improvement in chemical water quality resulting from a reduction in ammonium and KjN concentration due to the installation of wastewater treatment plants. Habitat suitability modelling or ecological niche modelling is one possible way to investigate the potential invasive range of species and is increasingly used in risk assessment (Jiménez-Valverde et al., 2011). These models are often based on environmental and climatic conditions (Gallien et al., 2010). Habitats that meet these environmental constraints are identified as vulnerable for invasions. In the current model, only a limited set of physical-chemical variables and slope were included because other variables were not consistently monitored by the VMM. Nevertheless, the included factors were already pinpointed in other modelling studies as being important variables determining the prevalence of macroinvertebrates (D'heygere et al., 2003).

Although our integrated model was useful to predict the future increase in alien species, the performance (based on *K* and CCI) was rather low, which could be ascribed to several factors: (1) alien species are often characterised as very opportunistic species, being able to easily cope with changes in environmental conditions (Grabowski et al., 2007); (2) alien species may not yet have spread to all suitable habitats, making it difficult to determine species-environment relationships (Stohlgren et al., 2010) and (3) alien species may interact strongly with other environmental stressors, thereby modulating their effects (Früh et al., 2012). Although we internally validated our model with part of the data, it was not possible to validate the future predictions. In this respect, Araújo and Guisan (2006) suggest that evaluation strategies should be discussed in the context of three possible uses: description, understanding and prediction. Complexity of model evaluation increases from explanation to prediction to the point, where models that simply seek to describe a given pattern may not need to be evaluated, whereas the evaluation of models aiming at prediction is desirable, but not always conceptually possible.

The introduction and establishment of alien species usually takes place via several invasion stages (Colautti and MacIsaac, 2004). Introduced species have to pass all stages successful before they are established. In this respect, it is important to notice that, although the improvement of the chemical water quality might be beneficial for the establishment of alien macroinvertebrates, other factors like biotic resistance and vectors for introduction are important elements determining the prevalence of alien species. This prevalence will probably increase in the future, but decreasing nutrient values and the construction of more natural banks could enhance the competitive advantages of indigenous species and in this way, reduce the future rate of increase in alien species establishment. Moreover, also indigenous species will still benefit from the improvement in water quality and thus their prevalence and abundance will increase as well. The highest concentrations of KjN and NH_4 were measured in small streams with a low prevalence of alien species. The reduction of these nutrient concentrations could favour the competitive advantage of indigenous species, which currently still dominate small streams. Vermonden et al. (2010) found that in urban waters in the Netherlands, indigenous macroinvertebrates were able to coexist and even dominate alien species in nutrient-poor, densely vegetated systems. It is also expected that natural systems with a good ecological water quality and a high species diversity can act as a kind of buffer against the further spread of alien species. These systems will have a higher biotic resistance and will consequently be more difficult to invade. Based on our results, there is still a lot of expansion possible in large and small streams, which currently have the lowest biocontamination levels. By improving the physical habitat as well as the chemical water quality in these natural habitats, the biotic resistance could be increased, giving indigenous species a competitive advantage. Preventing alien species from entering new habitats via a good legislation regarding the treatment of ballast water of ships, stronger regulations in the trade of alien species and drafting watch lists for alien species could help to protect natural biodiversity.

3.5 Conclusion

A serious increase in the prevalence, the abundance and the diversity of alien macroinvertebrates was observed for Flemish watercourses in the period from 1999 to 2009. The highest biocontamination levels were encountered in rivers, polder waters and lakes.

Harbours, canals, brackish and estuarine regions were identified as hotspots for alien species introduction and establishment. The improvement of the chemical water quality in habitats, which were degraded in former decades, favoured the establishment of alien species. For waters with a good chemical water quality, a significant negative correlation was observed between the SBCI and the ecological water quality (expressed as MMIF). Habitats with a low diversity due to environmental degradation seemed to be favourable for the establishment of alien macroinvertebrate species. Based on an integrated model, an increase in the prevalence of alien macroinvertebrates is predicted as a result of the improvement of the chemical water quality due to the installation of planned wastewater treatment plants. A good monitoring program prioritising vulnerable habitats in combination with a practical method to assess the impact of invasive alien species can help invasive species management. To prevent the further worldwide introduction and spread of alien species several management measures, such as ballast water control, regulations regarding the trade of aquatic alien species and insight in species ecology will be necessary.

Chapter 4: Case study 1: Using long-term monitoring to investigate the changes in species composition in the harbour of Ghent (Belgium)

Adapted from:

Boets P., Lock K., Goethals P.L.M. (2011a). Using long-term monitoring to investigate the changes in species composition in the harbour of Ghent (Belgium). Hydrobiologia 663: 155-166.

Chapter 4: Case study 1: Using long-term monitoring to investigate the changes in species composition in the harbour of Ghent (Belgium)

Abstract

The macroinvertebrate community of the harbour of Ghent was studied by analysing 135 samples taken at different sampling locations from 1990 until 2008. The results showed that the current Crustacea and Mollusca communities are mainly represented, in terms of abundances, by alien species. In total, seven alien and four indigenous crustacean species were found. Mollusc diversity was higher, with a total of 14 species, four of which were alien. Macroinvertebrate diversity was very low at the beginning of the 1990s, but increased due to the improvement of the chemical water quality achieved by sanitation and stricter environmental laws. This is reflected by the dissolved oxygen concentration, which increased from an average of 2 mg l^{-1} to an average of 9 mg l^{-1}, allowing more sensitive species to establish. Since 1993, the number of alien taxa has augmented, whereas the number of indigenous taxa has remained stable. The improvement of the chemical water quality and the simultaneous increase in total number of species was also reflected in an increase of the Multimetric Macroinvertebrate Index Flanders, which is used to assess the ecological water quality in Flanders. Due to intensive international boat traffic and the low natural species diversity, the harbour of Ghent is highly vulnerable for invasions. Stronger regulations and a better understanding about the contribution of shipping, shortcuts via artificial water ways (e.g. canals), habitat degradation and environmental pollution are required to reduce the further spread of invasive alien species.

4.1 Introduction

In Flanders, numerous canals have been constructed since the seventeenth century for industrial and economic activities. One of these canals is the canal Ghent-Terneuzen, connecting the town of Ghent with the Scheldt estuary. This canal is of great economic importance to Ghent because it provides an indirect connection between the harbour of Ghent and the North Sea (Zajac and Deckmyn, 2008). Continuous pollution in former decades led to severe degradation of the water quality of the canal Ghent-Terneuzen. However, due to sanitation and stricter environmental laws, canals and rivers with a bad water quality substantially improved during the last fifteen to twenty years allowing recolonisation (MIRA-T, 2008).

Invasions usually proceed through a number of successive stages: after a successful introduction, a species will try to adapt to its new environment; in the next stage, the species will reproduce and complete its life cycle and finally, it will disperse and possibly become dominant (Kolar and Lodge, 2001). Many species establishments are reflections of changes caused by man, rather than being agents of change themselves. In such cases, biological invasions can be seen as indicators of environmental change (Vitousek et al., 1996). Sometimes, new niches are exploited because equivalent indigenous species are not present. Due to pollution and habitat degradation in former times, species diversity was low in many running waters in Flanders. Since the improvement of the water quality of these waters, niches have been restored, giving mainly alien species the opportunity to colonize these empty niches (Den Hartog et al., 1992). Although the opportunities for species introductions have increased, not all alien species are successful in their new environment. Among macroinvertebrates, crustacean and mollusc species are considered to be the most important and successful invaders (van der Velde et al., 2002; Devin et al., 2005a).

In order to assess the impact of alien species on indigenous macroinvertebrate assemblages, lab experiments are often used (Dick et al., 2002; Boets et al., 2010). However, although these experiments may give valuable information about the autoecology of alien species, it is difficult to convert these results to field situations. Therefore, long-term data sets of field observations can be used to unravel factors determining the success of biological invasions and their impact on indigenous species assemblages (Zettler and Daunys, 2007; MacNeil et al., 2009). In the present study, we focussed on the composition and diversity of the benthic macroinvertebrate community of the harbour of Ghent, a former heavily polluted water body.

Based on a twenty-year survey, possible changes in species composition were investigated. Furthermore, the relationship between the changes in water quality and the establishment of alien macroinvertebrates was examined in order to gain insight into drivers of changes in species composition.

4.2 Methods

4.2.1 Study area

The canal Ghent-Terneuzen connects the towns of Ghent (Belgium) and Terneuzen (The Netherlands) and has a length of approximately 31 km of which 17 km on Belgian territory. This canal provides a connection between the harbour of Ghent and the Scheldt estuary (Fig. 4.1). The canal has an average width of 200 m and a depth of 13.5 m. The water management (VMM) aims to counteract the salinisation with brackish water entering from the Scheldt estuary and thus the canal is fed with freshwater from the rivers Scheldt and Leie. Although there is a constant flow of 20 m^3 h^{-1} minimizing the increase in salinity, the development of a salt tongue at the bottom of the canal is inevitable, mostly during dry summers (Seys et al., 1990). Important sources of pollution are industrial plants and manufactories located along the canal, which caused severe degradation during the 1960s. At the end of the 1980s, high peak values of several heavy metals, PAHs and PCBs could be found, particularly in the contaminated sludge (Seys et al., 1990). Since the beginning of the 1990s, great efforts were made to reduce these peak values and to obtain a better water quality (MIRA-T, 2008).

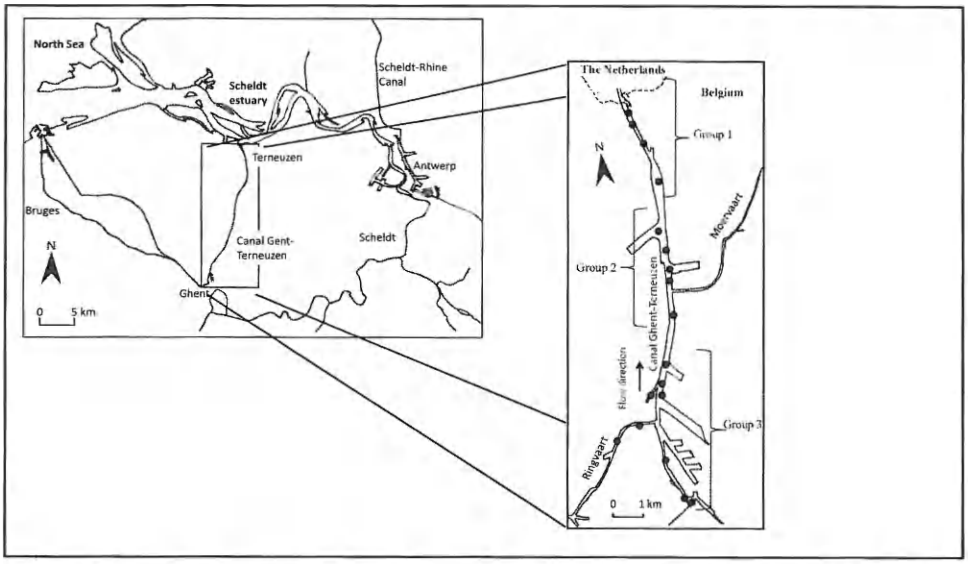


***Figure 4.1** Location of the study area showing sampling sites (n = 18, black dots) and the division in groups of sampling sites based on their salinity in the harbour of Ghent (group 1 = brackish water, group 2 = slightly brackish water, group 3 = fresh to slightly brackish water).*

4.2.2 Sampling and data analysis

The data analysis was based on the samples taken by the VMM. The samples were taken via artificial substrates as described by Gabriels et al. (2010). This sampling method is useful for deep waters like canals where handnet sampling is almost impossible (Gabriels et al., 2010). Three replicates of artificial substrates, which consist of polypropylene bags (5L) filled with bricks of different sizes, are left in the water for a period of three to four weeks after which they are retrieved. In this way, species can colonize the substrates. The database of the VMM contained information about the abundances of macroinvertebrates that were identified at family or genus level, depending on the identification level needed for the calculation of the biotic index (Gabriels et al., 2010). Besides biological data, also physical-chemical data measured by the VMM were available and used in our analysis.

Extra sampling was done in the harbour of Ghent in March 2009 in order to search for Talitridae and Mysidae, two families of macrocrustaceans that are often missed when artificial substrates are used. These samples were not quantitatively gathered and therefore not included in the average abundance calculations.

In the present study, we focussed on Crustacea and Mollusca because most alien species belong to these groups. Only Crustacea were identified to species level using several identification keys (e.g. Eggers and Martens, 2001), since these species could not be identified based on the database of the Flemish Environment Agency. Based on this database, however, it was possible to determine the alien and some indigenous molluscs, since the identified alien genera were only represented by one species. In total, 135 samples were studied from 18 different locations of which 16 are situated along the canal Ghent-Terneuzen and two on the Ringvaart (a canal around the city of Ghent that is directly connected with the canal Ghent-Terneuzen) (Fig. 4.1). Samples were available from 1990 to 2008. However, not every location was sampled each year. In 1990 and 2007, only one site was sampled thus giving some deviating macroinvertebrate abundance and species composition values. The average abundance was calculated as the total abundance per year divided by the number of sampling stations monitored in that year. The biological water quality was assessed for the period 1989-2008, based on the Multimetric Macroinvertebrate Index Flanders (MMIF) (Gabriels et al., 2010). The calculation of the MMIF without alien species was based on the same metrics except that all alien species were excluded from the analysis.

For the physical-chemical analysis, 2810 measurements from 18 different sampling locations spread over a period from 1989 until 2008 were taken into account. Standard parameters (DO, pH, phosphate and nitrate) were analysed in accordance to ISO 17025.

Non-parametric tests were carried out for all analyses, since these are generally applicable and do not have to meet the requirements of parametric tests. Since it is known that conductivity is influenced by salinity, the correlation between both was tested using a Spearman Rank correlation coefficient. Conductivity is seen as an important variable determining the presence or absence of macroinvertebrates (Gabriels et al., 2007; Boets et al., 2010a). Differences in species conductivity preferences were tested with species as independent and conductivity as dependent variables with Kruskal-Wallis ANOVA, and post-hoc multiple comparisons of mean ranks (Conover, 1980). Any possible differences in MMIF with and without alien species was tested using the Wilcoxon Matched Pairs Test. The significance level for hypothesis testing was set at $p \leq 0.05$. All statistical analyses were performed using Statistica 7.0 (Statsoft Inc., 2004).

4.3 Results

4.3.1 Chemical and biological water quality

The chemical as well as biological water quality was tested in order to gain insight in the relationship between changes in both water quality parameters and the establishment of alien species. Over the last 20 years, the chemical water quality has improved. The dissolved oxygen increased gradually from an average of 2.0 mg l^{-1} in 1989 to 9.1 mg l^{-1} in 2008 (Fig. 4.2a). Additionally, the decrease in orthophosphate concentration from 1.5 to 0.5 mg l^{-1} further indicated the improvement of the water quality of the canal Ghent-Terneuzen (Fig. 4.2b). In contrast, the nitrate concentration increased from 1.7 to 5.3 mg l^{-1} (Fig. 4.2c), whereas pH was neutral to alkaline and increased only slightly from 7.5 to 7.9 (Fig. 4.2d).

A strong positive correlation between conductivity and salinity could be observed ($r = 0.83$, $p = 0.03$), strong fluctuations in both conductivity (Fig. 4.3a) and in salinity (Fig. 4.3b) were closely related. However, this was not surprising, as conductivity is partially determined by salinity. In the canal Ghent-Terneuzen, a slight north-south gradient of salinity could be found. Sites closest to the Scheldt estuary were brackish, whereas sites closest to Ghent were almost freshwater as the sampling by the VMM confirmed. Due to this gradient, the samples were clustered in three groups based on similarities in salinity (Fig. 4.1). Group 1 consisted of four sampling sites having brackish water conditions, group 2 consisted of five sampling sites having intermediate salinity levels, whereas group 3 consisted of 9 sampling sites situated within the slightly brackish to freshwater part of the canal. This division in groups was made to be able to detect changes in yearly average salinity related to temporal or spatial differences. Besides the spatial differences in salinity levels, temporal differences in salinity between the groups and the average were obtained (Fig. 4.3a and 4.3b). Peaks in conductivity in 1997 and 2004 were congruent with peaks of salinity. These peaks could be observed in all groups and were different from conductivity and salinity levels of other years.

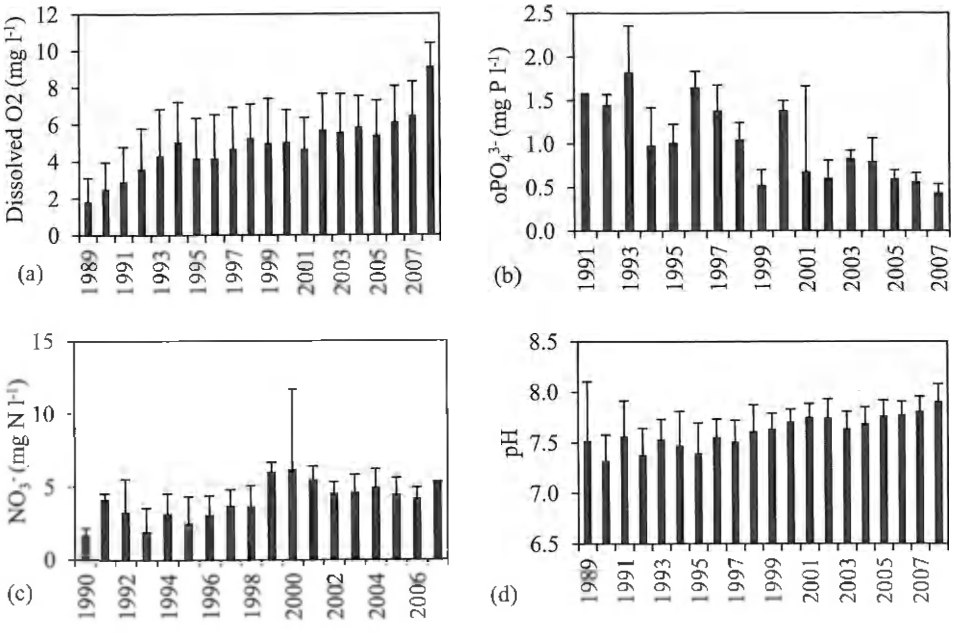


Figure 4.2 Physicochemical characteristics of the harbour of Ghent. Annual average values (± 1 SD) from 1989 to 2007 are shown for (a) dissolved oxygen, (b) orthophosphate, (c) nitrate and (d) pH.

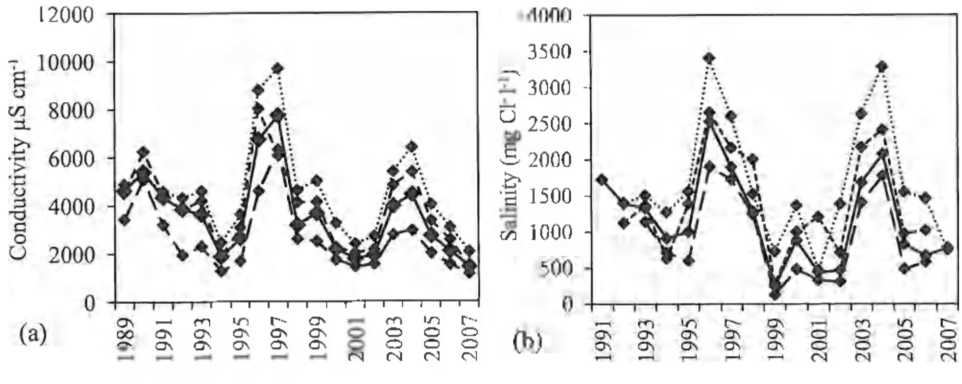


***Figure 4.3** (a) Average conductivity and (b) average salinity in the harbour of Ghent. The average for all sampling sites and the average per group of sampling sites according to the geographic location and the salinity (group 1 = brackish water, group 2 = slightly brackish water, group 3 = fresh to slightly brackish water) (···· group 1; --- group 2; – –group 3; ——average).*

With chemical improvement of the water quality, the biological water quality improved as mirrored by the MMIF (Fig. 4.4). The average MMIF increased during time and some relatively sensitive taxa appeared in the waters (Fig. 4.4, Table 4.1). However, the water quality still remained far below the good water quality (MMIF $\geq$ 0.7) as premised by the European Water Framework Directive, and the index value significantly (p = 0.04) decreased further, when alien species were excluded from the analyses.

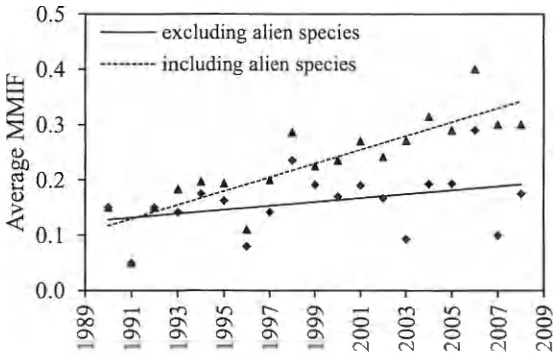


Figure 4.4 *Yearly average values of the Multimetric Macroinvertebrate Index Flanders (MMIF) (as described by Gabriels et al. (2010)) calculated when including (squares) and excluding alien species (circles) from 1990 to 2008.*

Table 4.1 *Overview of most sensitive taxa (n = 10) found in the harbour of Ghent, their tolerance score, as well as the year the species was first recorded in the harbour of Ghent. The tolerance scores range from 1 = non-sensitive to pollution to 10 = very sensitive. Alien species are shown in bold.*

Taxa	Phylum/Order	Tolerance score	First record
Ferrissia clessiniana	Mollusca	7	2001
Acroloxus lacustris	Mollusca	6	2001
Armiger	Mollusca	6	2005
Gyraulus	Mollusca	6	2001
Segmentina nitida	Mollusca	6	1999
Valvata	Mollusca	6	2006
Caenis robusta	Ephemeroptera	6	2006
Cloeon dipterum	Ephemeroptera	6	1998
Coenagrion puella	Odonata	6	2000
Ischnura elegans	Odonata	6	2001

4.3.2 Macroinvertebrate community

In total, 11 different macro-crustacean species, seven of which were alien, were found in the harbour of Ghent from 1990 to 2008 (Table 4.2). During the first three monitoring years (1990, 1991 and 1992), the macro-crustacean community consisted of only one indigenous species: *Asellus aquaticus*. Since 1993, the alien amphipod *Gammarus tigrinus* was recorded and only a few years later (since 1996), *G. tigrinus* reached higher abundances than *A. aquaticus* and became the dominant species. Since 1998, a third species, *Palaemon macrodactylus*, occurred. During the first years of its occurrence, this species was only present in the downstream part of the canal close the Dutch border. After three years, the species was present almost 10 km upstream and another two years later, it occurred in the canal near the centre of Ghent. A species that arrived almost simultaneously with *P. macrodactylus* was *Rhithropanopeus harrisii*. *R. harrisii* colonised the harbour of Ghent much faster than *P. macrodactylus* and was already present in 1999 from the Dutch border to the centre of Ghent. There was a significant difference in salinity preference between *G. tigrinus* and *P. macrodactylus* ($p = 0.01$), between *A. aquaticus* and *R. harrisii* ($p = 0.007$) as well as between *A. aquaticus* and *P. macrodactylus* ($p < 0.0001$). *A. aquaticus* and *G. tigrinus* occurred at lower salinities, whereas *P. macrodactylus* and *R. harrisii* preferred higher salinities. Nevertheless, all species occurred at the whole salinity range (from the city of Ghent to the border to the Netherlands), but sometimes at lower densities (< 10 individuals per sample). After 1998, several additional indigenous as well as alien Crustacea species occurred, albeit in low numbers (Table 4.2). One individual of *D. villosus* was recovered for the first time in 2005 in the Ringvaart. In 2008, this species reached high abundances (> 300 individuals per sample) at the sampling point closest to the Dutch border. During an extra sampling effort in March 2009, another alien amphipod (*Orchestia cavimana*) was found in very large numbers (> 200 Individuals m^{-2}) under rip-rap, litter and other debris near the border of the canal. Because information about the first occurrence of this species in the canal was lacking and our samples were not quantitatively gathered, this species was not included in the species assemblage analysis. When only macrocrustaceans (class Malacostraca, superorder Peracarida and Eucaridea) were taken into account, the average diversity increased over the years from one in 1990 to five species in 2008. Most of the recently established Crustacea are aliens. In terms of average abundance, indigenous Crustacea fluctuated around the same number as the average abundance of alien Crustacea (Fig. 4.5).

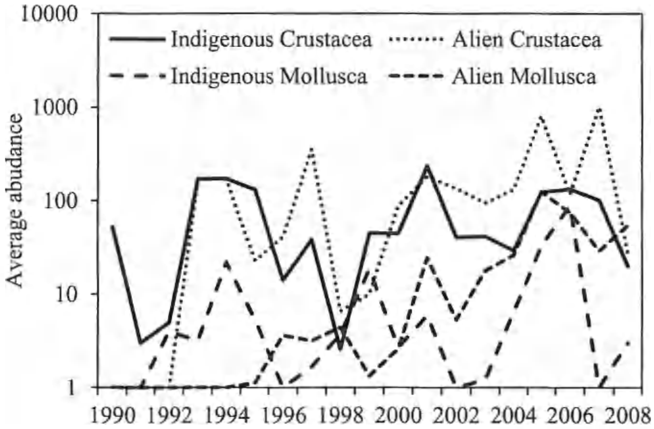


Figure 4.5 *Average abundance of sampling sites (logarithmic scale) of indigenous and alien Crustacea and Mollusca in the harbour of Ghent from 1990 to 2008.*

The Mollusca community was more diverse compared to the crustacean community with a total of 14 different species recorded from 1990 to 2008, four of which were alien (Table 4.2). During the first three monitoring years, no mollusc species were present. *Dreissena polymorpha* was the first alien species to colonise the harbour of Ghent in 1995. Since 2005, *D. polymorpha* was abundantly present together with *Potamopyrgus antipodarum* and *Physella acuta*, two other alien species. The abundance of the alien species *Ferrisia clessiniana* remained low compared to the other alien mollusc species. Maximum number of species within the mollusc community was reached in 2006 with six indigenous and four alien species. The abundance of indigenous Mollusca fluctuated strongly, while the abundance of the alien molluscs gradually increased since their occurrence in 1995 (Fig. 4.5).

Table 4.2 *Annual average abundance of Crustacea (n = 11) and Mollusca (n = 13) from 1990 to 2008. Abundance was calculated by dividing the total abundance by the number of sampling sites per year. Alien species are shown in bold.*

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
CRUSTACEA																			
Crangonyx pseudogracilis												0.07							
Dikerogammarus villosus																0.07			50
Gammarus tigrinus				169	168	22	40	354	4.0	5.8	76	172	110	23	84	796	79	1000	11
Corophium multisetosum																0.07			
Corophium lacustre																	0.20	100	3.0
Asellus aquaticus	51	2.0	4.0	170	171	130	13	37	1.6	44	44	233	40	40	28	123	132		16
Proasellus coxalis										0.17									
Neomysis integer															0.57				
Palaemon macrodactylus									1.57		0.20	0.47	15	48	39	0.87	33		
Eriocheir sinensis										0.17									
Rhithropanopeus harrisii										2.83	11	8.2	8.7	23	7.3	5.6	4.6	7.0	5.0
MOLLUSCA																			
Acroloxus											0.1	0.07			1.6	6.7	10		
Armiger																8.1	3.4		
Bithynia					0.13										1.9	16	70		
Dreissena polymorpha						0.13	2.6	2.2	3.4		0.4	5.2	0.67	3.1	13	39	25	25	20
Ferrissia clessiniana												0.26		0.21	0.43	0.4	9	1	
Gyraulus												0.13					4		
Lymnaea												0.07							
Physa				2.2	21	4.4		0.67	1.1	18	1.6	4.7		0.21	1.9				
Physella acuta																17	4		3
Pisidium									1.6										
Potamopyrgus antipodarum										0.33	1.2	18	3.7	14	12	67	36	2	32
Sphaerium										0.17					0.14	1.7			
Valvata																	0.6		2

When analysing the total macroinvertebrate community, Crustacea and alien Crustacea represented on average 55 ± 33% and 40 ± 28%, respectively, of the total macroinvertebrate abundance. At the beginning of the1990s, the pollution tolerant taxa Oligochaeta and Diptera, were still present at high densities (> 1000 individuals per sample), but as the water quality improved, their abundance decreased gradually. The average number of taxa of the total macroinvertebrate community assessed via artificial substrates increased from five taxa in 1990 up to 13 taxa in 2006 (Fig. 4.6). However, whereas the average number of indigenous taxa remained stable, the average number of alien taxa increased year by year since 1993 to an average number of five alien taxa in 2007.

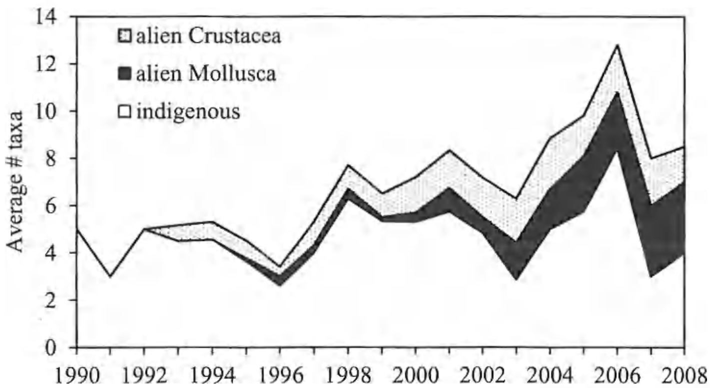


Figure 4.6 *Average number of taxa for indigenous macroinvertebrates (white area), alien Mollusca (black area) and alien Crustacea (dotted area) per year from 1990 to 2008.*

4.4 Discussion

The macroinvertebrate community of the harbour of Ghent showed a development towards higher taxa richness during the time period of our study. At the beginning of the 1990s almost no species occurred except for some pollution tolerant taxa like Chironomidae, Asellidae, *Helobdella* and Tubificidae. Nowadays, more different taxa occur, including many alien species. Alien Crustacea represented about 50% of the total crustacean abundance of the harbour of Ghent. This is comparable with the results obtained in the rivers Meuse (Josens et al., 2005), the Rhine (van der Velde et al., 2000; Bernauer and Jansen, 2006) as well as the Moselle (Dhur and Massard, 1995), where alien species dominate the crustacean community.

Gammarus tigrinus, originating from North-America, was the first to colonize the harbour of Ghent. *G. tigrinus* shows a high tolerance towards different/varying environmental conditions allowing the establishment and maintenance of stable populations in fresh as well as brackish waters. The species is now widely distributed in rivers, canals and ponds throughout Flanders (Boets et al., unpublished data, chapter 2). Two more crustacean species originating from North-America were introduced only a few years later. However, besides *Proasellus coxalis*, also *Crangonyx pseudogracilis* was not able to establish a stable population in the harbour of Ghent and was only recorded in one sample in 2001. MacNeil et al. (2000) found that *C. pseudogracilis* and *Gammarus* sp. are able to survive under the same physicochemical conditions, but that probably other factors including biotic ones play a role in the exclusion of *C. pseudogracilis* in *Gammarus* sp. dominated rivers. Laboratory experiments showed that *C. pseudogracilis* suffered heavily from predation by *G. tigrinus* (Dick, 1996). Therefore, intraguild predation, coupled with the ability of *G. tigrinus* to adapt easily to changing environmental conditions could have favoured the latter one. Another alien species restricted to brackish and marine environments, but being quite tolerant towards low levels of temperature and salinity, is *Palaemon macrodactylus*. This species, originating from Southeast Asia, was recorded for the first time in Belgium in 2003 from the harbour of Antwerp (Soors et al., 2010) and in 2004 from the harbour of Zeebrugge (d'Udekem d'Acoz et al., 2005). Similar to *Rhithropanopeus harrisii*, the larvae of this species were most likely transported via ballast water of ships. The first record of occurrence in Flanders showed a high abundance of the species, therefore, it is not surprising that the species was already present in 1998 as found at our sampling sites closest to the Dutch border. Although the first European occurrence of *P. macrodactylus* was recorded in 1999 (Spain; Cuesta et al., 2004), the species was already present in Flanders one year earlier. d'Udekem d'Acoz et al. (2005) stated that *P. macrodactylus* is most likely to occur in the mesohaline parts of estuaries, albeit this species was also recorded in the upstream part of the canal in water with a low salinity (1.9‰), where it reached high abundances (> 100 individuals per sample) in 2006. According to Ashelby et al. (2004), *P. macrodactylus* is known to be present in water with fluctuating salinities as in San Francisco Bay, but can also be found there in freshwater habitats. According to our results, this species invaded the canal at the most favourable niche (downstream) and migrated slowly upstream with an average of four kilometres per year. Dedecker et al. (2006) indicated that the upstream migration rate of the amphipod *Gammarus pulex* is higher in rivers with a low stream velocity. The low stream velocity of the canal Ghent-Terneuzen could have favoured the upstream migration of *P. macrodactylus*. Monitoring of the Thames showed that *P. macrodactylus* was possible to co-occur with the indigenous *P. longirostris* in British estuaries, potentially occupying overlapping ecological niches (Worsfold and Ashelby, 2006). Despite the co-occurrence of *P. macrodactylus* with other carrideans, a dietary overlap is possible with species of the same size sharing similar resources. Together with *R. harrisii*, *P. macrodactylus* can have a negative impact on the abundance of other macroinvertebrates.

During the extra sampling effort in 2009, *Orchestia cavimana* was found under stones and organic debris near the border of the canal. Although this species was not present in the samples of the Flemish Environment Agency, it can be assumed that it was already established for a long time. *O. cavimana* hides under stones and rip-rap along rivers banks and is seldom sampled with artificial substrates. Another family that is often missed when using artificial substrates is Mysidae. *N. integer*, a indigenous species of Mysidae, was sampled only once, because the species is a fast swimmer which occurs in swarms near the bottom of the river during daytime.

The crustacean species that most recently invaded the canal was the Ponto-Caspian species *Dikerogammarus villosus*. Since the opening of the Main-Danube canal in 1992, many species originating from the Ponto-Caspian area invaded rivers in Germany, the Netherlands and also Belgium. Especially in the canals situated in the east of Flanders (Belgium) many Ponto-Caspian species were found (Messiaen et al., 2010), including *D. villosus*. The establishment of the Ponto-Caspian species *D. polymorpha* (present in the harbour of Ghent since 1995) prior to the introduction of *D. villosus* could have favoured the establishment of latter one. Ricciardi et al. (1997) showed that organic enrichment caused by mussel deposition, together with the development of spatially complex surfaces, acting as refugia, can increase the abundance of amphipod communities. As both species originate from the same area and co-evolved over a long period of time, this association fits the Invasional Meltdown Theory (Simberloff and Von Holle, 1999). In this way, non-indigenous species can facilitate one another's invasion and increase the probability of a successful establishment. Once a species is adapted to the local environmental conditions and dominantly present, it can have an impact on the macroinvertebrate community causing a general decline in diversity and abundance of the natural system (van der Velde et al., 2000; van Riel et al., 2006a). *D. villosus* is an euryhaline species and a very successful invader throughout Europe (Bruijs et al., 2001, Bij de Vaate et al., 2002; Bollache et al., 2004). Due to the introduction of *D. villosus*, the abundance of *G. tigrinus* decreased enormously, especially at the sampling site closest to the Dutch border, from 2007, when it was the most abundant species at this site to

2008, where it was absent. This may indicate that *G. tigrinus* was displaced by *D. villosus* via intraguild predation. Lab studies showed that *D. villosus* predates on indigenous as well as alien species (Dick and Platvoet, 2000; Dick et al., 2002; Boets et al., 2010a). On the other hand, *D. villosus* is able to collect, consume and digest micro-algae as indicated by Platvoet et al. (2006), although the results showed that not adults but juveniles had algae in their gut. Gammaridae used to be classified as shredders feeding with dead organic material and plant material with some recognition of omnivory (MacNeil et al., 1997). Results of recent research based on stable isotope analysis ($\delta^{15}N$) and numerous lab experiments indicated that Gammaridae are opportunistic omnivores predating also on species belonging to the same guild. Therefore, being generalists probably contributes to the success of these invasive amphipods.

Drastic changes of the macroinvertebrate community were observed when comparing our results of the macroinvertebrate diversity with those of a study conducted in the 1980s about the macrozoobenthos of the canal Ghent-Terneuzen (Seys et al., 1990). Their study revealed that the macroinvertebrate community in the Belgian part of the canal was dominated by Oligochaeta and that no other taxa occurred. Only on Dutch territory, some other taxa like Asellidae, Polychaeta and Chironomidae were present. If we compare this to the most recent results, we do not only see an increase in the number of taxa belonging to the Crustacea and Mollusca, which include all alien species, but there is also an increase in the number of taxa belonging to the Diptera, Hirudinea, Odonata and several other taxa. This increase is mainly due to the improvement of the chemical water quality, which is demonstrated by the changes in oxygen content and orthophosphate. Where in 1989, the average oxygen content fluctuated around 2.0 mg l^{-1}, far below the limit allowing a healthy and diverse macroinvertebrate community, the oxygen concentration increased to a maximum of 10.8 mg l^{-1} in 2008. Conductivity strongly fluctuated, primarily due to changes in salinity: the high peaks of conductivity always co-occurred with high peaks in salinity and are probably caused by dry and hot summers when the inflow of fresh water diminished. With the improvement of the water quality, not only some indigenous species colonized the new vacant niches, but also many alien species established. This phenomenon was already observed earlier in the river Rhine, where after the catastrophic environmental Sandoz accident, populations of *Chelicorophium curvispinum* and *Corbicula* sp. reached a dominant position within two years (Den Hartog et al., 1992). Although at an early stage the degradation of the canal could have eased the invasion by alien species, the improvement of the water quality during the last 15 years made it possible for some alien species to establish stable populations with high densities. Many alien macroinvertebrates are seen as pollution tolerant species (e.g. *Gammarus tigrinus*). However, as the water quality improved, the number of alien species increased. This seems to go against the traditional idea that degradation of the habitat in combination with pollution favours the success of alien species (e.g. Grabowski et al., 2007). However, when pollution levels become too high (e.g. orthophosphate > 4.7 mg l^{-1}), (alien) species may not be able to survive.

When assessing the water quality based on the macroinvertebrate community, the MMIF score generally increases when including alien species, whereas the MMIF did not increase when excluding these alien species. Thus alien species seems to have an influence on the outcome of the water quality assessment. Also Gabriels et al. (2005) found that alien species can have an influence on the calculation of the Belgian Biotic Index. Therefore, they propose the use of a semi-fixed taxa list, including a tolerance class for each taxon in order to avoid inconsistencies in the calculation of the Belgian Biotic Index. We should be aware that when invasive species are able to colonise low degraded rivers with a good water quality and a high abundance of indigenous taxa, they may cause a decline in the diversity resulting in lower values of biotic indices (MacNeil et al., 2013). However, the increase in the biological water quality is in this case not caused by a decrease of the chemical water quality. It is advisable to make use of alternative methods, such as modelling techniques, to assess their impact on local species assemblages. Another alternative would be to use the site-specific biocontamination index next to the traditional biotic indices, as this could indicate sites which are dominated by alien species.

In conclusion, the macroinvertebrate species composition of the formerly polluted and degraded harbour of Ghent changed due to the improvement of the chemical water quality and the introduction of alien species. Due to the increasing trade and the inland location of the harbour of Ghent, more alien species are to be expected. Generally, brackish water ecosystems, which tend to have a low species diversity, in combination with intensive boat traffic, have a high potential for biocontamination. Moreover, the harbour of Ghent and Flanders in general, can act as a secondary source of dispersal throughout Europe. Therefore, it is advisable to monitor and control, via stronger regulations for shipping and control on trade, the further spread of invasive alien species in European waters.

Chapter 5: Case study 2: Macroinvertebrate composition in brackish polder waters

Adapted from:

Boets P., Lock K., Goethals P.L.M. (2011b). Shifts in the gammarid (Amphipoda) fauna in brackish polder waters of Flanders (Belgium). Journal of Crustacean Biology 31: 270-277.

Chapter 5: Cast study 2: Shifts in the gammarid (Amphipoda) fauna in brackish polder waters

Abstract

The macrocrustacean community of brackish polder waters in Flanders was investigated based on a twenty year survey comprising 430 biological samples taken at 218 different locations. A clear shift in the gammarid community could be observed. After its introduction, the alien species *Gammarus tigrinus,* originating from North-America, reached high abundances and became widely spread in the polder waters within a few years. Simultaneously, a decrease in the prevalence of the indigenous brackish water gammarids *G. duebeni* and *G. zaddachi* occurred. However, at the same time a decrease in the salinity of the polder waters also was observed. Uni- and multivariate data analysis revealed a clear difference in the environmental preferences of *G. tigrinus, G. duebeni,* and *G. zaddachi*. The alien species preferred lower salinities, lower orthophosphate concentrations, and a higher oxygen concentration compared to the two indigenous species. Besides the decrease in prevalence of the indigenous gammarids, a decrease was also observed in prevalence of two other indigenous brackish water crustaceans: *Palaemonetes varians* and *Neomysis integer*. It appears that the decrease in salinity is the most important factor causing the decline of the indigenous gammarids and not the introduction of the alien species *G. tigrinus*.

5.1 Introduction

Although alien species can cause major shifts in community composition, there is no consensus about the factors that explain their success in brackish water ecosystems. According to Wolff (1999), the macroinvertebrate diversity in brackish waters is usually low and the many empty niches that are present can be colonised by alien species. The low species diversity is well illustrated by the classical curve of Remane (1958), where the lowest species richness falls in the salinity range from 5 to 10 psu and most species living within this range are typically mixohaline. However, Ricciardi and MacIsaac (2000) and Jazdzewski et al. (2004) suggest that species diversity is not an important factor to resilience against species introductions, but that aquatic invasions are mediated more by dispersal opportunity and the favourability of the abiotic habitat conditions. Nehring (2006) pointed out that low indigenous species richness in aquatic communities might facilitate invasions, but that the frequency and intensity of introductions are critical components in the invasion process. Although according to Williamson (1996) all communities are susceptible to invasion, it is expected that they are not equally so. Biodiversity, species composition, energy flow, physical-chemical habitat, and climate conditions are considered major controllers of population and ecosystem dynamics. We often lack a good understanding of these dynamics and the inter-relationships of indigenous and alien biota. It is known that changes in abundance of indigenous and alien biota occur as a result of displacement and competition (Devin et al., 2005b; van Riel et al., 2007). In addition, changes in physical-chemical conditions and habitat deterioration could ease the introduction and establishment of alien species (Den Hartog et al., 1992; Didham et al., 2005).

Amphipods of the superfamily Gammaroidea are widespread and often play an important functional role in fresh and brackish water ecosystems (Jazdzewski, 1980). In many aquatic communities, gammarids are the dominant macroinvertebrates in terms of both biomass and density (MacNeil et al., 1997). Consequently, in these environments, they contribute significantly to the energy flow by decomposing organic material and serving as food for other organisms, such as fish (Grabowski et al., 2007). Numerous species belonging to Gammaroidea are considered successful invaders (van der Velde et al., 2000; Grabowski et al., 2007). Several life history traits, such as a short generation time, early sexual maturity, high fecundity, and a high tolerance towards changes in salinity and stress in general are put forward as important traits contributing to their success (Grabowski et al., 2007). Another factor promoting the successful invasion of the alien gammarids may be predation and competition (Kelly et al., 2006). Gammarids used to be classified as shredders or collectors, but MacNeil et al. (1997) and Mayer et al. (2008) showed that a wider food base is exploited than was previously thought. Furthermore, cannibalism and inter- and intraguild predation seem to be common and important factors contributing to species exclusions and replacements (Polis et al., 1989; Dick et al., 1993).

Several authors indicated that the success of an alien species cannot be attributed to one particular factor, but rather to a combination of biotic and abiotic conditions of the recipient habitat (Sakai et al., 2001; MacNeil et al., 2004). Variability in conditions, such as water quality and complexity of the habitat influences the outcome of the competition for a niche, depending on differences in the species' tolerance to these conditions (MacNeil et al., 2004). Studies that focus on changing biological factors, such as food supply, competition and predation and changing physical-chemical factors, such as nutrients, salinity and water quality in relation to the occurrence of a species may explain community shifts. These studies of change in community structure and function are important for the understanding and the management of ecosystems. Moreover, a good insight into the ecology and behaviour of alien species can help to decrease their further spread.

Based on literature, it is known that the original gammarid composition of the brackish polder waters consisted in Flanders of two indigenous species: *Gammarus duebeni* Lilljeborg, 1852 and *Gammarus zaddachi* Sexton, 1912. At the beginning of the nineties, *Gammarus tigrinus* Sexton, 1939 invaded Belgian waters (Messiaen et al., 2010). Since this species is known to be a successful invader (Daunys and Zettler, 2006; Packalén et al., 2008), the species was supposed to have an impact on the local gammarid community. Therefore, the aim of the present study was to investigate whether changes in the prevalence of gammarid species has occurred during the last twenty years in brackish polder waters in Flanders. Possible reasons for the observed shifts are discussed.

5.2 Materials and Methods

5.2.1 Study Area

Polders are a low-lying land, enclosed by embankments known as dikes that form artificial hydrological entities. The polders, which refer to a geographical area, consist in Flanders (Belgium) of the coastal polders, which are mainly situated behind the dunes and continue about 15 km inland and the polders that flank the river Scheldt (Fig. 5.1). The polders comprise the hinterland of the Belgian coast and continue in the north of East Flanders up to northwest of Antwerp. The soil of the polder area consists mainly of clay. Water enters the low-lying polders due to water pressure of groundwater, rain fall, and transportation of water by streams. This usually means that the polder has an excess of water that needs to be pumped out or drained by opening sluices at low tide. Three different water types can be distinguished in brackish polders based on salinity: oligohaline (0.5-5 psu), mesohaline (5-20 psu), and polyhaline (20-30 psu) water.

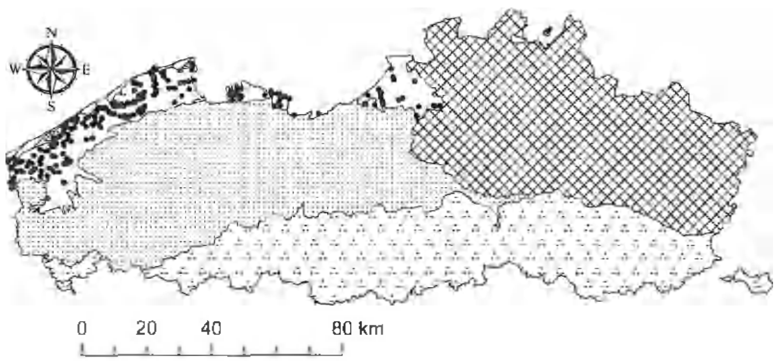


Figure 5.1 *Map of Flanders with indication of the different eco-regions: dune area (oblique stripes), polder area (white), sandy region (dots), campine region (cross-stripes) loamy region (dots in triangle) and the different sampling locations (black dots) situated in the polders.*

5.2.2 Data Collection and Analysis

Data from 1989-2008 were obtained from the Flemish Environment Agency (VMM), which monitors the physical-chemical and biological quality of surface waters since 1989 on a regular basis. Not every location was sampled each year. Samples from 218 different locations situated within these polders and distributed over several types of running and stagnant waters were investigated (Fig. 5.1). Only those years containing more than 10 samples were included for analysis of changes in prevalence of macroinvertebrates. From those samples containing Gammaridae, all crustaceans were identified to species level. In total, 430 biological samples containing gammarids were investigated. Since physical-chemical parameters were measured more frequently, a total of 15,000 physical-chemical samples were available for sites that contained gammarid species. Measured parameters are listed in Table 5.1, but not every parameter was measured for each water sample. At least four standard parameters: pH, temperature, conductivity, and dissolved oxygen were recorded in the field when the biological samples were taken; these samples were taken with a standard hand-net or by means of artificial substrates if it was not possible to perform the kick-sampling method (Gabriels et al., 2010). Sampling effort was proportionally distributed over all accessible aquatic habitats. In addition to the hand-net sampling, macro-invertebrates were manually picked from stones, leaves, or branches along the same stretch (Gabriels et al., 2010). The biotic index, expressed as the Belgian Biotic Index, was calculated according to Gabriels et al. (2010). The abundance equals the number of individuals caught in one sample. The prevalence was calculated as the number of presences in a year divided by the number of sampled stations.

Non-parametric tests were carried out, since these are generally applicable and do not have to meet the requirements of parametric tests. The relationship between the different chemical parameters was checked using Spearman's Rank Correlation Coefficient. Kruskal-Wallis ANOVA was performed to check for significant differences in preference of gammarids for chemical habitat conditions, followed by post-hoc multiple comparisons of mean ranks (Conover, 1980). Gammarid species was the independent variable, whereas the different abiotic factors were the dependent variables. The significance level for hypothesis testing was set at $p \leq 0.05$. All statistical analyses were performed using Statistica 7.0 (Statsoft Inc., 2004). Besides the basic statistics, multivariate data analysis was performed using CANOCO for Windows 4.5 (ter Braak and Šmilauer, 2002) to determine which environmental parameters might be responsible for differences in species composition. Direct gradient analysis was done, since environmental variables were explicitly incorporated in the analysis. To test whether a linear or unimodal method was needed, a Detrended Correspondence Analysis (DCA) was performed. If the Length of Gradient (LoG) > 4, a unimodal method is needed, whereas if the LoG < 3 a linear method is designated. In our case, a Canonical Correspondence Analysis (CCA) was used since the LoG = 4.4. Averages were used for missing data and a log-transformation was performed prior to CCA to normalise the data.

Table 5.1 *Overview of the different parameters used for analysis with indication of their abbreviation and unit.*

Parameter	Abbreviation	Unit
Ammonium	NH_4	mg N l^{-1}
Biological Oxygen Demand	BOD	mg BOD l^{-1}
Belgian Biotic Index	BBI	0= bad; 10=very good
Chemical Oxygen Demand	COD	mg COD l^{-1}
Salinity	Chloride	mg Cl^- l^{-1}
Transparency	Trans	depth (in cm)
Total Phosphorous	Ptot	mg P l^{-1}
Conductivity	EC	μS cm^{-1}
Hardness	H	French degrees
Kjeldahl Nitrogen	KJN	mg N l^{-1}
Nitrate	NO_3^-	mg N l^{-1}
Nitrite	NO_2^-	mg N l^{-1}
Orthophosphate	oPO_4^{3-}	mg P l^{-1}
pH	pH	(-)
Temperature	T	°C
Dissolved Oxygen	Dissolved_O_2	mg O_2 l^{-1}
Oxygen Saturation	O_2_saturation	% O_2
Suspended Solids	SS	mg l^{-1}

5.3 Results

5.3.1 Chemical and Biological Water Quality

In total, 18 different biological and chemical parameters were analysed (Table 5.1). The following variables were strongly correlated: Biochemical Oxygen Demand (BOD) with Chemical Oxygen Demand (COD) (r = 0.74, p = 0.03), Conductivity (EC) with salinity (chloride) (r = 0.84, p = 0.01), total phosphorous (Ptot) with orthophosphate (oPO_4) (r = 0.81, p = 0.02) and dissolved O_2 (dissolved oxygen) with oxygen saturation (r = 0.98, p = 0.001). Therefore, only one of both parameters was used to assess the difference in habitat preference of the macro-crustaceans. The yearly average salinity decreased since the beginning of the measurements (Fig. 5.2). There was a significant decrease in ammonium (p = 0.001), BOD (p = 0.03), orthophosphate (p = 0.02) and nitrite (p = 0.01) (Fig. 5.2). On the other hand, no significant alterations were observed for nitrate and dissolved oxygen between 1990 and 2008 (Fig. 5.2).

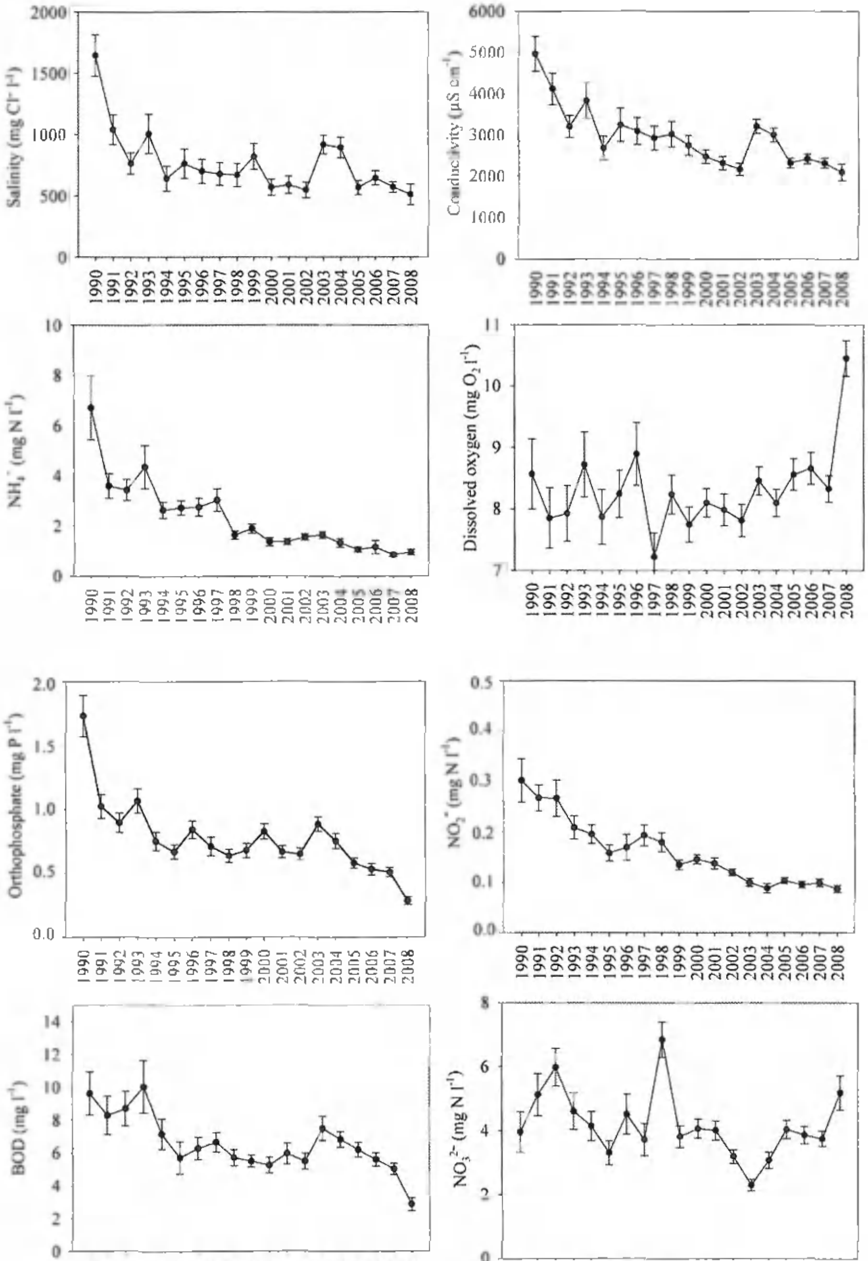


Figure 5.2 *Evolution in yearly average values and 95% confidence intervals of salinity, conductivity, ammonium (NH_4^+), Biological Oxygen Demand (BOD), orthophosphate, nitrate (NO_3^{2-}), nitrite (NO_2^-) and dissolved oxygen.*

5.3.2 Macro-Crustacea

In total, 23 different species of macro-Crustacea were found, four of which belonged to Gammaridae (Table 5.2). Two indigenous gammarids, *G. duebeni* and *G. zaddachi*, and one alien gammarid, *G. tigrinus* were common in the polder waters. A fourth invasive alien species, *Dikerogammarus villosus* (Sowinsky, 1894), was found only at two locations in brackish waters situated near the River Scheldt and was therefore not included in the further analysis of the gammarid prevalence. The alien species *G. tigrinus* occurred in waters with significantly lower salinities (p = 0.01), lower orthophosphate concentrations (p = 0.007) and higher oxygen concentrations (p = 0.001) compared to the two indigenous species *G. duebeni* and *G. zaddachi*. *Gammarus zaddachi* occurred in waters with significantly lower values of the Belgian Biotic Index compared to both other species (p = 0.005). Minimum and maximum values for salinity indicated that *G. tigrinus* was able to withstand lower salinities compared to the indigenous gammarids and even higher salinities than *G. duebeni* (Table 5.2). *Gammarus tigrinus* showed a wide tolerance towards low and high levels of orthophosphate concentration and dissolved oxygen (Table 5.2).

The prevalence of the indigenous brackish water gammarid species (*G. duebeni* and *G. zaddachi*) decreased since the end of the nineties (Fig. 5.3a). At the beginning of the nineties, *G. tigrinus* was absent from all samples, whereas in 1999, the species was already present in most of the samples and widely distributed in the polders. Also a decrease in prevalence of the indigenous brackish water species *Palaemonetes varians* (Leach, 1814) and *Neomysis integer* (Leach, 1814) was observed since the beginning of the nineties, whereas the prevalence of isopods belonging to Asellidae, *Asellus aquaticus* (Linnaeus, 1758), *Proasellus meridianus* (Racovitza, 1919), and *Proasellus coxalis* (Dollfus, 1892), increased since 1992 (Fig. 5.3b).

In a bi-plot of the species and the environmental variables, the first and the second axis had an Eigenvalue of 0.629 and 0.469, respectively. The first and second axis explained 81% and 59% of the variance in species composition, respectively. Conductivity, pH and the biotic index explained most of the variance in the species composition of Crustacea found in the polders, while nitrate, nitrite and ammonium were less important (Fig. 5.4). There was a clear separation along the first axis based on the chemical parameters between the species that are exclusively found in brackish water and those that have a wide tolerance towards salinity and are commonly found in freshwater habitats.

***Table 5.2** Overview of the macrocrustaceans (*=alien species) found in the polder waters with indication of the number of samples (out of a total of 430) in which the species was recorded. Mean as well as minimum and maximum values of salinity, orthophosphate (oPO_4^{3-}) and dissolved oxygen (DO) are given for each species (na=not available).*

Order	Family	Species	# samples	Salinity (mg $Cl^- l^{-1}$)	oPO_4^{3-} (mg P l^{-1})	DO (mg O_2 l^{-1})
Amphipoda	Crangonictidae	*Crangonyx pseudogracilis**	1	141 (141-141)	1.00 (1.00-1.00)	22.0 (22.0-22.0)
	Corophiidae	*Chelicorophium curvispinum**	13	41 (28-470)	0.15 (0.10-0.20)	7.6 (4.2-21.4)
		Corophium lacustre	3	1000 (938-1730)	0.42 (0.09-0.75)	16.0 (5.6-21.0)
		Corophium multisetosum	1	1730 (1730-1730)	0.42 (0.42-0.42)	16.0 (16.0-16.0)
	Gammaridae	*Dikerogammarus villosus**	7	42 (28-70)	0.13 (0.10-0.20)	8.0 (4.2-9.0)
		Gammarus duebeni	100	647 (92-5100)	0.73 (0.01-4.58)	6.3 (0.7-20.7)
		*Gammarus tigrinus**	335	434 (28-5860)	0.54 (0.01-5.10)	7.6 (0.4-28.3)
		Gammarus zaddachi	56	1131 (73-7640)	0.97 (0.24-3.20)	6.3 (0.7-14.0)
	Talitridae	*Orchestia cavimana**	10	510 (170-1510)	1.35 (0.33-2.80)	6.7 (0.7-14.0)
Decapoda	Cambaridae	*Orconectes limosus**	2	40 (40-41)	0.15 (0.10-0.20)	7.2 (7.2-7.2)
	Crangonidae	*Crangon crangon*	1	na	na	na
	Palaemonidae	*Palaemon elegans*	2	886 (772-1000)	0.74 (0.72-0.75)	7.7 (5.6-9.7)
		Palaemonetes varians	123	1131 (60-5860	0.65 (0.01-3.20)	6.6 (0.7-20.7)
	Panopeidae	*Rhithropanopeus harrisii**	3	53 (42-436)	0.15 (0.15-0.18)	7.8 (7.2-9.0)
	Portunidae	*Carcinus maenas*	1	7640 (7640-7640)	2.16 (2.16-2.16)	na
	Varunidae	*Eriocheir sinensis**	2	230 (34-426)	0.63 (0.20-1.10)	4.9 (4.9-4.9)
Isopoda	Asellidae	*Asellus aquaticus*	205	395 (28-3100)	0.59 (0.01-4.58)	6.9 (0.8-27.0)
		*Proasellus coxalis**	41	393 (28-3100)	0.48 (0.04-1.50)	1.45 (7.6-25.3)
		*Proasellus meridianus**	29	170 (28-1700)	0.58 (0.02-3.10)	8.0 (21.6-2.1)
	Sphaeromatidae	*Lekanesphaera hookeri*	12	998 (419-5860)	0.53 (0.12-0.92)	9.1 (3.3-20.3)
Mysida	Mysidae	*Hemimysis anomala**	1	528 (528-528)	0.62 (0.62-0.62)	7.80 (7.80-7.80)
		Neomysis integer	96	668 (45-5860)	0.71 (0.10-3.60)	6.8 (0.7-20.4)
		Praunus flexuosus	1	817 (817-817)	2.00 (2.00-2.00)	19.0 (19.0-19.0)

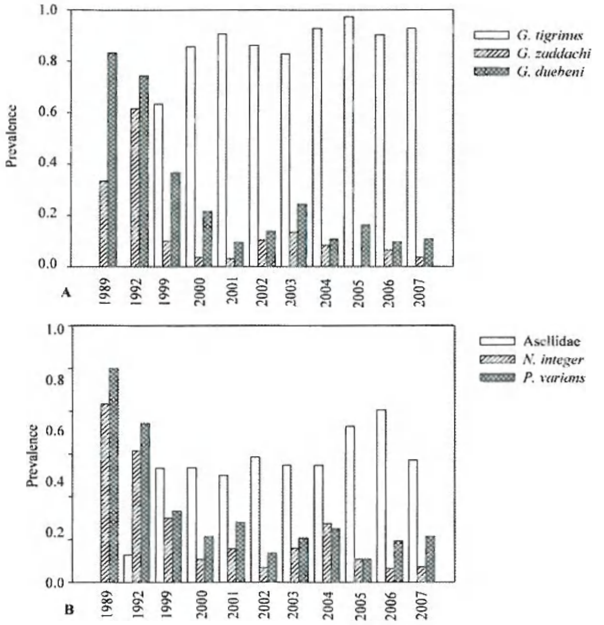


Figure 5.3 *(a) Prevalence (calculated as the number of ssites where the species was recorded relative to the total number of sites) of two indigenous (Gammarus duebeni and Gammarus zaddachi) and one alien (Gammarus tigrinus) gammarid and (b) prevalence of two indigenous brackish water species (Palaemonetes varians and Neomysis integer) and one indigenous freshwater taxon (Asellidae).*

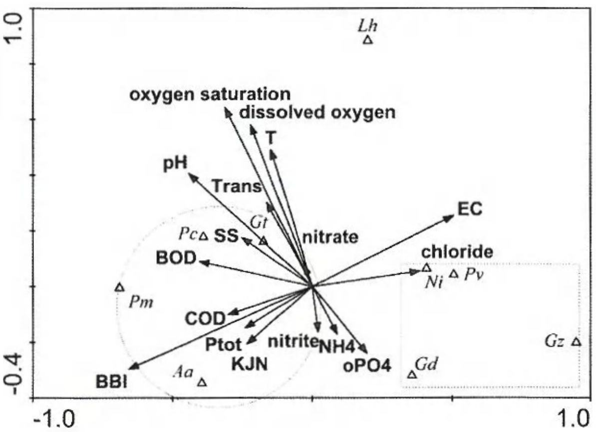


Figure 5.4 *Bi-plot of species (Gt = Gammarus tigrinus, Gd = G. duebeni, Gz = G. zaddachi, Lh = Lekanesphaera hookeri, Aa = Asellus aquaticus, Pm = Proasellus meridianus, Pc = Proasellus coxalis, Pv = Palaemonetes varians, Ni = Neomysis integer) and environmental variables (codes are explained in Table 1) with indication of two clusters: the circle comprises the freshwater taxa, whereas the rectangle comprises the brackish water taxa.*

5.4 Discussion

Gammarus tigrinus, originating from North-America, was recorded for the first time in the eastern part of Flanders in 1991 (Messiaen et al., 2010). This species rapidly expanded towards the end of the nineties and is currently widely distributed throughout the polders of Flanders. A simultaneous decrease of the indigenous brackish water gammarids occurred leading to a dominant position of this alien species within the amphipod community in most polder waters. Only at a few sampling locations, a temporary co-occurrence between *G. tigrinus* and one of the indigenous species was observed. Similar observations were made in the mid sixties in the Netherlands where *G. tigrinus* rapidly invaded all oligohaline waters and replaced the indigenous species that formerly inhabited these waters (Pinkster and Stock, 1967; Pinkster et al., 1992). More recently, *G. tigrinus* has invaded the Baltic Sea, causing changes in the indigenous fauna composition and consequently altering species composition and interspecific interactions within these communities (Szaniawska et al., 2003; Daunys and Zettler, 2006; Grabowski et al., 2006; Packalén et al., 2008). The impact of *G. tigrinus* on local communities is not at all a unique phenomenon: also several other alien gammarids impacted local communities (Jazdzewski et al., 2004; Josens et al., 2005).

Gammarus tigrinus is a euryhaline species that shows a wide tolerance towards salinity. Under laboratory conditions, the species can survive in waters varying in salinity from fresh (180 mg $Cl^- l^{-1}$) to brackish (7100 mg $Cl^- l^{-1}$) (Pinkster, 1975; Pinkster et al., 1977; Savage, 1982). This is comparable to our results, although the minimum and maximum values for salinity were lower: 28 mg $Cl^- l^{-1}$ and 5860 mg $Cl^- l^{-1}$, respectively. On the other hand, the presence of both indigenous species is restricted to more saline waters and both are less tolerant towards freshwater conditions. Based on our results, a possible reason why *G. tigrinus* became so successful in these polders is due to a decrease in salinity. These new environmental conditions probably facilitated the spread of *G. tigrinus*. Normant et al. (2007) found that although *G. tigrinus* exhibits a wide tolerance towards salinity, the energy maintenance was highest in fresh and oligohaline water. In addition, Wijnhoven et al. (2003) found a high resistance of *G. tigrinus* for increased temperatures and pollution compared to other gammarids. However, our results also indicated that a decrease of nutrients favoured the prevalence of this alien species. We think that favourable environmental conditions can contribute to the establishment success of an alien species, which was also indicated by Jazdzewski et al. (2004). Our findings are supported by previous work on the macroinvertebrate composition in polder lakes in Flanders (Everaert et al., 2011). Based on the use of data-driven modelling techniques they found that *Gammarus tigrinus* tolerates a wide range of environmental conditions. In addition, the species also benefits from an increase in water quality since it was mainly present in polder lakes that are characterised by a relatively high biotic index.

To maintain itself in a certain habitat, a population not only has to survive but also has to reproduce. Pinkster (1975) found that *G. tigrinus* reproduced under laboratory conditions throughout the year except when temperatures were below 5°C and/or salinities were below 180 mg Cl- l^{-1}. It seemed that also the length of the reproductive period was influenced by both salinity and temperature. In addition, Pinkster (1975) found that *G. tigrinus* had a reproductive peak during summer months when the water temperature was relatively high. The average incubation time of *G. tigrinus* was generally lower than that of the indigenous gammarids and was shortest in summer (Pinkster, 1975). Another factor that is important for the success of an alien species is the time to reach sexual maturity. Females of *G. tigrinus* start to produce eggs at a total length of 4 mm. The time to reach this length depends on the temperature and reaches an optimum at a temperature of 20°C. *Gammarus duebeni* and *G. zaddachi* could reproduce throughout the year when salinities were high enough. However, when salinities became too low (< 400 mg Cl^{-} l^{-1}) these species could only reproduce during the unfavourable winter months and thus at lower water temperatures, resulting in a longer incubation time of the eggs and a lower reproductive output (Dennert et al., 1969; Pinkster et al., 1977). This could possibly explain why *G. duebeni* and especially *G. zaddachi* were only found in polder waters with a high salinity. *Gammarus duebeni* is only able to withstand the competition by *G. tigrinus* in extremely variable habitats, where the water level and salinity fluctuate constantly (Pinkster et al., 1977). Some other studies also indicate the importance of salinity on the reproduction and brood size of amphipods. A reduction in brood size with decreasing salinity was observed for *Eulimnogammarus obtusatus* (Dahl, 1938) under laboratory conditions (Pinkster and Broodbakker, 1980) and for wild populations of *Corophium multisetosum* Stock, 1952 (Cuhna et al., 2000). *Gammarus tigrinus* can be seen as an opportunistic r-strategic species that is able to increase its density over a short period, becoming the dominant species in the recipient community. The difference in time to reach sexual maturity, together with the short incubation time and the favourable reproductive period possibly contributed to the success of *G. tigrinus* in its competition with the indigenous species. Besides the aforementioned characteristics, another element that could have played a role in the success of *G. tigrinus* is its predatory behavior (Dick, 1996; Dick and Platvoet, 1996; Bailey et al., 2006). Gammaridae used to be classified as shredders or gatherers/collectors (Cummins and Klug, 1979). However, intraguild predation is more common than previously thought and may be a powerful force in determining species replacements and community shifts (Polis et al., 1989).

Although the chemical water quality of the studied polder waters improved, the prevalence of Crustacea indigenous to the polders declined. Besides the decrease in prevalence of *G. duebeni* and *G. zaddachi*, also a decrease in the prevalence of *N. integer* and *P. varians* was observed. However, it is unlikely that the expansion of the alien *G. tigrinus* caused the decline of all these species. It is more probable that the decrease in salinity caused the decline of the indigenous brackish water species and the increased prevalence of freshwater tolerant species, such as Asellidae and the alien *G. tigrinus*. A previous study performed by Everaert et al. (2011) concluded that conductivity and ecological water quality were the main factors determining the occurrence of alien and indigenous macroinvertebrates in the polder lakes, which are part of the polder waters. They concluded that applying multi-target approaches (e.g. relating physico-chemical characteristics to the macroinvertebrate composition) gives insight in a potential alien macroinvertebrate community subgroup, what is in particular relevant for water managers to protect and restore surface waters.

In conclusion, *G. tigrinus* invaded the polder waters during the nineties and is now widely distributed in them. It was thought that the invasion by *G. tigrinus* could have played an important role in the decreased prevalence of indigenous gammarids. However, the indigenous and alien gammarids clearly show different preferences with regard to environmental conditions. In addition, not only for the two indigenous gammarids, *G. zaddachi* and *G. duebeni*, but also for other brackish water species indigenous to the polders a decline was observed, which indicates that the decreasing salinity probably was the main cause of their reduction.

6.1 Introduction

Intercontinental shipping across the oceans and aquaculture of non-indigenous species in coastal areas contributed to the spread and establishment of many alien species especially in coastal and brackish water environments (Eno et al., 1997; Reise et al., 1999; Ruiz and Hewitt, 2002; Streftaris et al., 2005). At the end of the nineties, about 80 alien species established in the North Sea, which tends to be lower compared to other estuarine regions in the world (Reise et al., 1999). This number is probably an underestimation since it only concerns macro-organisms. Moreover, defining the number of alien taxa is a real challenge because there are a lot of differences in the spatial and taxonomic effort addressed to the study of alien species. This is illustrated by Streftaris et al. (2005), who found 141 alien species in the North Sea only a few years after Reise et al. (1999) reported 80 alien taxa. It has been found that the diversity of alien species in North Sea biota increases from offshore towards the coast and a further increase is observed from the open coast towards the estuaries (Wolff 1999). The majority of the alien species encountered are invertebrates, primarily crustaceans, molluscs, polychaetes and hydroids (Reise et al., 1999). Most alien invertebrates colonised the North Sea via shipping, either as larvae in ballast water tanks or as adults attached to hulls. Although there is no clear evidence that alien species have driven indigenous species to extinction in the North Sea (Reise et al., 1999; Nehring, 2006), alien species are known to have irreversibly modified certain functions of the North Sea ecosystem (Streftaris et al., 2005). As Williamson (1996) states that most of the successful invaders are generalists, those generalists transported by ships can become abundant in harbours, because these species are often well adapted to a varying salinity and human modified environments (e.g. hard substrates). Besides a high physiological tolerance to survive the transport with ballast water, successful invaders are usually characterised by a high fecundity, the capacity to adapt to a new ecological niche and good means of dispersal (Streftaris et al., 2005). In addition to species characteristics, the susceptibility to invasions of the recipient ecosystem plays a crucial role in the invasion success. In this respect, several arguments have been proposed to explain the success of non-indigenous species in estuaries and thus brackish water ecosystems. Based on data of aquatic habitats in the Netherlands, Wolff (1999) formulated three main hypotheses to explain the high number of non-indigenous species in Dutch estuaries. Brackish waters are typically characterised by low densities and species diversity, hence it is easier for an alien species to establish (Wolff, 1999; Nehring, 2006). It has been shown for marine ecosystems that species-rich communities appear to be better buffered against invasions (Stachowicz et al., 1999). Since most harbours with intensive international ocean ship traffic are situated in these brackish waters, it is likely that many alien species have the potential to occur at these locations.

The aims of the present study were fourfold: (1) to investigate the presence of indigenous and alien macro-Crustacea in the four Belgian coastal harbours (Nieuwpoort, Oostende, Zeebrugge and Blankenberge), (2) to map the degree of biocontamination, which was expressed as the biocontamination index (Arbačiauskas et al., 2008), (3) to test the importance of several abiotic factors for the occurrence of these macro-Crustacea, (4) to assess the importance of shipping and yachting for the introduction and dispersal process of alien macro-Crustacea.

6.2 Materials and Methods

The study was conducted at the four coastal harbours in Belgium: Nieuwpoort, Ostend, Blankenberge and Zeebrugge (Fig. 6.1) and sampling lasted from September until November 2009. Each sampling location was sampled once during this sampling campaign and consequently there was no repetition in sampling of the sampling stations. Zeebrugge and Ostend are international maritime ports, which are of high economic value to Belgium (Mathys, 2009). More than 300 companies operate within the harbour area of Brugge-Zeebrugge. Zeebrugge can be characterised as a polyvalent harbour with a good geographic location, a high productivity and the possibility to enter for ships up to 55 feet depth. The harbour of Ostend is an important area for shipping, yachting and fishing. Nieuwpoort harbours mainly yachts and fishing boats, whereas Blankenberge is only visited by yachts.

Samples were taken along a salinity gradient starting in the harbour and continuing inland. In total, 43 different sampling locations including channels, docks, canals, reservoirs and small watercourses were selected of which 17 in Nieuwpoort, 11 in Ostend, three in Blankenberge and 12 in Zeebrugge-Brugge (Fig. 6.1). Biological samples of macroinvertebrates were collected by means of a handnet or with artificial substrates if it was not possible to perform the kick-sampling method (Gabriels et al. 2010). For a detailed description of these techniques we refer to previous chapters. Both sampling methods were used to sample the macrobenthos communities living in or near the surface of the substrate.

Besides the abovementioned sampling techniques, a trawl net with a circular diameter of 100 cm, a length of 3 m and a mesh size of 200 µm was used to sample the hyperbenthos. This sampling method was used for qualitative analysis only and the samples were used to assess the species present in the water column between 0.2 m and 1.2 m above the bottom. This sampling technique was used to catch mobile species, such as Mysida, since these are often missed when using a handnet or artificial substrates. The trawl net was with a long rope attached to a zodiac and left into the water to the appropriate depth for sampling. Samples were taken by sailing within a radius of 100 m from a predetermined fixed sampling location (GPS determined) within the harbour for 10 minutes at an average ship speed (4 km h^{-1} relative to the bottom). All samples were taken during day time when hyperbenthic organisms are known to be concentrated near the bottom. The samples gathered by trawl net were not included in the analysis of abundance, number of species and biomass data, because the sampling method could not be compared to the other sampling techniques used since the quantities sampled differed between the trawl net on the one hand and the handnet and artificial substrates on the other hand.

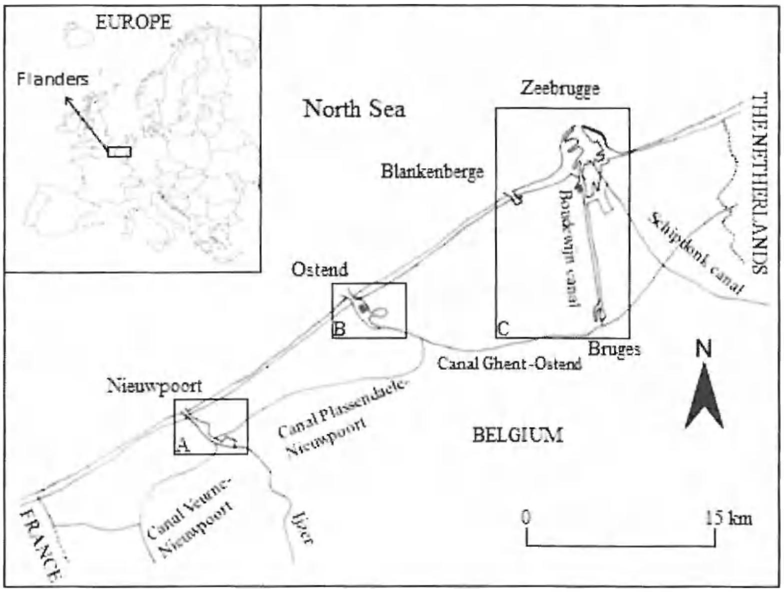


Figure 6.1 *Map of the Belgian coast with indication of the different harbours and the sampled areas (A, B, C), which are shown in detail in Figure 6.5.*

All macroinvertebrates were preserved in 90% alcohol and afterwards identified in the laboratory to species level. All individuals of each species were measured based on the standard length (distance from base of the rostral tip to the end of the last abdominal segment for most species, but carapace width for crabs) and their biomass was derived from length-ash-free dry weight regressions (Mees, 1994).

One water sample for chemical analysis was taken at each sampling location. Dissolved oxygen (wtw oxi 330), conductivity (wtw cond 315i) and pH (HI 9210N) were measured in the field by means of a hand electrode. Salinity was measured with a refractometer (DIGIT ATC 100). Ammonium, nitrite, nitrate and total phosphorous were determined in the laboratory using test kits (Merck Spectroquant) specifically designed for the analysis of water with a high chloride content.

Since alien species constitute a significant biological pressure, the assessment of such a pressure should be integrated within the context of the ecological status assessment of water bodies required by the European Water Framework Directive (Cardoso and Free, 2008). In order to provide an integrated ecological assessment of alien species, a biocontamination index was established (Arbačiauskas et al., 2008). This "biocontamination" could be defined as the presence of alien species regardless of their abilities to cause negative ecological and/or socio-economic impacts (Panov et al., 2009). Five classes of biocontamination ranging from zero (no contamination) to four (severe contamination) could be defined. The classes of SBCI correspond to the five ecological quality classes as defined by the European Water Framework Directive (2000/60/EC; European Community, 2000) and allow status ranking of water bodies from high to bad quality. For the calculation of the SBCI, all taxa (macro-Crustacea and other macroinvertebrate taxa) were included. The relationship between shipping, the SBCI and the number and abundance of alien species was analysed using Spearman's Rank Correlation Coefficient. The relative abundance, number of species and biomass was calculated as the total value of alien species divided by the total value of all species. A non-parametric Kruskal-Wallis ANOVA was used to check for differences in number of species, abundance and biomass between harbours. Post-hoc multiple comparisons of mean ranks were used to assess differences between specific harbours (Conover, 1980). The significance level for hypothesis testing was set at $p \leq 0.05$. All statistical analyses were performed using Statistica 7.0 (Statsoft Inc., 2004).

Data on shipping was obtained from several information sources depending on the type of shipping. Information on transcontinental shipping (ship movements as well as the origin of ships), which includes container traffic, transportation of goods, roll-on roll-off and transportation of passengers, was obtained from the annual reports made by the Flemish harbours (Merckx and Neyts, 2008). Information on recreational crafts originated from the logbooks of different marinas. For Ostend and Nieuwpoort the number of yachts entering the marina was documented in logbooks, whereas for Blankenberge and Zeebrugge no information on the number of recreational craft movements was available. Therefore, estimations were made about the number of yachts entering these marinas. The number of anchorages in each of the marinas was counted based on aerial photographs. Based on that number and information available of the yacht movements of Ostend and Nieuwpoort, an extrapolation for Zeebrugge and Blankenberge was made. Finally, information on the number of fishing vessels entering Zeebrugge, Ostend and Nieuwpoort on an annual base was retrieved from the Department of Ocean Fisheries (www.vlaanderen.be/landbouw).

6.3 Results

6.3.1 Physical-chemical data analysis

Maximum concentrations of ammonium, nitrite and total phosphorous were found in Nieuwpoort (Table 6.1). In Blankenberge, Nieuwpoort and Ostend, most samples were evenly distributed over a salinity gradient ranging from almost fresh (2 PSU) to saline (35 PSU) water. In Zeebrugge, all samples were taken in the range of 28 to 35 PSU, although they were located up to 15 km inland. The water was alkaline with a pH ranging between 7.22 and 9.12. Dissolved oxygen was rather low for Ostend with a minimum of 1.09 mg l^{-1} and a maximum of 5.62 mg l^{-1}. Maximum values for dissolved oxygen were around 10 mg l^{-1} for Nieuwpoort, Blankenberge and Zeebrugge (Table 6.1).

Table 6.1 *Measured ranges for dissolved oxygen (DO), salinity, conductivity (EC), ammonium (NH_4), nitrate (NO_3), nitrite (NO_2), total phosphorous (P total) and pH in the Belgian harbours.*

Harbour	DO (mg l^{-1})	Salinity (PSU)	EC (mS cm^{-1})	NH_4 (mg N l^{-1})	NO_3 (mg N l^{-1})	NO_2 (mg N l^{-1})	P total (mg P l^{-1})	pH
Blankenberge	7.00-10.30	7-35	6.43-49.60	0.05-0.16	0.60-1.49	0.01-0.05	0.14-1.05	7.85-9.12
Nieuwpoort	2.98-9.91	3-35	1.78-51.80	0.10-1.02	0.12-1.61	0.01-0.70	0.20-4.86	7.82-8.44
Ostend	1.09-5.62	2-35	1.92-43.90	0.05-0.59	0.98-5.64	0.01-0.10	0.20-1.33	7.64-8.72
Zeebrugge	4.50-9.80	28-35	36.30-47.70	0.05-0.21	1.03-1.91	0.01-0.17	0.20-1.40	7.22-7.78

6.3.2 Shipping

Only two out of four harbours received transcontinental commercial ship traffic: Zeebrugge and Ostend with 9405 and 4868 ships entrances reported during the year 2008, respectively.

For Zeebrugge, 55% of the ships originated from Europe, 30% from Asia and 2% from North-America, whereas for Ostend, almost all ships originated from within Europe (99.5%). Information on the traffic of recreational crafts was directly available for Ostend and Nieuwpoort with 1255 and 1391 yachts entering the marina in 2009, respectively. About 25% were yachts coming from neighbouring marinas in Belgium, the other 75% of the yachts entering the marinas originated from other harbours situated in Europe. Since no detailed data on traffic of yachts was available for Blankenberge and Zeebrugge estimations were made on the number of yachts entering these marinas, which resulted in 570 yachts for Zeebrugge and 1140 yachts for Blankenberge. Based on data of 2009, it was found that most fishing vessels entered in Ostend (n=882), followed by Nieuwpoort (n=612) and Zeebrugge (n=240). There were no fishing vessels entering the harbour of Blankenberge. A significant correlation was found between the total number of ship entrances and the number of alien species recorded per sample ($r = 0.405$; $p = 0.04$), which increased with increasing ship traffic.

6.3.3 Biological data analysis

A total of 41 macro-Crustacea was found, 11 of which were alien to Belgium (Table 6.2). *Hemigrapsus takanoi* Asakura and Watanabe 2005, *Gammarus tigrinus* (Sexton 1939) and *Caprella mutica* Schurin 1935 were the most frequently encountered alien macro-Crustacea. The other alien macrocrustaceans were recorded in less than 10% of the samples. Almost 50% of the alien species originated from North-America, 27% from East Asia, 18% from the Ponto-Caspian region and 9% from New Zealand. The most common indigenous species were *Microdeutopus gryllotalpa* Costa 1835, *Melita palmata* (Montagu 1804) and *Carcinus maenas* (Linnaeus 1758), which were all recorded in almost half of the samples.

In general, about 25% of all macrocrustacean species were alien and they accounted for 30% of the total macrocrustacean abundance in the Belgian coastal harbours (Fig. 6.2). These alien species accounted for more than 65% of the total relative biomass of macrocrustaceans, which is primarily due to the alien crab *H. takanoi* and the amphipod *G. tigrinus*. When analysing the presence of alien macro-Crustacea per harbour, Ostend had the highest total relative abundance of alien species, whereas Zeebrugge the lowest (Fig. 6.2). However, with Kruskal-Wallis ANOVA, no significant difference in abundance of alien macro-Crustacea was detected between the harbours ($\chi^2 = 1.69$, df = 3, $p = 0.64$). The total relative number of alien species was more or less similar among the large harbours, with the highest number of alien species found in Zeebrugge (Fig. 6.2).

6.3.4 Number of species and biocontamination

The salinity range was divided based on the Venice system (1959), which consist of an oligohaline (0.5-5 PSU), mesohaline (5-18 PSU), polyhaline (18-30 PSU), euhaline (30-40 PSU) and hyperhaline zone (>40 PSU). The average number of indigenous species found per sample was highest at salinities above 30 PSU (Fig. 6.4). The average number of alien species found per sample was highest at the oligo- (0.5-5 PSU) and mesohaline (5-18 PSU) zone. The number of alien macroinvertebrates seemed to reach a maximum at salinities below 5 PSU, whereas the number of indigenous species slightly increased with increasing salinity (Fig. 6.4). The lower number of indigenous species observed at lower salinities was probably related to the low water quality in many of the canals and rivers situated in the oligohaline zone.

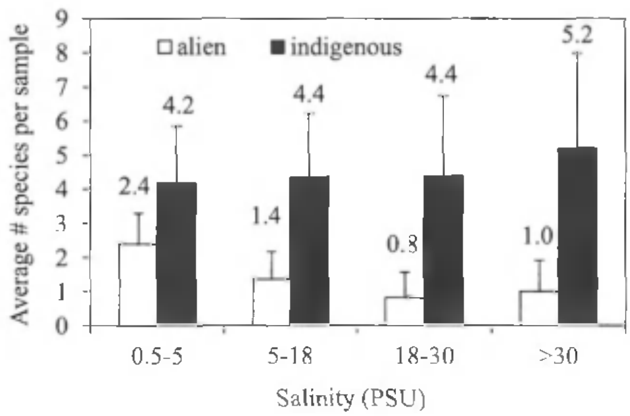


Figure 6.4 *Average number (with standard deviation) of alien (white) and indigenous (black) species per sample for the different salinity zones (based on the Venice system, 1959).*

The presence of alien species and the biocontamination at the different sampling points has been mapped (Fig. 6.5). Most sampling locations situated within the harbour of Nieuwpoort and Ostend had a low biocontamination index and consequently a good to moderate status (Fig. 6.5a, b). One sampling point situated in the marina of Nieuwpoort had a SBCI of 4, which was due to high densities of *Crassostrea gigas* (Thunberg 1793) and *H. takanoi*. It was observed for Nieuwpoort and Ostend that sampling locations situated more inland were categorised in class 3 or 4 and thus were characterised by a high number or high densities of alien species. In Zeebrugge, most sampling locations scored bad regarding biocontamination.

while bio-uncontaminated sites were lacking (Fig. 6.5c). Some sampling locations situated within the harbour docks of Zeebrugge and the harbour of Blankenberge were characterised by high abundances of *H. takanoi* and *C. mutica* and were consequently categorised in class 3 or 4. The SBCI was positively correlated with the number of alien species ($r = 0.69$; $p = 0.031$), the abundance of alien species ($r = 0.82$; $p = 0.01$) and the biomass of alien species ($r = 0.61$; $p = 0.04$).

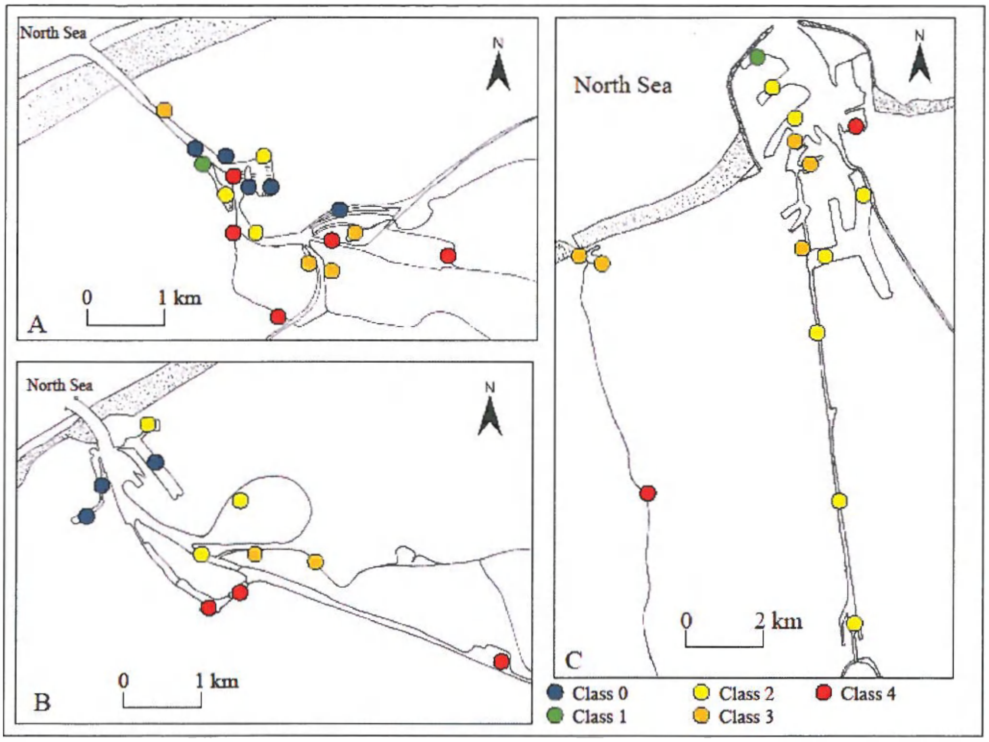


Figure 6.5 *Map with the site-specific biocontamination index (SBCI) (Arbačiauskas et al. 2008) for the sampling sites situated in (A) Nieuwpoort, (B) Ostend and (C) Zeebrugge -Blankenberge. SBCI classes: 0 (no biocontamination), 1 (low biocontamination), 2 (moderate biocontamination), 3 (high* biocontamination) and 4 (severe biocontamination).

6.4 Discussion

A total of 11 alien macrocrustacean species was found during a recent sampling campaign at the Belgian coastal harbours. Most of these species were discovered during the last two decades (Table 6.2), which is similar to the general findings in other parts of the North Sea and the Baltic Sea (Gollasch and Nehring, 2006). Only a few species were abundantly present alien species mentioned above thrive very well in a brackish water environment and can therefore also be expected to occur in the near future along the Belgian coast. On top of the already introduced species, more alien species are to be expected from other parts of the world, whereby secondary dispersal via other European harbours can be an important vector.

The highest number of alien species was found in the oligo- and mesohaline zone. This was also observed by Paavola et al. (2005), who found that several brackish water seas (Baltic Sea, Black Sea, Caspian Sea and Sea of Azov) have been invaded frequently resulting in a maximum number of alien species present in the oligohaline zone. The natural species minimum in brackish water as reported by several authors (Remane, 1958; Wolff, 1999; Paavola et al., 2005; Nehring, 2006) was confirmed during our study. Consequently, the hypothesis stating that the low indigenous species richness in brackish water leaves many empty niches favouring the establishment of alien species could be confirmed. Most sampling locations in the oligohaline zone were situated in canals and rivers with a low water quality, which could contribute to the low indigenous species diversity. Alien species are often more tolerant to a low water quality and hence more successful in these degraded habitats (Kennard et al., 2005; Boets et al., 2011a). Salinity is often considered to be a good parameter for risk assessment of future invasions of brackish water areas (Paavola et al., 2005). Areas with similar salinities can act as a potential donor region since genuine brackish water species can easily adapt to new habitat conditions. The fact that brackish waters have a higher potential for invasions due to intensive international ship traffic in combination with the physiological adaptations of brackish water species, enabling them to survive the transportation via ballast water, could explain the high number of alien species encountered in the mesohaline zone (Paavola et al., 2005). In addition, brackish waters are subjected to a two-sided invasion pressure by alien species, via the ocean and via inland waters, which could contribute to the high number of alien species encountered (Nehring, 2006). The presence of empty niches, suitable environmental conditions and availability of proper vectors might be the most effective predictors to determine the susceptibility to invasions of brackish waters (Paavola et al. 2005).

When investigating the number of alien species, Zeebrugge was the harbour with the highest number of alien macro-crustaceans, which is reflected by a high site-specific biocontamination index. The harbour of Zeebrugge received the highest number of transcontinental commercial ships compared to the other harbours. It is known that many alien species are being transported via ballast water of ships (Ruiz et al., 1997). A positive correlation between shipping and the number of alien species established confirmed that shipping is probably the most important vector of species introductions. The so-called propagule pressure, which is related to the introduction of alien species, is seen as an important element in the establishment success of alien species, although it is not always taken into account in studies about biological invasions (Williamson and Fitter, 1996). Propagule pressure is not always easy to measure, but can be related to the intensity of unintentional introductions, such as the estimated quantities of discharged ballast water, or the number of ships that might carry fouling on hulls (Occhipinti-Ambrogi, 2007). Whereas the number of alien species is related to propagule pressure, the high abundance of certain alien species can be ascribed to favourable environmental conditions (e.g. habitat suitability) (Crooks and Soulé, 1999). Despite the fact that most observed alien species originated from North-America, most ships which anchor in Zeebrugge originated from European harbours and only a small part originated from North-America. Frequent commercial shipping between neighbouring countries in Europe or countries bordering the same sea might be responsible for secondary introductions and dispersal of alien species. Moreover, also secondary dispersal via yachts or fishing vessels originating from neighbouring harbours could have partially contributed to introduction and spread of these alien species. Besides North-America, also East Asia was an important donor region. According to Gollasch and Nehring (2006) and Kerckhof et al. (2007), three prime source regions of aquatic alien species could be distinguished for the North Sea: Northwest Atlantic, Indo-Pacific and North Pacific.

Although there are no clear examples of species extinctions in the North Sea due to the introduction of alien species, the distribution area of indigenous species may have been reduced (Reise et al., 1999). Moreover, there exists the danger that the introduction of alien species can cause the spread of associated organisms or diseases, which may affect indigenous species and cause economical damage (Ruiz et al., 1997). In addition, there is the ongoing threat of global warming, which may be beneficial for certain alien species to spread and establish. Since the underlying mechanisms of species introductions are still not fully understood and their impact is very unpredictable, we should be aware of new species introductions (Gollasch and Nehring, 2006). Therefore, an active management with control of ballast water and an effective legislation regarding the introduction of species is an essential element to prevent more introductions of alien species.

Chapter 7: Establishment and future spread of alien macrocrustaceans

Adapted from:

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Chapter 7: Establishment and future spread of alien macrocrustaceans

Abstract

Among the invaders, alien macrocrustaceans are known to be very successful invertebrates that colonise new habitats rapidly. Data from different fresh and brackish waters gathered by the Flemish Environment Agency (VMM) were used to build data-driven models predicting habitat preference, abundance and species richness of alien macro-Crustacea present in surface waters in Flanders. Different techniques, such as regression and classification trees in combination with several optimisation methods (e.g. pruning) were used to construct the models. The performance of the models was moderate, because a balance between performance, ecological relevance and complexity was strived for. When using a three-fold cross validation it was found that the variation between the folds was limited, which is an indication of the robustness and the good reliability of the constructed models. Based on a sensitivity analysis the importance of conductivity, Kjeldahl nitrogen and shipping were stressed as well as graphically illustrated. Alien macrocrustaceans were predicted as present under brackish water conditions as well as in fresh waters with intensive ship traffic and low levels of organic pollution. The alien species richness was higher in rivers with intensive ship traffic and increased with increasing conductivity. Especially in brackish waters, alien macrocrustaceans reached high abundances. In fresh water, the abundance of alien species was generally lower. An integrated model that combined our habitat suitability model with a water quality model was used to predict the future distribution of alien macrocrustaceans. The predictions indicated that the prevalence and species richness of alien macrocrustaceans is likely to increase with improving chemical water quality, whereas their abundance will probably decrease slightly. From our analysis, it is clear that models are a useful tool and that decision makers should focus on vulnerable areas, such as brackish water areas and areas with intensive ship traffic in order to prevent the further introduction and spread of invasive alien species.

7.1 Introduction

The introduction of invasive alien species often has negative influences on indigenous communities and ecosystems, with consequences, such as species loss, biotic homogenization and changes in nutrient cycling (Gurevitch and Padilla, 2004; MacNeil et al., 2011). Therefore, techniques for modelling species' potential distributions could support pro-active strategies to avoid the introduction of alien species or to help in risk analysis by revealing those regions which are seen as hotspots for alien species introductions (Worner and Gevrey, 2006; Giovanelli et al., 2008; Ba et al., 2010). Besides their power to predict the future distribution of alien species, models can be used to assess the impact of invasive alien species on indigenous species assemblages (Jaarsma et al., 2007). In this way, negative effects of invasive alien species on the environment (e.g. food web disturbance or habitat alteration) as well as on the economy (e.g. high costs for eradication and control) could be reduced and tackled in advance. Being able to determine which habitats are vulnerable for invasions is essential for a good management, because it is often either impossible or at least very expensive to eradicate invasive alien species after their establishment (Perrings et al., 2005).

Using conventional statistical multivariate methods to analyse data poses limits, because they are mainly applicable to linear data and have less flexibility in interpreting ecological data. Integrative and adaptive models that cover the non-linearity in a system are envisioned in information processing in ecology (Park and Chon, 2007). Machine learning methods offer an advantage over traditional analysis techniques, because they do not introduce any prior assumptions about the relationship between variables (Džeroski and Drumm, 2003). Several data-driven modelling techniques, such as Decision Trees, Artificial Neural Networks, Bayesian Belief Networks and Support Vector Machines have been proven to be successful in predicting the presence and distribution of species in aquatic ecosystems (Adriaenssens et al., 2004; Goethals et al., 2007; Boets et al., 2010a; Hoang et al., 2010; Dominguez-Granda et al., 2011; Everaert et al., 2011). Selection of modelling techniques may be based on specific study objectives or the format of response variables (i.e. presence-absence versus abundance) and the availability and resolution of predictor variables, such as climatic, physical-chemical and land-use data, which can be related to a species' occurrence and abundance. However, the selection among different modelling approaches is sometimes based on cost or convenience (Stohlgren et al., 2010). In this study, we opted to use classification and regression trees to predict the presence, abundance and species richness of alien macrocrustaceans in surface waters in Flanders, since these techniques are widely applied and yield results that are easy to interpret (Dakou et al., 2007; Vlacklavik and Meentemeyer, 2009; Boets et al., 2010a; Kampichler et al. 2010; Everaert et al., 2011). If we want to prevent the introduction and to reduce the impact of alien macrocrustaceans, a strict policy is needed. In this context, models could help to support decision-making in water management by inducing measures for those regions which are at high risk.

In the present study, our goal was: (1) to predict in which habitats alien macrocrustaceans are likely to establish, (2) to determine which parameters positively or negatively influence the species richness of alien macrocrustaceans, (3) to assess which environmental conditions are favourable for alien macrocrustaceans to build up high densities and become dominant and (4) to make predictions on the future distribution of alien macrocrustaceans based on an integrated modelling approach. For the latter, habitat suitability models were combined with water quality models, which predict changes in chemical water quality due to the installation of planned wastewater treatment plants.

7.2 Materials and Methods

7.2.1. Data collection

The dataset consisted of biological and chemical data collected by the Flemish Environment Agency (VVM). The model development was based on all samples collected during the year 2004, because this year was characterized by intensive sampling (over 800 samples) spread over Flanders. Macroinvertebrates were collected by standard handnet sampling or via artificial substrates if it was not possible to use the kick sampling method (Gabriels et al., 2010). Macroinvertebrates were identified to the level needed for the calculation of the Multimetric Macroinvertebrate Index Flanders (MMIF; Gabriels et al., 2010). Indigenous and alien species can belong to the same family and therefore, it was not clear from the VMM database if alien macrocrustaceans occurred in the samples. Since we wanted to make predictive models for alien macrocrustaceans, only species belonging to this group were identified to species level. In this way, data on the presence/absence, the abundance and the species richness of alien macrocrustaceans present per sampling location was available. Conductivity, pH and dissolved oxygen were always measured in the field during macroinvertebrate sampling. Other chemical parameters were retrieved from monitoring data. As the chemical monitoring, which was usually performed on a monthly basis, was not carried out simultaneously with the macroinvertebrate sampling, measurements from the last date before macroinvertebrate sampling were used. The slope of a watercourse was determined based on the difference in height between two points 1000m apart, using GIS-software (version 9.3.1) applied on the Flemish Hydrographic Atlas (AGIV, 2006). The same data were used to determine the sinuosity on a stretch of 100m. River morphology was evaluated based on pictures of the sampling sites: pool-riffle pattern and meandering were both quoted from 0 (absent) to 5 (well developed) and summed, which yielded a score from 0 to 10. Information on the number of passing ships on navigable waterways originated from the annual reports by nv De Scheepvaart and the River Information Services. For each sampling point, it was indicated whether ships passed or not and if so, how many ships passed on an annual base (based on the report of the year 2009). The complete dataset consisted of three response variables (presence/absence, number of alien macrocrustaceans and abundance of alien macrocrustaceans) and 16 predictor variables, two of which were discrete and 14 continuous (Table 7.1).

7.2.2 Model development and validation

Two types of decision trees were used to construct the models: classification and regression trees. A decision tree is called a classification tree (CT) if the response variable is qualitative (e.g. presence/absence of alien macrocrustaceans) and a regression tree (RT) if the response variable is quantitative (e.g. alien species richness or abundance). Decision trees were grown with a recursive partitioning algorithm from a training set of records, which is known as 'Top-Down Induction of Decision Trees' (Quinlan, 1992). For each step, the most informative input variable is selected as the root of the sub-tree and the current training set is split into subsets according to the values of the selected input variable. In this way, rules are generated that relate the predictor variables (e.g. river morphology) with the response variables (e.g. presence/absence of alien macrocrustaceans). For discrete predictor variables, a branch of a tree is typically created for each possible value of that particular variable. For continuous predictor variables, a threshold is selected and two branches are created based on that threshold. Tree construction ends when the variance of the class values of all examples in a node is within a certain range. Such nodes are called leaves and are labelled with a regression equation in case of regression trees or with the corresponding value of a class in case of classification trees (e.g. presence or absence).

Table 7.1 *Average as well as minimum and maximum values (the range is indicated between brackets) of the assessed environmental parameters for the three constructed models: habitat preference, species richness and abundance of alien macrocrustaceans (BOD: Biological Oxygen Demand; COD: Chemical Oxygen Demand).*

Variable	Unit	Habitat preference	Alien species richness	Abundance of alien species
Ammonium	mg N l^{-1}	1.82 (0.04-48.6)	1.38 (0.06-10.6)	1.49 (0.08-10.8)
BOD	mg l^{-1}	5.71 (1.0-354)	4.44 (1.06-30)	4.70 (1.0-30)
COD	mg l^{-1}	34.7 (2.5-680)	32.9 (8.0-132)	33.7 (2.5-132)
Dissolved Oxygen	mg l^{-1}	7.0 (0.2-28.3)	7.07 (1.0-21.6)	7.11 (0.5-28.3)
Conductivity	µS/cm	1109 (90-17570)	1612 (90-17570)	1397 (90-17570)
Kjeldahl Nitrogen	mg N l^{-1}	3.9 (0.8-163)	2.66 (0.8-119)	2.89 (0.80-12.2)
Nitrate	mg N l^{-1}	3.3 (0.1-31.4)	2.7 (0.1-15.7)	3.1 (0.2-18)
Nitrite	mg N l^{-1}	0.16 (0.002-3.0)	0.14 (0.002-0.87)	0.15 (0.002-0.87)
Orthophosphate	mg P l^{-1}	0.46 (0.003-16.0)	0.38 (0.004-2.38)	0.38 (0.005-3.33)
Total Phosphorus	mg P l^{-1}	1.06 (0.05-100)	0.82 (0.06-6.17)	0.82 (0.05-6.17)
pH		7.7 (6.0-9.4)	7.7 (6.4-9.2)	7.7 (6.0-9.4)
Sinuosity		1.04 (0.0-1.98)	1.03 (0.0-1.74)	1.05 (0.0-1.89)
Slope	m $1000m^{-1}$	2.24 (0.0-42.5)	1.33 (0.0-20.5)	1.67 (0.0-20.5)
Number of ships		934 (0-32772)	2032 (0-32772)	1840 (0-32772)
River Morphology	classes (0-10)	3 (0-10)	3 (0-10)	3 (0-8)
Number of alien Crustacea	species/sample	0.44 (0-5)	1.2 (0-5)	1 (0-5)
Abundance of alien Crustacea	individuals/sample	10 (0-224)	25 (0-202)	22 (0-202)
Shipping	class (0,1)	present (n=107); absent (n=776)	present (n=30); absent (n=111)	present (n=51); absent (n=222)
Alien Crustacea	class (0,1)	present (n=299); absent (n=584)	present (n=94); absent (n=47)	present (n=182); absent (n=91)

Pruning was performed to prevent trees from over-fitting data (Džeroski and Drumm, 2003) and to make them easily interpretable (Dakou et al., 2007). Pruning can be used during tree construction (pre-pruning) and/or after the tree has been constructed (post-pruning). Pre-pruning is achieved when a minimum number of instances are needed before branching continues. Post-pruning on the other hand, implies that by changing the pruning confidence factor (PCF) some of the ending sub-trees of a highly branched tree can be replaced by leaves. In our case, pre- as well as post-pruning were performed as optimisation techniques.

The model training and evaluation was based on a three-fold cross validation. The dataset was, after reshuffling, randomly split in three subsets: two thirds were used for training and one third for validation. For each training and validation set a model was build and in this way, a performance value for each of the three different models was obtained. Average performance was used as final criterion for model evaluation. Model performance was based on the percentage Correctly Classified Instances (CCI) and Cohen's Kappa Statistic (K) for classification trees and the multiple correlation coefficient (R) for regression trees. In order to reach a satisfactory model performance, the CCI should be at least 70% and K should be at least 0.4 (Gabriels et al., 2007). For the multiple correlation coefficient, the closer the value is to 1, the better the model predicts the data (Everaert et al., 2010).

For the construction of the classification trees, the J48 algorithm was applied (Hall et al., 2009), which is a re-implementation of the C4.5 algorithm. Regression trees were built using M5' (Wang and Witten, 1997), a re-implementation of the M5 algorithm (Quinlan, 1992). For both techniques, the standard settings from the machine learning package WEKA were applied (Witten and Frank, 2005), except for the PCF (pruning confidence factor) and the minimum number of instances required for further splits, which were adapted in order to obtain the most optimal model. The most optimal model was defined as a model with a good balance between a good technical performance (CCI, K) on the one hand and a high ecological relevance and reduced complexity on the other hand.

In total, 882 samples of the year 2004 from different sampling locations scattered over surface waters (different water types) in Flanders and comprising biological as well as physical-chemical and shipping data were used to build the models. All used response and predictor variables are listed in Table 7.1. Three different datasets were compiled, which were either used to predict habitat preference, abundance or species richness of alien macrocrustaceans.

Classification trees, which were used to model habitat preference of alien macrocrustaceans, can deal very well with missing data and outliers (Pham, 2006). Therefore, all data (882 instances) were used when applying this technique. When using regression trees, missing data and outliers (based on all values exceeding three times the standard deviation) were removed and the database was stratified, since this generally yields more consistent and robust performances (Everaert et al., 2010). Stratification implied that each possible outcome was represented by the same number of instances in the database. This resulted in total in 273 instances that were used for the abundance model and 141 instances that were used for the model predicting the species richness of alien macrocrustaceans.

7.2.3 Sensitivity analysis

Sensitivity analysis was done for the regression tree models to determine the weight of each variable in the regression equations as well as to check the robustness of the constructed models. For each parameter the minimum, maximum and average values were determined (Table 7.1). Afterwards, the outcome for each of these equations of the selected model was calculated by keeping all parameters constant (averages) except for the one which we wanted to analyse the sensitivity off, which ranged from its minimum to its maximum value. Dividing the maximum by the minimum outcome of the regression equation yielded a factor indicating the importance of each parameter. In addition, the effect of conductivity on the species richness and abundance of alien macrocrustaceans in relation to the other parameters was graphically illustrated for each of the three folds by keeping all other parameters constant (average values) except for conductivity which ranged from low (fresh water conditions) to high values (brackish water conditions).

7.2.4 Future dispersal

Predictions on the future prevalence, species richness and abundance of alien macrocrustaceans were made based on an integrated modelling approach. The constructed classification and regression tree models were combined with predictions on the improvement of the chemical water quality (PEGASE water quality model) due to the installation of planned wastewater treatment plants (Ronse and D'heygere, 2007). With the PEGASE model, physical-chemical data were modelled for three years: 2006 (reference data), 2015 and 2027, according to the deadlines set by the European Union Water Framework Directive (European Union, 2000). Based on previous research regarding the distribution of alien macrocrustaceans in Flanders (Boets et al., 2011a) and for practical reasons (e.g. due to the fact that not for all catchments in Flanders PEGASE data were available), we opted to investigate the distribution of alien macrocrustaceans in a selected catchment in Flanders (the canal Ghent-Terneuzen and its tributaries). Data generated per segment by the water quality model on physical-chemical water quality parameters were used as input for our habitat suitability models to make predictions on the future distribution. We assumed that shipping and number of ships remained constant, as we did not have predictive data on the future shipping intensity. Conductivity was kept constant as this parameter is not included in the PEGASE water quality model and no predictions on possible changes could be made. Finally, sinuosity and river morphology were kept constant as well, as these parameters are not expected to change in this timeframe. All other physical-chemical water quality parameters changed according to the water quality model. The final outcome for the different models was calculated and afterwards visualised in ArcMap (version 9.3.1).

7.3 Results

7.3.1 Habitat preference

The presence or absence of alien macrocrustaceans could be accurately predicted based on the physical-chemical variables and the information regarding shipping. Only this tree that had an acceptable reliability in combination with a low complexity and a high ecological relevance was selected and presented (Fig. 7.1). After pruning (PCF = 0.25), a classification tree with four leaves was constructed. With this tree, 72 ± 4% of the instances were correctly classified and K = 0.28 ± 0.06. The model revealed that alien macrocrustaceans are present at high conductivities (>1740μS cm^{-1}), which could be ascribed to brackish waters. If the conductivity was lower than or equalled 1740 μS cm^{-1}, other factors determined whether alien macrocrustaceans were present or not. In fresh water, alien macrocrustaceans were present in water with a low conductivity, where ships passed and where the orthophosphate concentration was lower than 0.2 mg l^{-1} (Fig. 7.1). This indicates that under freshwater conditions, shipping in combination with a good chemical water quality promote the occurrence of alien macrocrustaceans.

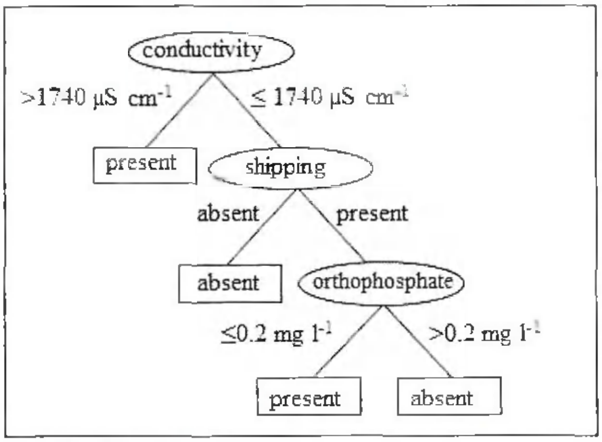
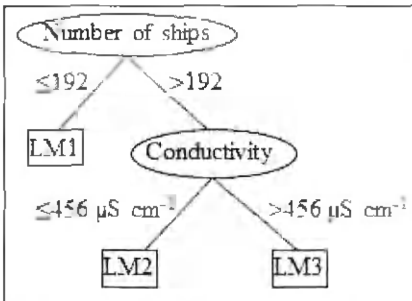


Figure 7.1 *Classification tree predicting the presence or absence of alien macrocrustaceans in surface waters in Flanders (Pruning Confidence Factor = 0.25; Correctly Classified Instances = 72 ± 4%; Cohen's Kappa = 0.28 ± 0.06).*

7.3.2 Species richness

Similar to the model for habitat preference, only the model having an acceptable reliability in combination with a low complexity and a high ecological relevance is given in the results (Fig. 7.2). The model predicting the species richness of alien macrocrustaceans consisted of a regression tree with three leaves and an average performance of $R = 0.59 \pm 0.06$ (Fig. 7.2). The species richness of alien macrocrustaceans could be predicted as follows: if the number of ships was lower than or equal to 192 per year, the linear model 1 (LM1) was used. LM1 consisted of the variables chemical oxygen demand (COD), total phosphorus, conductivity, Kjeldahl nitrogen and slope. According to LM1, the number of alien macrocrustacean species present in the surface waters increased with increasing conductivity. If the number of ships was higher than 192 and the conductivity was lower than 456 µS cm^{-1}, the model LM2 was applied. LM2 used the same variables as LM1 and they contributed in a similar way to the increase or decrease of alien species richness. Finally, if the number of ships was higher than 192 and the conductivity was higher than 456 µS cm^{-1}, the alien species richness was determined by LM3. LM3 used the same variables as LM1 and LM2, but in this case, increasing conductivity as well as an increasing phosphorus concentration positively contributed to the established species richness of alien macrocrustaceans. Based on these regression equations, the average alien species richness was calculated for the different linear models. This resulted for LM1 (based on 112 sites) in 1.1 species, for LM2 (based on 5 sites) in 2.7 species and for LM3 (based on 21 sites) in 1.9 species.



(LM1): Species richness = - 0.002 * COD - 0.295 * Total Phosphorus + 0.0001 * Conductivity - 0.106 * Kjeldahl Nitrogen - 0.080 * Slope + 1.660
(LM2): Species richness = - 0.005 * COD - 0.132 * Total Phosphorus + 0.0001 * Conductivity - 0.242 * Kjeldahl Nitrogen - 0.035 * Slope + 3.065
(LM3): Species richness = -0.005 * COD + 0.208 * Total Phosphorus + 0.0001 * Conductivity - 0.298 * Kjeldahl Nitrogen - 0.035 * Slope + 2.367

***Figure** 7.2 Regression tree with regression equations predicting the species richness of alien macrocrustaceans in surface waters in Flanders (Minimum Number of Instances = 4; Correlation Coefficient = 0.59 ± 0.06).*

Sensitivity analysis indicated that when intensive ship traffic (> 192 ships per year) was present, the alien species richness was generally higher compared to low levels of ship traffic (< 192 ships per year) (Fig. 7.3a, b). Conductivity was an important variable determining the alien species richness, especially at higher conductivities, since after a certain threshold (456 $\mu S\ cm^{-1}$), the alien species richness increased with increasing conductivity (Fig. 7.3b). The highest species richness of alien macrocrustaceans was reached in freshwater with a good chemical water quality and intensive ship traffic. Sensitivity analysis of the linear regression equations pointed out that Kjeldahl nitrogen had a major contribution in determining the alien species richness. High levels of Kjeldahl nitrogen (> 10 mg l^{-1}) reduced the species richness of alien macrocrustaceans substantially.

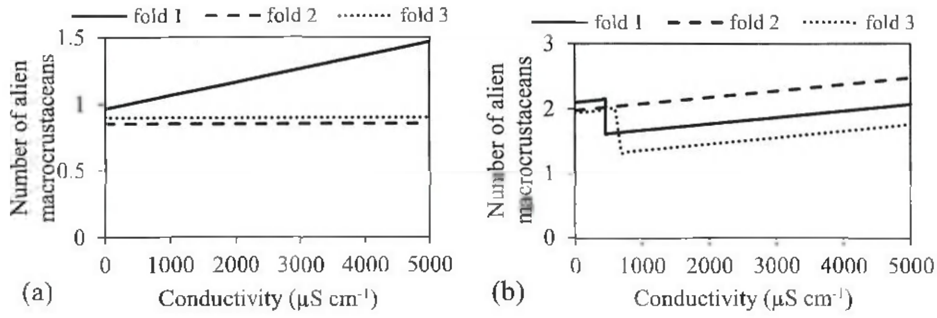
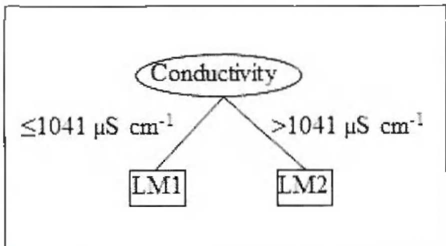


Figure 7.3 *Sensitivity analysis illustrating the effect of changing conductivity on the species richness of alien macrocrustaceans (a) at low (< 192 ships per year) and (b) high levels of ship traffic (> 192 ships per year) for all three folds.*

7.3.3 Abundance

The most reliable and ecological relevant model yielded a regression tree with two leaves and a correlation coefficient (R) of 0.56 ± 0.08 , which predicted the abundance of alien macrocustaceans in surface waters in Flanders (Fig. 7.4).



(LM1): Abundance = - 0.251 * Ammonium + 0.024 * COD - 0.013 * Conductivity + 8.412 * pH + 0.002 * Number of Ships - 49.994

(LM2): Abundance = - 0.666 * Ammonium + 0.697 * COD + 0.002 * Conductivity - 7.929 * Kjeldahl Nitrogen + 30.739 * Orthophosphate + 3.926 * pH + 0.0005 * Number of Ships + 0.4635

Figure 7.4 *Regression tree with regression equations predicting the abundance of alien macrocrustaceans in surface waters in Flanders (Minimum Number of Instances = 4; Correlation Coefficient = 0.56 ± 0.08).*

If the conductivity was lower than or equal to 1041 μS cm^{-1}, the linear model LM1 was used. LM1 predicts the abundance of alien macrocrustaceans as a function of the variables ammonium, COD, conductivity, pH and the number of ships. Ammonium and conductivity have a negative influence on the abundance, whereas the abundance increases with increasing COD, the number of ships and pH. The average number of individuals calculated based on the 209 sites to which the equation could be applied was nine. In fresh waters, conductivity and ammonium negatively affect the abundance, which indicates that with increasing nutrient content, the abundance decreased. If the conductivity was higher than 1041 μS cm^{-1}, the linear model LM2 was used. LM2 used the variables ammonium, COD, conductivity, Kjeldahl nitrogen, orthophosphate, pH and number of ships to determine the abundance of alien macrocrustaceans. Only ammonium and Kjeldahl nitrogen negatively contributed to the abundance, whereas for all other predictor variables, the abundance increased when some or all of these variables increased. LM2 was applied to 64 sites with on average 64 individuals. This indicates that alien macrocrustaceans could reach higher abundances in brackish waters (high conductivity) and that high levels of orthophosphate do not necessarily negatively influence the abundance. In fresh water, increasing conductivity lead to a decrease in abundance of alien macrocrustaceans, whereas in brackish water increasing conductivity influenced the abundance positively (Fig. 7.5). Sensitivity analysis revealed that the regression equation for freshwater (LM1) was mostly influenced by the number of ships, whereas for brackish water (LM2) Kjeldahl nitrogen exerted an important negative effect on the abundance of alien macrocrustaceans.

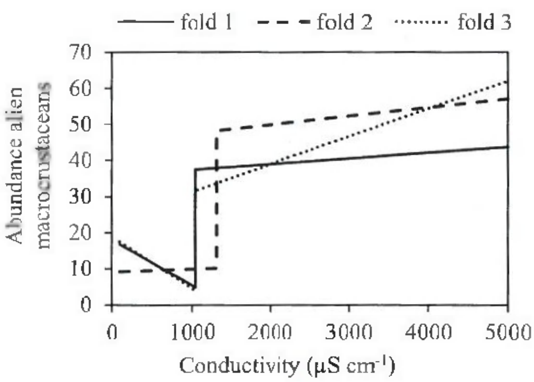


Figure 7.5 *Sensitivity analyses illustrating the effect of changing conductivity on the abundance of alien macrocrustaceans for all three folds.*

7.3.4 Future dispersal

Based on our integrated model, we made predictions on the future prevalence, alien species richness and abundance of alien macrocrustaceans for the canal Ghent-Terneuzen and its tributaries. The model predicts an increase in prevalence of alien macrocrustaceans of 10% by the year 2027 (Fig. 7.6). The small canals around the city of Ghent as well as a tributary in the east are likely to be colonised by alien macrocrustaceans. There is also an increase in predicted alien species richness over the years (Fig. 7.7). Compared to the reference situation

7.4 Discussion

7.4.1 Habitat modelling

Habitat suitability models can be applied to predict the potential distribution of alien species and to reveal their ecological niche preferences (Peterson, 2003; Pitt et al., 2009; Drake and Bossenbroek, 2009). These models can identify habitats at risk of invasion, which can help subsequent management efforts to maximize the efficacy of preventive measures to stop the spread of invasive alien species. Although these models are not 100% accurate in their predictions, they offer information regarding species preferences and their potential for invasion (Ba et al., 2010; Boets et al., 2010a). Our developed habitat suitability models revealed that alien macrocrustaceans especially occur in brackish waters or freshwater with intensive ship traffic and low nutrient levels. Both salinity and shipping are known to be important parameters influencing the establishment and spread of alien macroinvertebrates (Bij de Vaate et al., 2002; Grabowski et al., 2009; Ba et al., 2010; Everaert et al., 2011).

Brackish waters are typically characterised by a low density and diversity of indigenous species, hence it is easier for alien species to establish (Remane, 1958; Wolf, 1999). These waters with unsaturated ecological niches have a high potential to be invaded by alien macroinvertebrates (Paavola et al., 2005). Many alien macrocrustaceans are tolerant towards high salinities and therefore, they can easily establish in brackish water (Grabowski et al., 2007). In addition, it is assumed that brackish water species have a better chance of being transported alive than marine or freshwater species (Wolff, 1999). Grabowski et al. (2009) found that in the two largest rivers in the Baltic basin in Poland, alien amphipods were mostly found at conductivities above 800 $\mu S\ cm^{-1}$, where they reached high population densities. Moreover, brackish waters are subjected to a two sided 'invasion pressure', since species introduced in the freshwater as well as in the marine environment have the opportunity to migrate to these brackish waters (Nehring, 2006). Indigenous species inhabiting rivers with an increased conductivity risk to be displaced by alien ones, since alien species often have a competitive advantage over indigenous species in polluted rivers (Grabowski et al., 2009). Organic discharges should therefore be minimized at all time, since these could ease the further spread of alien species (Grabowski et al., 2009).

Shipping is recognised as the most important vector of aquatic alien species introductions to Europe (Bij de Vaate et al, 2002; Gollasch and Nehring, 2006). Improved ship design allows larger and faster ships, resulting in more frequent ship arrivals and larger amounts of ballast water being released. The construction of faster ships resulted in shorter voyages and consequently improved survival of alien species (Gollasch and Nehring, 2006). Harbours and rivers with intensive ship traffic can be seen as hotspots for species introductions. Once an alien species is introduced into its new habitat, secondary dispersal via ballast water or hull fouling of ships can account for a substantial part of alien species dispersal (Bij de Vaate et al., 2002).

The model predicting species richness of alien macrocrustaceans indicated that intensive ship traffic in combination with conductivity were the main factors determining a high alien species richness. As mentioned earlier, shipping is a key vector of species introductions (Bij de Vaate et al., 2002). Shipping activity is seen as an important proxy variable of propagule pressure (Ricciardi, 2006). The 'propagule pressure' concept focuses on the number of invading propagules for a given introduction and the frequency with which they are introduced (Williamson and Fitter, 1996). The more ships pass in a water body, the higher the chance that alien species become established and the higher the chance of an increased alien species richness. This phenomenon was also observed in the river Rhine, where the number of alien taxa decreased upstream with decreasing cargo transport (Wirth et al., 2010). Shipping is most intensive in man-made river ecosystems (e.g. canals) with artificial or semi-natural embankments (reinforced by stones and concrete). These habitat conditions are often very attractive for alien species to establish and to become dominant. Boets et al. (2010) found that the invasive alien species *Dikerogammarus villosus* thrives very well in canals with artificial concrete riverbanks. Many alien macroinvertebrates can easily colonise and establish stable populations on these hard substrates (van Riel et al., 2006b) and are therefore preferred habitats.

Besides the possibility to reach a new habitat and become established, favourable environmental conditions are important to build up viable populations. The abundance of alien macrocrustaceans was mainly determined by conductivity. The average abundance calculated based on the linear regression equations was lower in freshwater (conductivity $\leq$1041 $\mu S\ cm^{-1}$) compared to brackish water (conductivity >1041 $\mu S\ cm^{-1}$). It was found that in urban and densely populated areas, where high amounts of nutrients end up in river systems, the abundance of indigenous species can be reduced and that of alien species increased (Vermonden et al., 2010). At their initial introduction stage, alien species can often have a competitive advantage at high nutrient concentrations (contributing to a low water quality) compared to indigenous species (Grabowski et al., 2007; Strayer, 2010). With increasing chemical as well as biological water quality, indigenous species might again be able to compete with alien species. Leuven et al. (2009) found that in urban waters in the Netherlands, indigenous macroinvertebrates were able to coexist and even dominate alien species in nutrient-poor, densely vegetated systems. However, our models indicate that also alien species, at least under freshwater conditions, can benefit from an improving water quality and that high nutrient concentrations can negatively affect the alien species richness. The high abundances detected at higher conductivities could be attributed to the presence of species like *Gammarus tigrinus*, which especially occurs in brackish waters in Flanders, where it can reach high abundances (Boets et al., 2011b). This species shows a wide tolerance towards low or high levels of salinity, but is generally present in waters with a conductivity between 1200 and 3200 $\mu S\ cm^{-1}$. The high abundances of alien macrocrustaceans found at high conductivities could be ascribed to the high abundance of *G. tigrinus* present in these brackish waters.

7.4.2 Integrated modelling

Integrated models are often based on a combination of environmental and climatic conditions (e.g. Ficetola et al., 2007; Gallien et al., 2010). Habitats that meet these environmental and climatic constraints are identified as vulnerable for invasions. In this paper, a somewhat different approach was used: a habitat suitability model was coupled with a predictive water quality model. Based on such an integrated model, it is expected that in 15 years, there will be an increase in prevalence and species richness of alien macrocrustaceans, but the abundance is predicted to decrease at some locations and to remain stable between 10 to 100 individuals per sample. At an initial stage, alien macrocrustaceans can have a competitive advantage in habitats with a low water quality and high nutrient levels. However, alien macrocrustaceans can also benefit from an improvement in the chemical water quality, especially in those watercourses that evolve from a bad to a moderate water quality (Boets et al., 2011a). Due to the installation of wastewater treatment plants in Flanders, the chemical water quality is predicted to improve during the coming decades and therefore, our integrated model can give more accurate predictions on the future distribution of alien macrocrustaceans compared to simple habitat suitability models. Via this integrated model, valuable insight in the future potential invasive range of alien macrocrustaceans is given.

It has been suggested to incorporate species migration, population dynamics, biotic interactions and community ecology into species distribution models at multiple spatial scales (Guisan and Thuiller, 2005). In the next step, our integrated model could be optimized by including also dispersal of alien macrocrustaceans via a migration model. In our current model, species can only be present if an appropriate vector (shipping) is present, which imposes some limitations. The fact that species can actively colonise new areas and the time needed for this could be included in future models and give more accurate predictions. Recently, Gallardo et al. (2012) combined a large scale bioclimatic model with a local-scale migration model to predict the future distribution of *Dikerogammarus villosus* in Great Britain. Based on different scenarios of the annual migration speed, they were able to predict the dispersal of this alien macrocrustacean within the Great Ouse River catchment. They concluded that this approach helps to prevent and control the spread of invasive alien species and consequently can provide managers with a powerful spatial and temporal basis for informed decision-making.

7.4.3 Model performance

The performance of the habitat preference model was fair to moderate according to Gabriels et al. (2007). The variation on the different folds that were used was limited, which is an indication of the robustness of the constructed models. The fact that the performance was not very high could be due to some factors inherent to alien species. First of all, alien species may not yet have spread to all suitable habitats, making it difficult to determine species-environment relationships (Stohlgren et al., 2010). Secondly, alien species are often characterised as very opportunistic species, being able to easily cope with changes in environmental conditions (Williamson and Fitter, 1996; Nehring, 2006). Alien species can be seen as generalists, invading those niches which are available. Most alien species are omnivores and consequently, they do not pose any specific requirements regarding food availability (Strayer, 2010). All these elements make it difficult to accurately predict the habitat suitability and the distribution range of alien macrocrustaceans. Araújo and Guisan (2006) suggest that evaluation strategies should be discussed in the context of three possible uses: description, understanding and prediction. Complexity of model evaluation increases from explanation to prediction to the point where models that simply seek to describe a given pattern may not need to be evaluated, whereas the evaluation of models aiming at prediction is desirable but not always conceptually possible. Even though the accuracies of the models were not very high, Dzeroski and Drumm (2003) state that, when using these techniques, we should bear in mind that the primary goal of such an analysis is to pinpoint the essential site characteristics rather than to predict the exact number of species. A major difficulty in applying data mining techniques for modeling invasive alien species is related to settings selection to obtain the most optimal model. The performance of models can be assessed from different perspectives, usually accounting for technical reliability, ecological relevance and user convenience. However, it is difficult to find a balance between these criteria, which are moreover to some extend both synergistic and antagonistic. Consequently, to compare and select optimal settings, there is a need for frameworks that can guide model developers in this selection, based on the specific characteristics of the data as well as the needs and interest of the model users (Willems, 2010). Ensemble forecasting models (Araújo and New, 2007; Stohlgren et al., 2010), using presence as well as absence data (Phillips et al., 2009), incorporating dispersal using estimates of dispersal rates (Midgley et al., 2006) or developing spatially-explicit species distribution models (Harris et al., 2009; Iverson et al., 2009; Smolik et al., 2010) have been suggested to overcome the shortcomings of traditional modelling techniques. Nevertheless, we can conclude that our models are useful and understandable for determining those environmental parameters and conditions that are important for alien macrocrustaceans to establish and become dominant. These models could be used by decision makers to pinpoint those regions within the aquatic environment that are under severe threat of invasion by alien species.

7.5 Conclusion

Alien macrocrustaceans have a preference for waters with a high conductivity. Shipping in combination with a good chemical water quality promote the occurrence of alien macrocrustaceans. A maximum species richness of alien macrocrustaceans was reached in freshwater with a good chemical water quality and intensive ship traffic. In fresh water, increasing conductivity leads to a decrease in abundance of alien macrocrustaceans, whereas in brackish water increasing conductivity influenced the abundance positively. The predictions based on our integrated model approach indicated that the prevalence and species richness of alien macrocrustaceans is likely to increase with improving chemical water quality, whereas their abundance will probably decrease slightly. From this study, it is clear that models are a useful tool for decision making and that policy makers should focus on vulnerable areas, such as brackish water areas and areas with intensive ship traffic in order to prevent the further introduction and spread of alien species.

Chapter 8: Risk assessment of The Killer shrimp, *Dikerogammarus villosus*

Adapted from:

Boets P., Pauwels I.S., Lock K., Goethals P.L.M. (accepted). Using an integrated modelling approach for risk assessment of the 'killer shrimp' *Dikerogammarus villosus*. River Research and Applications.

Chapter 8: Risk assessment of The Killer shrimp, *Dikerogammarus villosus*

Abstract

The aim of this study was to predict the future distribution of an invasive alien macroinvertebrate species, *Dikerogammarus villosus*, under changing environmental conditions. For this, we used an integrated modelling approach. First, a habitat suitability model (HSM) was constructed based on a regression tree model, to determine the preferred chemical water quality conditions. Subsequently, this HSM was combined with a chemical water quality model that makes predictions on future water quality scenarios for the year 2015 and 2027. It was expected that the area of suitable habitat of *D. villosus* would increase with improving water quality conditions in the future. Finally, migration speed was incorporated to model the spatial-temporal spread of *D. villosus* based on a network analysis. Based on monitoring data of Flanders (Belgium), it was calculated that *D. villosus* is able to spread with an average speed of 5 km year^{-1}.The model simulations indicate that the species is primarily present in large rivers and canals with a good chemical water quality. With improving water quality the species will be able to colonise additional watercourses, mainly due to a decrease in chemical oxygen demand and orthophosphate concentration. A validation based on the observed occurrence shows that the model accurately predicts areas with a high suitability which are most likely to be invaded by *D. villosus*. Our integrated modelling approach is useful as a practical method to perform risk assessment for watercourses that are vulnerable to invasions not only in Flanders, but in the whole world.

8.1 Introduction

Future global biodiversity scenarios highlight potentially dramatic increases in biological invasions in European ecosystems as a consequence of rising atmospheric carbon concentrations, higher temperatures, altered disturbance regimes and increased habitat deterioration (Sala et al., 2000). Aquatic ecosystems are especially considered to be highly susceptible for invasions due to strong anthropogenic pressures (Gherardi, 2007b).

Recently, habitat suitability models (HSMs) have been used in risk assessment to predict the future distribution of invasive alien species (Ficetola et al., 2007; Ba et al., 2010; Boets et al., 2010a; Jiménez-Valverde et al., 2011; Boets et al., in press a). However, the large variability inherent to alien species and the limited ability to reach their maximal distribution in the invaded area make the prediction of biological invasions very complex. Consequently, simple HSMs sometimes fail in accurately predicting a species distribution (Sutherst and Bourne, 2009). Due to the complexity of biological invasions, an integrated and interdisciplinary approach is required to support the risk assessment and understanding of the processes involved. In this respect, combinations of modelling techniques at different spatial scales have recently been successfully applied (Ficetola et al., 2007; Smolik et al., 2010; Gallardo et al., 2012). Despite this, future changes in water quality or habitat structure may undermine the validity of projections based on the current situation. Climate change scenarios, taking into account changes in environmental conditions, are spatially still too coarse to provide accurate patterns of distributional changes at the local scale. Incorporating additional information on species dispersal, water quality and biotic interactions are required to accurately assess whether a species will be able to establish in a new niche.

Dikerogammarus villosus (Sowinsky, 1894) is a species originating from the Ponto-Caspian region that has been spreading throughout Western Europe. Recently, the species has also been detected in Great Britain in 2010 (MacNeil et al., 2010a) and is expected to invade North-America in the future (Ricciardi and Rasmussen, 1998). The species is one of the most successful invaders of freshwater systems (Pöckl, 2007; MacNeil et al., 2010a; MacNeil et al., 2012). Characteristics, such as predatory behaviour, a high reproductive output, relatively broad physical-chemical tolerance to environmental conditions and an omnivorous diet (Devin et al., 2004; Pöckl, 2007), have allowed it to become an abundant macrocrustacean in several European watercourses (e.g. van Riel et al., 2006a; Messiaen et al., 2010; MacNeil et al., 2010a). The species is known to have a large ecological and economic impact on aquatic ecosystems. Several papers report the decline of macroinvertebrate densities as well as fish with the arrival of this invader (Casellato et al., 2007; MacNeil et al., 2012, MacNeil et al., 2013).

In former decades, many watercourses were degraded, resulting in a decreased ecological water quality in Flanders. Thanks to the construction of wastewater treatment plants and stronger environmental regulations, the water quality has been improving substantially in Flanders during the nineties (MIRA, 2011). With the improvement in chemical water quality, also the ecological water quality has improved, which is not only beneficial for indigenous species, but also for invasive species, such as *D. villous* (Boets et al., 2011a). Therefore, it was expected that the area of suitable habitat of the species would increase with improving water quality. Additionally, a combination of changing favourable climatic, biotic and abiotic habitat conditions can ease the spread and determine the success and dominance of the species.

In this study, we used an integrated modelling approach to assess the future distribution of *D. villosus* under changing environmental conditions, resulting from expected investments in wastewater treatment infrastructure (MIRA, 2009). First, a HSM was constructed to determine the preferred chemical water quality conditions based on a regression tree model. Subsequently, this HSM was combined with an existing chemical water quality model that predicts the changes in water quality variables for the year 2015 and 2027. Finally, migration speed was calculated based on field data and subsequently incorporated to model the spatial-temporal spread of *D. villosus*. With this study, we aimed to evaluate the applicability of such a coupled modelling approach for decision makers to perform risk assessment for invasive species and to take preventive measures against the further spread of highly invasive aquatic species.

8.2 Material and methods

Our study was conducted in Flanders (northern part of Belgium), which is situated in the west of Europe and is bordered in the northwest by the North Sea (Fig. 8.1). Flanders comprises a total area of 13,521 km^2 and is a typical lowland area that is characterised by a dense network of navigable and non-navigable watercourses. Three main catchment districts are represented in Flanders of which two are international (the Scheldt district and the district of the Meuse). Due to its central position, several of the large watercourses connect Flanders with neighbouring countries, such as The Netherlands, Germany and France, which make it an important acceptor as well as donor region for aquatic alien species. Additionally, two large Flemish harbours: the harbour of Antwerp and the harbour of Zeebrugge, are hotspots for the introduction and secondary dispersal of aquatic alien species (Soors et al., 2010; Boets et al., 2012a). The killer shrimp, *Dikerogammarus villosus* was recorded for the first time in Belgium in 1997 in the Albert canal, situated in the east of Flanders (Messiaen *et al.*, 2010). The species quickly dispersed via large waterways and colonised the canal Ghent-Terneuzen in 2005 and only one year later the canal Ghent-Ostend, situated in the west of Flanders. Currently, the species has been recorded at 49 different locations, spread throughout Flanders

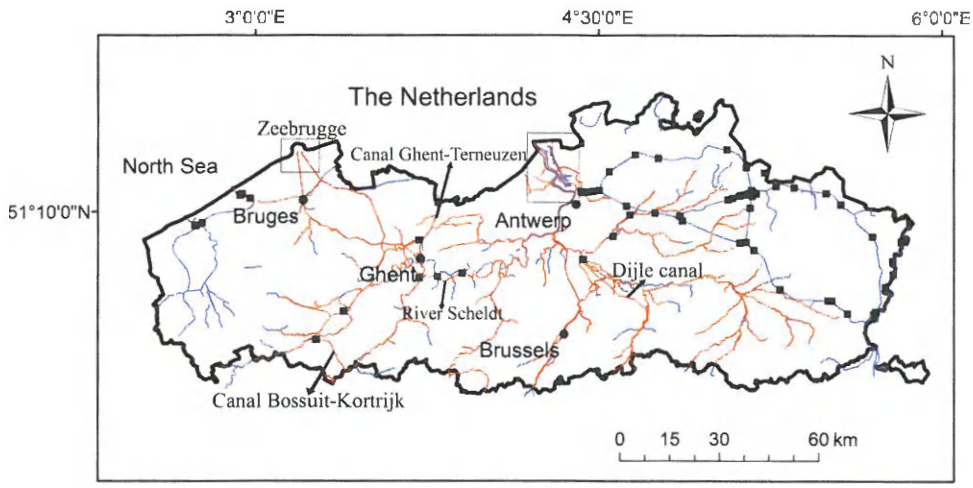


Figure 8.1 *Map of the study area (Flanders) with indication of the different locations where Dikerogammarus villosus was recorded from 1997 to 2010 (black squares). Watercourses for which chemical water quality data (PEGASE) are available are indicated in red, other watercourses are* indicated in blue. The harbours of Antwerp and Zeebrugge are indicated by a rectangle.

8.2.1 Habitat suitability model

The development of our habitat suitability model (HSM) was based on biological and physical-chemical data collected by the Flemish Environment Agency (VMM). For our model development we used a selection of 493 biological samples from 64 different sampling locations including presences and absences of *D. villosus* collected during the period 1990-2010. The selection of samples was based on previous research investigating the impact and spread of alien macrocrustaceans in Flanders (Messiaen et al., 2010; Boets et al., 2010a, 2011a, 2011b, Fig. 8.1).The biological samples were collected by standard hand net sampling or by artificial substrates if it was physically impossible to use the kick sampling method (Gabriels et al., 2010). Macroinvertebrates were identified by the VMM to the level (family or genus level) needed for the calculation of the Multimetric Macroinvertebrate Index Flanders (Gabriels et al., 2010). Since both indigenous and alien species can belong to the same family Gammaridae, it was not clear from the VMM database whether *Dikerogammarus villosus* occurred in the samples. Therefore, we identified all 493 biological samples collected by the VMM, containing Gammaridae, to species level.

Standard physical-chemical parameters (conductivity, pH and dissolved oxygen) were always measured in the field by the VMM (by means of hand electrodes) during macroinvertebrate sampling. Other chemical parameters (ammonium (NH_4), Kjeldahl nitrogen (KjN), nitrite (NO_2), nitrate (NO_3), orthophosphate (oPO_4), total phosphorous (P) and chemical oxygen demand (COD)) were retrieved from the VMM database. As the chemical monitoring, which was usually performed on a monthly basis, was not carried out simultaneously with the macroinvertebrate sampling, measurements from the last date before macroinvertebrate sampling were used. To avoid collinearity, we performed a correlation analysis in Statistica 7.0 based on a Spearman Rank Correlation analysis (Statsoft Inc., 2004). Ammonium, total phosphorous and nitrite were not included in the HSM development due to their high correlation coefficient with Kjeldahl nitrogen or orthophosphate that were used in the HSM: NH_4-KjN ($r = 0.75$, $p < 0.001$), P-oPO_4($r = 0.71$, $p < 0.001$) and NO_2-KjN ($r = 0.70$, $p < 0.001$). A selection of five chemical water quality variables (COD, dissolved oxygen, NO_3, KjN and oPO_4; Table 8.1), were finally included as predictor variables in our HSM. No other variables could be included because it would become impossible to couple the HSM with the available water quality model and to make future predictions afterwards. The incorporated chemical parameters, such as dissolved oxygen concentration, ammonium and COD are known to be important factors determining the occurrence of Gammaridae (Dedecker et al., 2005; Gabriels et al., 2007; Boets et al., 2010a).

To further optimise the HSM we included river type into the analysis, because it is known that *D. villosus* has a preference for large rivers and canals, which promote its quick dispersal (Josens et al., 2005; Leuven et al., 2009; Boets et al., 2010a; Messiaen et al., 2010). Therefore, river type (based on catchment area and salinity, see appendix 8.1) was used as a factor limiting the dispersal of *D. villosus*, although it was not directly included as a variable in our HSM. The following rivers types were selected: very large, large and small rivers.

Consequently, the HSM was applied only on the selected river types. It has been shown that hydro-morphological characteristics related to river type, such as substrate, water depth and bank structure can pose restrictions to the occurrence of *Dikerogammarus villosus* (Lods-Crozet and Reymond, 2006; van Riel et al., 2006a; Boets et al., 2010a).

Table 8.1 *Measured physical-chemical variables of the study area (Flanders) used as input variables to construct the habitat suitability model of Dikerogammarus villosus. Values are mean ± SD for sites where the species is present and sites where the species is absent (COD=chemical oxygen demand, KjN=Kjeldahl nitrogen).*

Physical-chemical variable (mg l^{-1})	Presence (n=237)	Absence (n=256)
COD	16.3±13.0	32.5±24.3
Dissolved oxygen	8.8±2.0	7.8±3.5
KjN	1.68±1.0	3.75±3.0
Nitrate	3.19±0.86	3.57±1.61
Orthophosphate	0.20±0.15	0.47±0.39

Data-driven regression trees were used to develop the HSM. Regression tree models were induced with a recursive partitioning algorithm from a training set of records, which is known as 'Top-Down Induction of Decision Trees' (Quinlan, 1992). For each step, the most informative input variable is selected as the root of the sub-tree and the current training set is split into subsets according to the values of the selected input variable. In this way, rules are generated that relate the predictor variables (e.g. dissolved oxygen) with the response variables (e.g. occurrence of *D. villosus*). For continuous predictor variables, a threshold is selected and two branches are created based on that threshold. Tree construction ends when the variance of the class values of all examples in a node is within a certain range. Such nodes are called leaves and are labelled with a regression equation. Regression tree models were built using M5', a re-implementation of the M5 algorithm (Quinlan, 1992). The standard settings from the machine learning package WEKA (version 3.6.6; Hall et al., 2009) were applied, except for the pruning parameters, which were adapted in order to obtain the most optimal tree. Pruning was performed to prevent trees from over-fitting data and to make them easily interpretable (Dakou et al., 2007). Pruning can be used during tree construction (pre-pruning) and/or after the tree has been constructed (post-pruning). In our case, pre- as well as post-pruning were used as optimisation techniques.

The model training and evaluation was based on a three-fold cross validation. The dataset was, randomly split in three subsets: two thirds were used for training and one third for validation. Model performance was based on the multiple correlation coefficient (R), the mean absolute error (MAE) and the root mean squared error (RMSE). A high R, a low MAE and a low RMSE imply good model performance and vice versa (Diamantopoulou et al., 2011). The most optimal regression tree was defined as a tree with a good balance between a good technical performance (high R, low MAE, low RMSE) on the one hand and a high ecological relevance and limited complexity on the other hand.

8.2.2 Water quality model

To simulate the changes in water quality, we made use of the PEGASE water quality model that was developed by Deliège et al. (2009) and that is used in Flanders to predict the chemical water quality by the years 2015 and 2027 (Ronse and D'heygere, 2007). The PEGASE model is a detailed hydrodynamic, deterministic water quality model that consists of three sub models: a hydrological and hydrodynamic sub model, a thermal sub model and a biological sub model (Ronse and D'heygere, 2007).

The flow (m^3 day^{-1}) is indispensable when determining the water quality because pollution loads are characterised by the flow and the concentration of pollutants in the water. The average flow per watercourse was calculated based on series of field measurements. Based on the measured flow, the natural flow could be determined by means of the mass balance. Besides flow, also the width of the watercourse (m), the slope (mm m^{-1}) and the coefficient of Manning (s $m^{-1/3}$) were incorporated. In addition, a digital elevation model and the land use were used to determine the natural flow. An important parameter of the thermal submodel was water temperature (°C), since this parameter influences the kinetics of processes in the water. Besides series of measurements of the water temperature, also measurements of solar radiation were used as input for the thermal model. Four types of processes were taken into account for the biological submodel: transportation and dilution, biochemical processes in the water column, interactions of the water column with the air near the water surface and interactions of the water column with the soil. Regarding the biochemical processes, the biomass of phytoplankton and –benthos, zooplankton and benthic filterers and heterotrophic bacteria were modelled. The integrated PEGASE model was calibrated and validated with measurements of BOD, COD, oxygen concentration, ammonium, nitrate, nitrite, Kjeldahl nitrogen, total nitrogen, orthophosphate and total phosphorous. The validation of the model with measured values generally resulted in accurate predictions of the different parameters (Ronse and D'heygere, 2007; Deliège et al., 2009; MIRA, 2009).

The data off the PEGASE model are restricted to the Flemish part of the Scheldt district (Fig. 8.1). The district of the river Yzer (southwest of Flanders) as well as part of the canals and watercourses situated in the east of Flanders (Meuse district) were not included. Consequently, no predictions on the future dispersal of *D. villosus* could be made for these districts. In addition, no PEGASE data on small streams were available. The dataset therefore only contained data on head watercourses, tributaries that had high inputs from pollution loads and important discharge and bypass canals (Ronse and D'heygere, 2007).

With the PEGASE model, physical-chemical data were generated for three years: 2006 (reference data), 2015 and 2027, according to the deadlines set by the European Water Framework Directive (European Union, 2000). The results are obtained for stretches of approximately 200 m. In the first scenario (2015), the standard policies as well as the proposed measures in the first period of the sanitation plans are implemented. In the second scenario (2027), all proposed restoration measures are implemented and the receiving water of the neighbouring countries is expected to be of good quality (for more details see MIRA, 2009).

8.2.3 Migration model

Several dispersal velocities have been reported for *D. villosus*. Josens et al. (2005) found that *D. villosus* spread with an average speed of 30-40 km year^{-1} in the River Meuse, while Leuven et al. (2009) reported a speed between 40 and 461 km year^{-1}, with a mean of 112 km year^{-1} in the River Rhine. In their analysis on the spread of *D. villosus* in the river Great Ouse (U.K.), Gallardo et al. (2012) distinguished between up- and downstream dispersal. They took 100 km year^{-1} as the maximum scenario and 20 km year^{-1} as the low speed scenario for downstream dispersal and chose different scenarios between 2 and 10 km year^{-1} for upstream dispersal. In the present study, dispersal velocity was determined based on the recorded occurrences of the species, since its first observation in Flanders in 1997. The hydrological network of Flanders, which was available in a format that was readily applicable in ArcGIS was retrieved from the VMM. A coherent route system was developed and the typology of the river network was checked before analysis. The distance between the different points of occurrence was calculated in ArcGIS using the linear referencing toolbox (©ESRI, ArcGIS

10). The mean dispersal value expressed as km $year^{-1}$ was implemented in the model to make the final predictions.

Using ArcGIS Network Analyst (©ESRI, ArcGIS 10), it is possible to dynamically model realistic network conditions, such as river networks. Before creating a network dataset using the network analyst tool, the quality of the hydrological network was checked and optimised using a topological validation with a correction of 1 m in Arcmap.

When integrating dispersal velocity, the spatial-temporal modelling depended on the distance between the occurrence of the species in 2006 (reference situation) and its maximal distribution that could be reached within a certain time frame. We made some simplifications of the environmental conditions, because we assumed that the flow was homogenous and that there were no physical barriers. We made no distinction between up- or downstream dispersal, because the flow rate of the rivers for which the potential spread was assessed is quite low (0.0-0.12 m s^{-1}, www.hydronet.be) and because, we found no significant difference between up- and downstream dispersal rate based on our distribution data. This is not surprising, since Flanders is situated in a typical lowland area (highest point 287 m a.s.l.) with many artificial waterways (e.g. canals) and streams and rivers with a small slope and thus a low flow velocity.

8.2.4 Integrated modelling

The developed HSM was combined with simulations on the changes in chemical water quality (PEGASE model; Deliège et al., 2009). Data generated per segment (200 m) by the water quality model were used as input for our HSM to make predictions on the future distribution of *D. villosus*. This type of model development is referred to as integrated modelling (Brandmeyer and Hassan, 2000). The following output parameters of the water quality model were included in the HSM: COD, dissolved oxygen, Kjeldahl nitrogen, nitrate and orthophosphate concentration. The output variables of the water quality model for the reference data of 2006 (different chemical water quality variables) were plotted in function of the habitat suitability to get insight in the relationship between these variables and the habitat suitability of *D. villosus*. Finally, migration speed was taken into account to predict the future distribution of *D. villosus* in 2015 and 2027. Migration speed was not included in the simulations for 2006, since 2006 was taken as a reference (initial points of introduction).

By coupling all three models discussed above, we assessed the future spread of *D. villosus* in Flanders based on the habitat suitability, the changes in environmental conditions and the migration speed. All final predictions were visualised in ArcGIS. The final integrated model was validated by comparing the observed occurrences of *D. villosus* in Flanders until 2006 with the simulation results of 2006. The model performance was based on the multiple correlation coefficient (R), the mean absolute error (MAE) and the root mean squared error (RMSE). In addition, the validity of our simulations for 2015 was evaluated by plotting and comparing the recorded occurrences of *D. villosus* until 2012.

8.3 Results

The habitat suitability model consisted of nine different regression equations and had a good performance ($R = 0.6$, MAE = 0.32, RMSE = 0.40) (see appendix 8.2). Our HSM pointed out that COD, oPO4, KjN and dissolved oxygen concentration are important variables determining the occurrence of *Dikerogammarus villosus*. High values for COD, KjN and oPO_4 negatively influence the habitat suitability of *D. villosus*, whereas high dissolved oxygen concentrations positively influence its habitat suitability (Fig. 8.2).

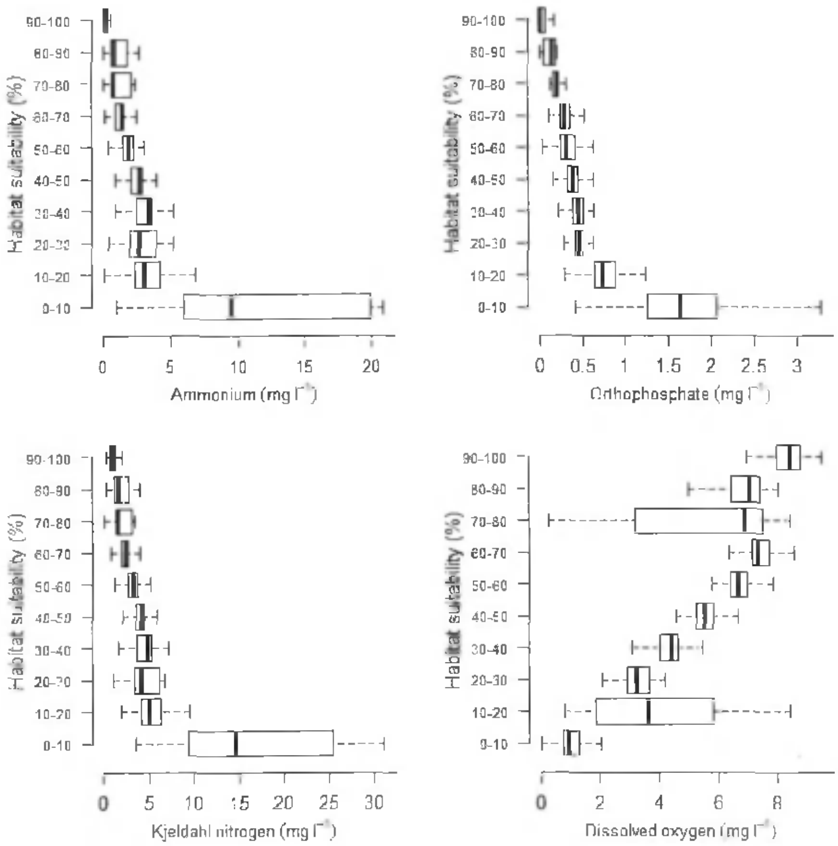


***Figure 8.2** Relationship between important physical-chemical variables (ammonium, orthophosphate, Kjeldahl nitrogen and dissolved oxygen), resulting from the water quality model and the predicted habitat suitability of Dikerogammarus villosus based on the constructed regression tree (habitat* suitability model).

Based on our integrated model, we simulated the habitat suitability of *D. villosus* for Flanders for three different years (Fig. 8.3). The reference data of 2006 indicate that 57 % of the rivers were still highly unsuitable (watercourses with less than 30% suitability) for *D. villosus*. The most vulnerable areas (watercourses with more than 70% suitability) were situated in the harbour of Antwerp, the river Scheldt and in the canal Bossuit-Kortrijk. Our calculations showed that *D. villosus* migrates in Flanders with an average speed of 5 km year^{-1}. Given this migration speed, predictions on the future distribution of *D. villosus* indicate a further dispersal by the year 2015 via the river Scheldt, the canal Ghent-Terneuzen and the canals connecting Ghent with the coast.

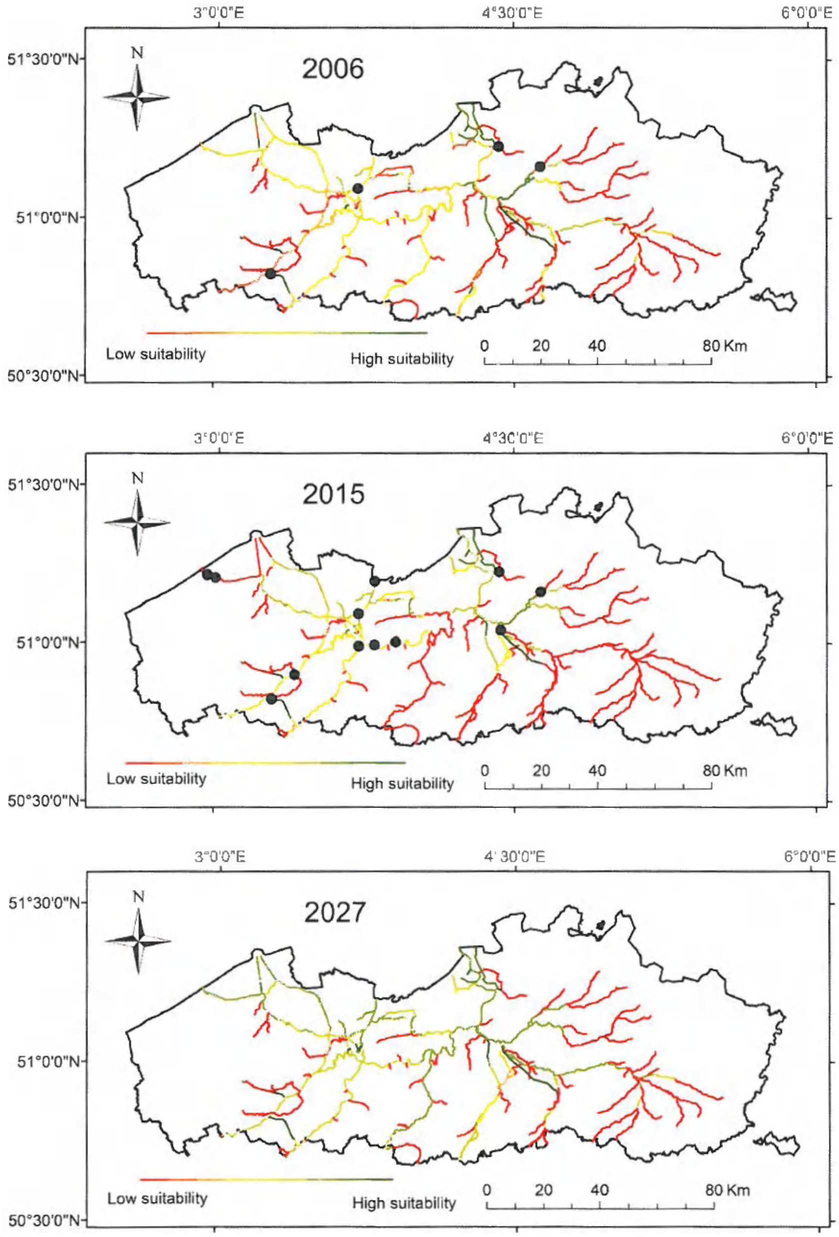


Figure 8.3 *Habitat suitability for Dikerogammarus villosus in the main rivers of Flanders based on an integrated modelling approach (integration of habitat suitability model, water quality model and migration model) for three different years 2006, 2015 and 2027. The recorded occurrences of D. villosus are plotted till 2006 on the reference map and till 2012 on the map showing the prediction of 2015. Colour code: dark red, not suitable to dark green, highly suitable.*

The harbour of Antwerp, the canal Bossuit-Kortrijk and the Dijle canal are most suited for the establishment of *D. villosus* (habitat suitability between 80 and 100%). Based on our migration model, it was clear that migration speed was not limiting the dispersal of *D. villosus* by 2027 since all watercourses were within the maximal migration distance (Fig. 8.3). Due to the improvement in water quality, most rivers and canals will be suitable for *D. villosus* by 2027. Hydro-morphological characteristics, such as river type, will be the key factors limiting its dispersal by 2027. Deep and large rivers with a hard bank structure and the presence of stones are the preferred habitat of *D. villosus*. Small rivers and brooks, with a high sinuosity, a natural bank structure and a loamy substrate will be more difficult to invade. The habitat suitability of watercourses with more than 70% suitability for *D. villosus* to establish increased from 7.7% in 2015 to 32.8% in 2027.

The performance of the integrated model was somewhat low: R = 0.21, MAE = 0.37 and RMSE = 0.45 for the simulations of 2006. Nevertheless, 3 out of 4 of the occurrence points were situated in a river that was predicted to have a high suitability (> 70%) for *D. villosus* to establish. When plotting the occurrence points recorded until 2012 on the outcome of the integrated model of 2015, six points of occurrence were situated in a river with a high suitability, four points were situated in a river with a moderate suitability (60%) and two points of occurrence were, although having suitable physical-chemical conditions, situated outside the possible migration range of *D. villosus*.

8.4 Discussion

The disappearance of geographic barriers due to construction of canals and the increase in global trade made it easier for *D. villosus* to invade new areas. Since the opening of the Danube-Main-Rhine canal in 1992, the killer shrimp has invaded many water bodies all over Europe. Currently, the species is found in several Western and Central European countries as well as the Baltic Sea and it is still continuing its spread. *D. villosus* was first recorded in the Albert canal situated in the east of Flanders (Belgium) in 1997. The species has been spreading gradually (Messiaen et al., 2010) and since 2002, some occurrences in the central and western part of Flanders have been reported. Its rapid spread in these canals can be ascribed to the high connectivity, intensive ship traffic and structural characteristics, such as hard substrates. In this study, we found that *D. villosus* spreads with an average speed of five km year^{-1} which is slower compared to earlier reports on the migration speed that are between 20 and 100 km per year for downstream dispersal and between 2 and 10 km for upstream dispersal (Leuven et al., 2009; Gallardo et al., 2012). The dispersal velocity may depend on a variety of biotic (e.g. density), abiotic (e.g. temperature, oxygen saturation) and hydro-morphological (e.g. stream velocity) factors and furthermore the occurrence of unsuitable patches may slow down its dispersal (Gallardo et al., 2012). Next to natural dispersal, also the dispersal through human mediated transport and human activities, such as fishing and shipping are important and could have facilitated its spread. This is supported by the differences in dispersal velocity reported in literature, which range between 2 to 461 km year^{-1}. In addition, despite models by Gallardo et al. (2012) predicted a progressive dispersal of *D. villosus* in the Great Ouse River catchment situated in southeast England, the species is after only two years already found in two completely different catchments isolated from the Great Ouse. Thus, although network models are a valuable tool to evaluate potential routes of invasion, they have some limitations with regard to the interference of human mediated dispersal. In this respect, the use of gravity models to predict long-distance dispersal has been proposed (Bossenbroek et al., 2001). They developed a production-constrained gravity model to forecast zebra mussel (*Dreissena polymorpha*) dispersal into inland lakes of Illinois, Indiana, Michigan and Wisconsin (USA). Taking into account human-related factors could improve the prediction accuracy and reduce the existing gaps between predicted invasion hot spots and actual occurrence.

Our integrated model demonstrated that *Dikerogammarus villosus* is likely to continue its spread in large rivers in Flanders due to an improvement of the chemical water quality caused by an increase in dissolved oxygen and a decrease in COD, nitrogen and phosphorous loads. Previous field observations in Belgium and Croatia found that the presence or absence of *D. villosus* is mainly determined by chemical water quality conditions, such as oxygen concentration and conductivity (Boets et al., 2010a; Boets et al., in press b). Dissolved oxygen concentration has been pinpointed as an important variable determining the habitat suitability of Gammaridae, including *Dikerogammarus villosus* (Kley and Maier, 2005; Boets et al., 2010a). Specifically, Dedecker et al. (2005) demonstrated that the abundance of the indigenous *Gammarus pulex* increased with an increasing level of dissolved oxygen. In a study conducted by Peeters and Gardeniers (1998), logistic regression as a function of several physical-chemical variables was used to define the habitat requirements of two common gammarids. They found that Kjeldahl nitrogen was, in addition to stream velocity, one of the main variables determining the habitat suitability.

Besides the chemical water quality also hydro-morphological factors, such as river type play an important role when determining the habitat suitability of *D. villosus*. Previous research in Flanders showed that the species mainly prefers rivers with an artificial bank structure (reinforced by stones and concrete) (Boets et al., 2010a). In Western Europe, the species is known to thrive very well in several large watercourses with a hard and stony bank structure. After its introduction in Germany, the species colonised the bare substrate of the river Rhine quickly and dominated the community within a few months (van Riel et al., 2006b). Also in other habitats, such as the lake Ijsselmeer in the Netherlands, the species preferred stones and complex habitats, where it co-existed with *Gammarus tigrinus* (Platvoet et al., 2009).

Other factors that might influence the dispersal of *D. villosus* are species interactions. According to the 'biotic resistance model' (Simberloff and Von Holle, 1999), a community characterised by high species richness is more resistant to the invasion of new species than is one with a low richness, due to limiting resources. However, this hypothesis can be violated since it has been frequently observed in aquatic ecosystems that previously introduced species facilitate the establishment of a species originating from the same endemic region. This facilitating mechanism seems to be more common than the classic biotic resistance theory (Simberloff and Von Holle, 1999). The earlier establishment by the zebra mussel (*Dreissena polymorpha* (Pallas, 1771)) is one of these interactions that may facilitate the establishment of *D. villosus*. Zebra mussel populations may facilitate the colonization of the killer shrimp by providing suitable habitat through the production of byssus threads and shells, which results in deposition of additional food material (Gergs and Rothaupt, 2008). Intraguild predation is another type of biotic interaction that may favour the establishment of *D. villosus* in gammarid communities. The predatory behaviour of *D. villosus* might partially explain its colonisation success of new areas since the species is able to eliminate both indigenous and alien macroinvertebrates (Dick et al., 2002; MacNeil et al., 2012).

When comparing the observed occurrence in 2012 with the predicted habitat suitability for 2015, 83% of the sites where *D. villosus* was recorded until 2012 were situated in rivers with a habitat suitability between 60 and 100%. This indicates the usefulness of our integrated model that could be used as a risk assessment tool. Although our integrated model was useful to predict the future habitat increase of *D. villosus*, the performance (based on *R*, MAE, RMSE) was rather low, which could be ascribed to several factors. When coupling all submodels into one final model, there is an additive increase in uncertainty. Next to the technical aspects that cause uncertainty, there are some characteristics specifically related to invasive alien species, which make it difficult to construct high accuracy models. *D. villosus* has been characterised as a very opportunistic species with an omnivorous diet (Platvoet et al., 2009), being able to easily cope with changes in environmental conditions (Wijnhoven et al., 2003). These are considered important traits explaining its invasion success, but at the same time impeding the prediction of its future dispersal. In addition, *D. villosus* may not yet have spread to all suitable habitats, making it difficult to determine species-environment relationships. To come up with more robust models, data gathered from different geographic areas could be combined to develop models that make predictions on the future distribution of invasive alien species. In this case, it is important that similar environmental conditions are present in the region where the model will be applied and that relevant variables are measured in both geographic areas (Boets et al., in press b). Finally, it is stated by Früh et al. (2012) that accurately predicting the spread of alien species and the corresponding consequences on biodiversity is difficult, since alien species may interact strongly with other environmental stressors, thereby modulating species-environment interactions. For example, in the beginning of the nineties, zebra mussels altered the water quality in the pelagic zone of Saginaw Bay, leading to decreased values in chlorophyll and phosphorous concentration, subsequently, affecting local communities (Fahnenstiel et al., 1995).

Based on our integrated model, several vulnerable areas for invasion by *D. villosus* could be discovered in Flanders. The Dijle canal and the canal Bossuit-Kortrijk are the most suitable watercourses for *D. villosus* to establish. Both canals have a high ecological water quality with a Belgian Biotic Index (BBI) of 9 (maximum BBI score is 10) and a Multimetric Macroinvertebrate Index (MMIF) of 0.6 and 0.8, respectively (maximum MMIF score is 1). Several pollution sensitive taxa, such as Leptoceridae, Caenidae and Ecnomidae can be threatened by the presence of the killer shrimp. Predation experiments have demonstrated that *D. villosus* predates on a whole range of macroinvertebrates, thereby leading to a decrease in abundance and diversity of indigenous and alien macroinvertebrates (Dick et al., 2002; Boets et al., 2010a; MacNeil et al., 2013). In addition, a study by MacNeil et al. (2011), demonstrated that *D. villosus* disrupts trophic dynamics of freshwater ecosystems by affecting leaf-litter processing and shredder efficiency. Invasive alien species have the potential to cause a shift in the 'keystone' ecosystem functions, changing energy flux and nutrient cycles, which together affect critical ecosystem services, such as biodiversity, fisheries yield and water quality. In this respect, it is important to prevent their further spread as this might lead to a decrease in biodiversity and finally to biotic homogenization. A controlled trade of alien species and a good legislation regarding ballast water control are measures that could prevent the further spread of alien macroinvertebrates, including *D. villosus*. Possible additional measures that could be taken to increase the public awareness are national and international risk assessment fact sheets showing the impacts and problems related to invasive alien species. Currently, a national framework including a preliminary species risk assessment protocol is set up by the Belgian government. The Belgian Forum on Invasive Species gathers scientific information on presence, distribution, auto-ecology, adverse impacts and management of invasive alien species. To date, most attention has been paid to plants and mammals and little is reported on the spread of invasive alien macroinvertebrates in Flanders, including *D. villosus*. In this respect, our integrated model could be of assistance to policy makers to perform risk assessment for alien species living in aquatic habitats.

In conclusion, based on our integrated modelling approach we predicted that large watercourses are vulnerable to invasion by *D. villosus* especially with the expected improvement in water quality. Within the next fifteen years the species will be able to colonise all main rivers in Flanders with possibly large effects on ecosystem functioning. Despite the degree of uncertainty related to the prediction of invasive alien species, we are convinced that our modelling approach can be used as a risk assessment tool to support the control and prevention of invasive alien species, as it reveals which sites and rivers are most vulnerable to invasion, and what are major water quality and habitat characteristics that influence this.

Chapter 9: General discussion and conclusions

The overall aim of this study was to get insight in (a) the ecology of alien macroinvertebrate species under different environmental conditions, (b) the drivers that cause changes in macroinvertebrate communities and (c) the impact of alien macroinvertebrates on ecosystem structure and functioning. To this end, an integrated approach was used by combining the results and information obtained from lab studies, field data and data-driven modelling. This combination allowed assessing the impact of alien macroinvertebrates in surface waters in Flanders. Risk assessments for alien macroinvertebrates could be made and hot spots for alien species introductions were determined. Based on an integrated modelling approach predictions on the future distribution of alien macroinvertebrates under changing environmental conditions could be made. Altough the here proposed approach does not list possible actions that can be directly implemented, this approach can be used as a risk assessment tool to support the control and prevention of invasive alien species, as it reveals which sites and rivers are most vulnerable to invasion and what are major water quality and habitat characteristics that influence these processes.

Factors favouring the establishment and spread of alien macroinvertebrates

The overview on the distribution of alien macroinvertebrates (chapter 2) revealed that more than 60 alien macroinvertebrate species occur in Flemish surface waters and that many species were introduced during the last few decades, probably as a result of increased trade in the world, the disappearance of natural migration barriers and the degradation of habitats in former decades. Mainly macrocrustaceans and molluscs were represented by a high number of alien species. This is comparable to neighbouring countries like France, Germany, the Netherlands (Gollach and Nehring, 2006; Labat et al., 2011; Bij de Vaate et al., 2002) and other European countries like Ireland and the U.K (Minchin, 2007). Although molluscs and crustaceans were characterised as taxonomic groups making up a large part of the macroinvertebrate community, it was the purpose to assess the factors that favour the establishment and spread of alien macroinvertebrates in general. Therefore, over 1800 samples (chapter 3) taken by the Flemish Environment Agency during three consecutive time intervals (1999, 2004, 2009) originating from several aquatic habitat types in Flanders were analysed.

The analysis pointed out that there are three main elements favouring the establishment and spread of alien macroinvertebrates:

- environmental conditions
- traits of the species
- vectors of introduction and dispersal

Environmental conditions

It was found that large watercourses, such as canals, harbours and brackish polder waters are highly suitable for alien macroinvertebrates to establish. Many alien macroinvertebrate species seem to thrive well in these artificial, human modified aquatic systems. Canals are often characterised by reinforced embankments and hard substrates, which seem to be very favourable for alien macroinvertebrates to establish (van Riel et al., 2006b).

Based on a detailed case study about the harbour of Ghent, it was found that environmental degradation in former decades can, in the initial introduction phase, favour the establishment of alien macroinvertebrates. This phenomenon was already observed earlier in the river Rhine, where after the catastrophic environmental Sandoz accident, populations of *Chelicorophium curvispinum* and *Corbicula* sp. reached a dominant position within two years (Den Hartog et al., 1992). The improvement in chemical water quality due to the installation of wastewater treatment plants can be beneficial for alien as well as indigenous species. Predictions on the future distribution of alien macroinvertebrates in the harbour of Ghent indicated that the alien macroinvertebrate diversity is likely to increase with improving water quality, whereas the relative fraction of aliens in terms of abundance will remain stable. A detailed analysis of the relationship between the ecological water quality and the occurrence of alien macroinvertebrates revealed that there was a significant negative correlation between the ecological water quality and the number of alien species. This trend was only observed for waters with a good chemical water quality, since in waters with a bad ecological water quality species diversity (alien as well as indigenous species) was generally low. In this respect, it is thought that indigenous species can win the competition from alien macroinvertebrates in nutrient-poor, densely vegetated systems (Vermonden et al., 2010). It is also expected that natural systems with a good ecological water quality and a high species diversity can act as a kind of buffer against the further spread of alien species. These systems will have a higher biotic resistance and will consequently be more difficult to invade. Currently, small streams are still largely free from alien species, possibly due to their higher biotic resistance and the difficulty for alien macroinvertebrates to invade these systems.

Conductivity has been used as proxy for water quality. Grabowski et al. (2009) found that in large rivers, which usually have a higher conductivity, more alien species occurred at higher abundances compared to small rivers. The absence of alien amphipods in small rivers may also be explained by their ecological preference for a higher conductivity. In smaller streams, the indigenous fauna suffers thus a lower pressure posed by alien macroinvertebrates. Similar observations were made in Flanders, where large rivers and canals with a high conductivity were highly 'biocontaminated' with alien macroinvertebrates, whereas smaller streams are hardly invaded (chapter 3). Salinity and conductivity were several times pinpointed as important variables determining the presence or absence of alien macroinvertebrate species (chapter 3, 4, 5, 6). An analysis of the polder waters indicated that it is probably the decreasing salinity due to a reduced input of brackish water that caused the decline of the indigenous brackish water species and not only the introduction of the indigenous species *Gammarus tigrinus*. Based on data-driven classification and regression trees, it was found that salinity and conductivity are root variables determining the habitat suitability of alien macrocrustaceans in general and *Dikergammarus villosus* in particular. The case study on the Belgian coastal harbours (chapter 6) indicated that the salinity gradient was one of the most important factors explaining the gradient in biocontamination, with more alien macroinvertebrates occurring in inland waters compared to the marine harbours. The hypothesis that brackish waters (intermediate salinity) are characterised by a low natural species diversity and thus vulnerable to invasions, was confirmed during our study. Inland brackish waters harboured more alien macroinvertebrates compared to the harbours with marine conditions and rivers situated in fresh water.

Conclusion: chemical and hydro-morphological conditions of the invaded ecosystem clearly contribute to the successful establishment and spread of alien macroinvertebrates.

Traits of the species

Another element favouring the establishment and spread of invasive alien macroinvertebrates are the characteristics inherent to these invasive alien species. Based on laboratory experiments, it was found that the invasive alien amphipod *Dikerogammarus villosus* was able to predate on other macroinvertebrates, leading to a reduction of the abundance of species belonging to the same guild (Gammaridae) as well as taxa, such as Asellidae, Baetidae and Chironomidae (Boets et al., 2010a; MacNeil et al., 2013). Gammaridae used to be classified as shredders feeding with dead organic material and plant material with some recognition of omnivory (MacNeil et al., 1997). Results of recent research based on stable isotope analysis ($\delta^{15}N$) and numerous lab experiments indicated that Gammaridae are opportunistic omnivores predating also on species belonging to the same guild. Furthermore, cannibalism and inter- and intraguild predation seem to be common and important factors contributing to species exclusions and replacements (Polis et al., 1989). Therefore, being generalists probably contributes to the success of these invasive amphipods.

The tolerance to salinity is another factor put forward as an important trait enabling species to successfully colonise new habitats. Those species that are euryhaline have a higher chance of getting introduced via ballast water of ships. Piscart et al. (2011) found that species originating from outside Eurasia (introduced by the ballast or drinking water on ships) were more salt tolerant than indigenous species from France and alien species originating from Eurasia. *Dikerogammarus villosus* and *Gammarus tigrinus* are two species with a high tolerance towards changes in temperature and salinity (Wijnhoven et al., 2003). Other characteristics, such as a high reproductive output, early sexual maturity and tolerance towards environmental pollution in general can give a competitive advantage. Research in the Austrian Danube indicated that the reproductive potential of *D. villosus* is high compared to other amphipods, with large females carrying more than 100 developing embryos and juveniles in the brood pouch (Pöckl, 2007). In our study area, very high abundances of the amphipods *G. tigrinus* and *D. villosus* were found, especially in the polder watercourses and canals. It was concluded that *G. tigrinus* has a reproductive advantage compared to the two indigenous species present in the polder waters at higher temperatures and lower salinities. *Gammarus tigrinus* can be seen as an opportunistic r-strategic species that is able to increase its density over a short period, becoming the dominant species in the recipient community. The difference in time to reach sexual maturity, together with the short incubation time and the favourable reproductive period possibly contributed to the success of *G. tigrinus* in its competition with the indigenous species.

Conclusion: species characteristics contribute to the success of invasive alien species introduction and establishment and determine the outcome of competition with other species.

Vectors of introduction and dispersal

The importance of shipping as a vector of introduction and secondary dispersal of alien macroinvertebrates was investigated during this study in several aquatic habitat types. As mentioned under the discussion of environmental conditions, canals are highly suitable for alien species to establish. A second characteristic of canals besides their hydro-morphological characteristics is the presence of intensive ship traffic. Ship traffic can be connected with the concept of 'propagule pressure'. This concept states that the higher the number of alien species introductions, the higher the chance that a population gets established. A higher introduction rate consequently leads to a higher genetic diversity and more stable populations. An analysis of the shipping in the Belgian coastal harbours revealed that the diversity of alien macroinvertebrate species was highest in Zeebrugge, which is characterised by a high number of transcontinental commercial ship movements. It is known that many alien species are being transported via ballast water of ships (Ruiz et al., 1997). Despite the fact that most observed alien species originated from North-America, most ships which anchor in Zeebrugge originated from European harbours and only a small part originated from North-America. Frequent commercial shipping between neighbouring countries in Europe or countries bordering the same sea might be responsible for secondary introductions and dispersal of alien species. Moreover, also secondary dispersal via yachts or fishing vessels originating from neighbouring harbours could have partially contributed to introduction and spread of these alien species. The large variation found on the dispersal rate of *D. villosus* (chapter 8) indicates that human mediated transport probably contributes to the fast spead via large waterways and canals.

Conclusion: shipping contributes significantly to the establishment and secondary dispersal of alien macroinvertebrates, mainly in artificial aquatic ecosystems.

Impact assessment

After it was known which habitats are vulnerable to invasions and which species can be seen as high impact species, their impact on (a) macroinvertebrate communities and (b) biotic indices and water quality assessment was assessed. As discussed in Boets et al. (2010a) and MacNeil et al. (2013), changes in the macroinvertebrate composition could be observed based on laboratory experiments and field data analysis for different types of taxa. The arrival of *D. villosus* in Flanders coincided with the decrease of several indigenous taxa, but also a previously successful amphipod invader, the North American *Gammarus tigrinus*. Besides a decrease in several taxa, there was also an increase observed for other Ponto-Caspian invaders, such as *Dreissena polymorpha* and *Chelicorophium curvispinum*. Although there are changes observed in the macroinvertebrate composition, these cannot be solely explained by the introduction of alien macroinvertebrates as indicated above. Changes in macroinvertebrate communities were observed for the three different case studies as a consequence of changing environmental conditions. The opinion that alien species are passengers of changes in the aquatic environment rather than being the actors of change themselves is supported. In addition, Noordhuis et al. (2009) pointed out that while the introduction of *D. villosus* can cause a decrease in the abundance of other macroinvertebrate taxa, it may not drive a species to actual local extinction and that the decrease in abundance of certain taxa could be linked to other factors such as decreasing nutrient concentrations. The study by MacNeil et al. (2013) showed that the arrival and establishment of *D. villosus*, as well as other invaders, coincided with the decline of some resident taxa, despite improving chemical water quality. In addition, the field study data also indicates that substrate may play an important role in determining the severity of any impacts of *D. villosus* on resident assemblages, with stony/rocky substrates promoting the establishment of *D. villosus* and the decline of resident species, in contrast to sandy substrates, where it occurred less frequently and may have had a lower impact on resident taxa.

When assessing the impact of alien macroinvertebrates, we have to take into consideration the ruling environmental conditions, since alien species do not need to have a negative impact on the ecosystem per se. The impact is often depending on the state of the ecosystem. For example, in the harbour of Ghent, a former degraded aquatic ecosystem, it was noticed that the introduction of alien macroinvertebrates contributed to the increasing trend observed in the Multimetric Macroinvertebrate Index for Flanders. This could be attributed to a recolonisation by indigenous species simultaneously with a colonisation by alien species. However, we should be aware that when alien species are able to colonise low degraded rivers with a good water quality and a high abundance of indigenous taxa, they may cause a decline in the diversity resulting in lower values of biotic indices (MacNeil et al., 2013). Mesocosm experiments indicated that the BMWP score was consistently lower when *D. villosus* was present compared to when no amphipods or the indigenous *G. pulex* were present (MacNeil et al., 2013). Our study also emphasises that the presence of invaders, such as *D. villosus* may have a confounding effect on using macroinvertebrate assemblages as reliable bioassessment tools for WFD purposes (Arbačiauskas et al., 2008; Cardoso and Free, 2008). It would thus be theoretically possible to achieve a high biotic index score in a site greatly dominated by *D. villosus* or to achieve a low biotic index score in a high chemical water quality site, where *D. villosus* may have eliminated sensitive taxa, such as mayflies, stoneflies and caddis-flies. It was already highlighted that systems, such as the BMWP do not distinguish between indigenous and alien taxa in the scoring process and rely on the presence/absence of taxa rather than relative abundance in score generation. In Flanders, there was no significant decrease in the MMIF value observed for systems dominated by alien species. Nevertheless, it is advised to calculate the site-specific biocontamination index next to the biotic indices that are used to asses the ecological water quality as this might indicate sites that are highly invaded by alien macroinvertebrates.

The knowledge gathered during three case studies was used to make habitat suitability models analysing the habitat preferences on the one hand and predicting the future dispersal on the other hand. Several key elements (traits, vectors, biotic interactions and environmental conditions) play a crucial role when making predictions on the future distribution of alien macroinvertebrates in surface waters. Based on classification and regression trees, predictions were made on the diversity and abundance of alien macrocrustaceans in Flanders. A maximum species richness of alien macrocrustaceans was reached in freshwater with a good chemical water quality and intensive ship traffic. In fresh water, increasing conductivity lead to a decrease in abundance of alien macrocrustaceans, whereas in brackish water, increasing conductivity due to increasing salinity influenced the abundance positively (up to 5 PSU).

In a later phase, a model, integrating a habitat suitability model with a water quality model and a migration model, was developed to perform a risk assessment for the invasive alien amphipod *Dikerogammarus villosus* in Flanders under changing environmental conditions. It was predicted that large watercourses are vulnerable to invasion by *D. villosus*, especially with the expected future improvement in water quality. Within the next fifteen years, the species was predicted to colonise all main rivers in Flanders. Despite the degree of uncertainty related to the prediction of invasive alien species occurrence, it can be concluded that models are a useful tool for decision making. Given the large number of alien species introductions, it is not feasible to follow up all species and habitats. Policy makers should focus on vulnerable areas, such as brackish water areas and areas with intensive ship traffic and certain key species, which are known to cause high impacts in other countries in order to prevent or minimise the further introduction and spread and consequently the future impact of alien macroinvertebrates.

While our proposed models can be useful as a tool to support the management of invasive alien species there are a few shortcomings. Our models make predictions on the future increase in diversity and abundance of alien macroinvertebrates based on improvements in water quality. As mentioned before, also indigenous species can benefit from these improvements and biotic interactions between alien and indigenous species are not fully investigated in this work. Migration speed is incorporated based on a combination of the species own dispersal capacity and the transportation via shipping. Disentangling the importance of both factors would be very interesting to determine the importance of human mediated transport. Finally, only a limited number of water quality parameters were included in the PEGASE water quality model and consequently in the risk assessment models as well. Incorporating other variables could increase the performance and accuracy of our models as well.

Recommendations for management of invasive alien macroinvertebrate species

To date, European Union's response to the problems of alien species has been driven by commitments to international agreements, such as The World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures and the Convention on Biological Diversity (Hulme et al., 2009). These commitments have not always been supported by action and average annual rates of alien species establishment in Europe have progressively increased over the last century for many taxa (Hulme et al., 2009). Therefore, the European Commission has put forward a proposal to the European Council and Parliament for an EU strategy on invasive alien species. The strategy emphasizes prevention as the most cost-effective way forward and presents three new policy options: maximise the use of existing legal instruments; adapt existing legislation through specific amendments or establish a comprehensive, dedicated legal framework to address biological invasions. Following the 'Invasive Non-Native Species Strategy for Great Britain' (Defra, 2008), which was developed jointly by government and stakeholders and is the first of its kind in Europe, the response is now, once a new species is found, to slow the spread by applying better biosecurity through the 'Check, Clean, Dry' approach (www.nonnativespecies.org/checkcleandry). This is important, not only to help slow the spread of one particular species, but also other invasive species that might be present in the water. For more examples on legislation, regulations and codes of conduct that are relevant to the management of invasion pathways in Europe we refer to Hulme et al. (2008). However, most of these efforts focus on intentional introductions, whereas recent introductions are increasingly unintentional. Major gaps exist in the binding international regulatory framework, especially as relates to hull fouling, air transport, tourism, pets and aquarium and garden species, live bait and plant seeds and interbasin water transfers and canals.

The establishment of a 'black list' of species prohibited for import and sale in Europe that would prioritise those species that cause the most significant threats has been proposed to improve the existing legislation. However, it is not easy to quantify the impact of these species and it is often time and money consuming to officially blacklist these species. In addition, another problem is that a significant proportion of invasive alien species in Europe are indigenous elsewhere in Europe. Blacklists may therefore need a regional or national focus. There are preliminary guidelines for environmental impact assessment and classification of alien species in some European countries (Verbrugge et al., 2012) and in Belgium (Branquart, 2011). A good example of this is the framework for bioinvasion impact assessment proposed by Zaiko et al. (2011). The methodology is based on a classification of the abundance and distribution of alien species and the magnitude of their impacts on indigenous communities, habitats and ecosystem functioning aggregated in a 'Biopollution Level' index (BPL) which ranges from 'no impact' (BPL = 0) to 'massive impact' (BPL = 4).

In Belgium, the classification is based on a simplified environmental impact assessment protocol and the geographic distribution of alien species. Such a categorisation provides a scientific background to prioritise actions to prevent introduction and mitigate the impact of alien species, including the improvement of the legislative framework. Currently, little or no attention is addressed to the impact and spread of alien macroinvertebrates in Flanders. Most interest is attributed to large mammals and plants. Only for two species, *Procambarus clarkii* and *Eriocheir sinensis*, an environmental impact has been assessed (E. Branquart, pers. comm.). In this respect, the results and models obtained from this study could be used to set up an effective management to reduce the introduction, spread and impact of alien macroinvertebrate species in Flanders. This could be achieved by prioritising those species which are known to cause high impacts and monitoring those regions which are indicated as hot spots for alien species introductions. In our opinion, it is also important to assess the impact and risk of potential future invaders, since once a species is established, it is very difficult or nearly impossible to eradicate them. In addition, several management measures, such as ballast water control, regulations regarding the trade of aquatic alien species and further insight in invaded ecosystems will be necessary.

Recommendations for further research

The here developed models predicting the future dispersal of alien species did not always have a high performance. This could be attributed to different elements as discussed in chapter 7 and 8. To increase the performance of the models some elements which could be helpful are proposed. First of all many species were only recently introduced in Flanders and may not have reached their maximal distribution potential. Therefore, it would be interesting to re-evaluate the developed models with data from other regions and other countries. In this way models could be developed that are basically applicable anywhere where the species is alien. It was already demonstrated in Boets et al. (in press b) that the input data and the environmental conditions are important to be able to use a model that was developed in one region in another region. Secondly, alien species are very opportunistic and it is not always possible to incorporate this variability inherent to alien species. Many alien species can survive a wide range of environmental conditions. A possible solution could be found in Fuzzy Logic models. These models are based on simple rules derived from expert knowledge that can predict whether a species can establish a population or not under certain environmental conditions.

In this study an integrated approach was aimed for where the impact of different alien macroinvertebrates was investigated for different aquatic ecosystems based on a combination of field data, lab studies and data-driven modelling. The impact assessment mainly focussed on the structure of the macroinvertebrate community. In the next step, it would be interesting to analyse the impact of alien macroinvertebrates on ecosystem level. This means that the changes in structure as well as functioning of the ecosystem could be analysed. A food web model could be developed to investigate the impact on different trophic levels (plankton, fish, birds) in order to achieve a better mechanistic understanding of the introduction of invasive alien macroinvertebrates. The structure of the ecosystem could be modelled based on cohesion analysis, a technique that has been recently applied in ecology (Krause et al., 2003).

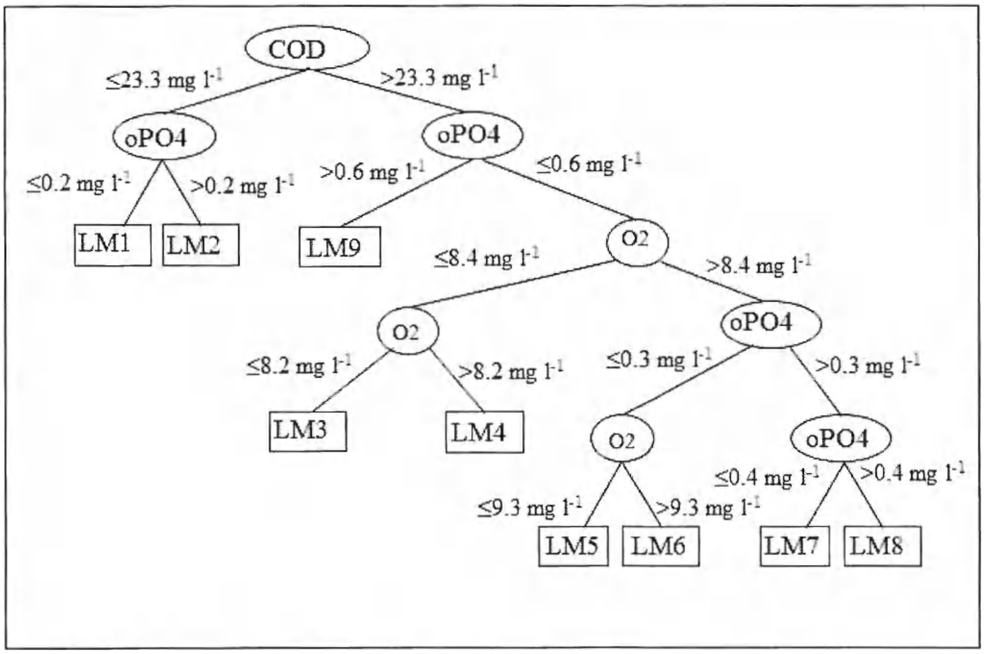
There are several main hypotheses, which are generally accepted, but not always supported by all taxonomic groups and habitats (Jeschke et al., 2012). To this end it would be interesting to investigate if these main hypotheses in invasion ecology are also applicable for macroinvertebrates and for the aquatic environment. Some examples like the tens rule and the biotic resistance were already brought up during this study, but not investigated in detail. A good experimental design considering the invader-ecosystem interactions would help to decide to accept or decline these major hypotheses. As science and the progress in invasion ecology is proceeding at a very high speed, new techniques and hypotheses will be discovered giving the opportunity to unravel the complexity of invasion ecology.

Appendices

Appendix 8.1 Main characteristics of different river types present in Flanders (based on Jochems et al., 2002)

River types	Hydro-ecoregion	Catchment area	Salinity PSU
Small stream	Sand/sandy loam/loam	< 50 km²	< 0.5
Small stream Kempen	Kempen	< 50 km²	< 0.5
Large stream	Sand/sandy loam/loam	50-300 km²	< 0.5
Large stream Kempen	Kempen region	50-300 km²	< 0.5
Small river	Any	300-600 km²	< 0.5
Large river	Any	600-10000 km²	0-5
Very large river	Any	> 10000 km²	< 0.5
Polder watercourse	Polder	Not applicable	0.5-5

Appendix 8.2 Regression tree model for *Dikerogammarus villosus*



Linear regression equations:

LM1 = -0.0012 * COD - 0.0067 * KjN - 0.0641 * oPO4 + 0.0227 * O2 + 0.6643

LM2 = -0.0014 * COD - 0.0797 * KjN - 0.0749 * oPO4 + 0.7768

LM3 = -0.0044 * COD - 0.0019 * KjN - 0.0399 * oPO4 + 0.0741 * O2 + 0.0944

LM4 = -0.0005 * COD - 0.0019 * KjN - 0.0399 * oPO4 + 0.142

LM5 = -0.0005 * COD - 0.0019 * KjN + 0.1338 * oPO4 - 0.0251 * O2 + 0.5126

LM6 = -0.0005 * COD - 0.0019 * KjN + 0.1338 * oPO4 - 0.0187 * O2

LM7 = -0.0005 * COD - 0.0019 * KjN - 0.6834 * oPO4 + 0.0138 * O2 + 0.7245

LM8 = -0.0005 * COD - 0.0019 * KjN - 1.4417 * oPO4 - 0.0129 * O2 + 1.074

LM9 = -0.0005 * COD - 0.0019 * KjN - 0.0832 * oPO4 + 0.1327

Figure *Regression tree model indicating the habitat suitability of Dikerogammarus villosus based on measured physical-chemical variables. COD = chemical oxygen demand, oPO4 = orthophosphate concentration, O2 = dissolved oxygen concentration. LM indicates the linear regression equation.*

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Summary

Besides habitat fragmentation, the introduction of invasive species is considered to be one of the greatest threats to global biodiversity. Due to increased global trade, habitat degradation and climate change the number of species introductions has increased spectacularly during the last decades. This has led to changes in structure and functioning of ecosystems worldwide.

In this study, the impact and spread of alien macroinvertebrates in surface waters in Flanders was investigated. To this end, a large database consisting of biological and physical-chemical data was used, which was collected by the Flemish Environment Agency and supplemented with own sampling campaigns. An integrated approach was aimed for, where the results from laboratory studies, long-term field data analysis and data-driven modelling were combined in order to gain insight in the ecology of alien macroinvertebrate species and the drivers that cause changes in macroinvertebrate community composition.

A detailed study on the distribution of alien macroinvertebrates in Flanders revealed that in total, 65 alien macroinvertebrates are established of which 40 are regularly encountered in fresh and slightly brackish inland waters. Most alien taxa belonged to the crustaceans and molluscs originating from North America and the Ponto-Caspian region. Many alien species were first discovered in the east of Flanders from where they started the colonisation of the central and western parts of Flanders. Changes in the macroinvertebrate composition were discovered during the last two decades as a result of changing environmental conditions and the introduction of alien species.

When analysing the factors that favoured the establishment and spread of alien macroinvertebrates it was found that shipping, hydro-morphological and physical-chemical factors were detrimental for the success of alien macroinvertebrates. Canals, harbours and the polder waters were hot spots for alien species introductions. Small streams were less invaded by alien macroinvertebrates probably because of a higher biotic resistance and the lack of proper vectors. Case studies of different aquatic ecosystems helped understanding the different factors contributing to successful invasions.

The case study on the harbour of Ghent indicated that previously degraded ecosystems are favourable for the early establishment by alien macroinvertebrates. With improving chemical water quality due to the installation of wastewater treatment plants and a stricter environmental legislation not only indigenous, but also alien macroinvertebrates colonised the

harbour of Ghent. In the polder waters the indigenous brackish water species decreased in abundance, whereas the American amphipod *Gammarus tiginus* quickly took in a dominant position after its establishment. These observed changes are probably caused by a decrease in salinity which coincided with an increase of freshwater asselids and the euryhaline species *G. tigrinus*. It was not only the introduction of the invasive amphipod, but the combination with changing environmental conditions that caused the changes in macroinvertebrate composition. The case study on the Belgian coastal harbours confirmed earlier findings that brackish waters are characterised by a low natural species diversity and a relatively high number of alien species. The harbour of Zeebrugge, which received most international ships, had the highest diversity of alien macroinvertebrates and was also characterised by a high site specific biocontamination index.

The knowledge gathered during the case studies was used when making predictions on the future distribution of alien macrocrustaceans in Flanders. Based on data-driven classification and regressions trees it was found that alien macrocrustaceans prefer large rivers and canals with a good chemical water quality and that with increasing conductivity the abundance and species richness of alien macrocrustaceans increases in the brackish water environment. When incorporating the improvements in water quality, it was found that the number of alien species (alien species diversity) will increase in the future, but that the fraction of alien species (alien species abundance) will remain stable.

In the last step, an integrated model coupling a habitat suitability model, a water quality model and a migration model was developed to predict the future distribution of a highly invasive alien amphipod species, *Dikerogammarus villosus*. It was found that *D. villosus* will invade more large watercourses in Flanders during the next fifteen years as a result of decreases in COD, nitrogen and phosphorous loads and an increase in oxygen concentration. It was calculated that *D. villosus* spreads with an average speed of five km per year and that given the relatively small size of Flanders, migration speed will not limit its maximal dispersal. The here developed model could be applied as an efficient tool by decision makers to perform risk analysis for (potential) invasive macroinvertebrate species to determine the future distribution range as this could help to reduce the number of species introductions and the impact they have on ecosystem functioning.

Besides performing risk assessment several management measures, such as ballast water control, regulations regarding the trade of aquatic alien species and further insight in invaded ecosystems are necessary to reduce the further spread and minimise the impact of invasive alien species.

Samenvatting

De introductie van invasieve soorten wordt naast habitatfragmentatie aanzien als een van de belangrijkste bedreigingen voor de globale biodiversiteit. Gedurende de laatste tientallen jaren werd een spectaculaire toename in nieuwe soorten waargenomen als gevolg van de stijging in internationale handel, toenemende habitatdegradatie en klimaatsveranderingen. Dit heeft een belangrijke invloed uitgeoefend op de structuur en functionaliteit van ecosystemen in de hele wereld.

In deze studie werd de verspreiding en impact van exotische macroinvertebraten in oppervlaktewateren in Vlaanderen (België) onderzocht aan de hand van een omvangrijke databank die zowel biologische als physico-chemische en hydro-mofologische data bevatte. Deze database werd samengesteld aan de hand van data verzameld door de Vlaamse Milieumaatschappij, aangevuld met eigen staalnames. Op basis van een geïntegreerde aanpak waarbij de resultaten van laboratorium testen, multivariate veld data analyses en data-gedreven modelering werden gecombineerd om zo inzicht te verwerven in de ecologie van exotische macroinvertebraten enerzijds en de sturende variabelen die veranderingen veroorzaken in de macroinvertebraten gemeenschap anderzijds.

Op basis van een getailleerde studie over het voorkomen en de distributie van exotische macroinvertebraten in Vlaanderen kon er worden vastgesteld dat er minstens 65 verschillende exotische soorten voorkomen, waarvan er 40 regelmatig worden aangetroffen in zoet en brak water en slechts een 25 tal in het mariene milieu. De meeste exotische taxa behoren tot de crustaceeën en mollusken afkomstig van Noord-Amerika en de Ponto-Kaspische regio. Vele van de exotische macroinvertebraten werden voor de eerste keer waargenomen in het oosten van Vlaanderen van waaruit de verdere kolonisatie plaatsvond. Veranderingen in de samenstelling van de inheemse macroinvertebraten gemeenschap werden waargenomen tijdens de laatste twee decennia als gevolg van een veranderende omgeving en de introductie van exotische macroinvertebraten.

Een analyse van de factoren die de introductie en verspreiding van exotische macroinvertebraten bevorderen duidt erop dat hydro-morfologische en fysico-chemische factoren in belangrijke mate het succes van nieuwe introducties bepaalt. Kanalen, havens en de brakke polderwaterlopen zijn 'hot spots' voor de introductie van exotische soorten. Kleinere rivieren en beken zijn in het algemeen minder geinvadeerd waarschijnlijk als gevolg van een hogere natuurlijke diversiteit en een gebrek aan introductie vectoren. Onderzoek op basis van case studies van de verschillende aquatische ecosystemen liet toe om inzicht te krijgen in de verschillende factoren die bijdragen aan een succesvolle invasie.

De case studie betreffende de haven van Gent toonde aan dat na een verontreinigingsgolf of verstoring rivieren gemakkelijker geinvadeerd worden door exotische macroinvertebraten in de daaropvolgende fase. Als gevolg van een betere chemische waterkwaliteit door de installatie van waterzuiveringsinstallaties en een striktere regelgeving aangaande de lozing van afvalwater koloniseerden niet alleen inheemse maar ook exotische macroinvertebraten de haven van Gent. De studie in de polders toonde aan dat inheemse brakwater soorten in abundantie daalden, terwijl de Noord-Amerikaanse amphipode *Gammarus tigrinus* na zijn introductie snel een dominante positie innam. De verandering in soortensamenstelling is waarschijnlijk het gevolg van een daling in de saliniteit wat aanleiding gaf tot een toename in abundantie van de zoetwatersoorten en de euryhaliene soort *G. tigrinus*. Bij gevolg is het niet alleen de introductie van de invasieve amphipode, maar een combinatie met de veranderende milieuomstandigheden die de verandering in de macroinvertebraten gemeenschap heeft veroorzaakt. De studie uitgevoerd in de Belgische zeehavens bevestigde de eerdere bevindingen door andere onderzoekers, namelijk dat brakwater wordt gekenmerkt door een lage natuurlijke soorten rijkdom en een relatief hoog aantal aan exotische soorten. In de haven van Zeebrugge, welke gekenmerkt werd door een groot aantal internationale scheepvaartbewegingen, werden de meeste exotische soorten teruggevonden en bijgevolg werd deze haven dan ook gekenmerkt door een hoge biocontaminatie.

De kennis die verzameld werd tijdens de verschillende case studies werd nadien gebruikt om accuratere modellen en voorspellingen te kunnen maken over de toekomstige verspreiding van exotische macrocrustaceeën in Vlaanderen. Op basis van data gedreven classificatie- en regressiebomen werd er aangetoond dat exotische macrocrustaceeën een voorkeur hebben voor grote rivieren en kanalen met een goede chemische waterkwaliteit en dat met toenemende conductiviteit de abundantie en soortenrijkdom van exotische soorten toenam in brak water. Als gevolg van de verbeteringen in de waterkwaliteit zullen er in de toekomst meer exotische soorten voorkomen, maar zal hun totale fractie (de abundantie aan exotische soorten) ongeveer gelijk blijven aan het huidige niveau.

In de finale fase van dit onderzoek werd er een geïntegreerd model ontwikkeld waarbij er een habitatgeschiktheidsmodel met een waterkwaliteitsmodel en een migratiemodel werden gekoppeld om de toekomstige verspreiding van de invasieve soort *Dikerogammarus villosus* te voorspellen. De analyses toonden aan dat *D. villosus* zijn areaal voornamelijk in grotere rivieren zal uitbreiden in de komende 15 jaar als gevolg van een daling in chemische zuurstofvraag, stikstof en fosfaat concentratie en een stijging in de zuurstof concentratie. Analyse van de veld data toonde aan dat *D. villosus* een gemiddelde migratiesnelheid heeft van 5 km per jaar en dat, gezien de relatief kleine oppervlakte van Vlaanderen, migratiesnelheid geen limitatie zal vormen voor de verdere verspreiding van deze soort. Het in deze studie ontwikkelde model kan gebruikt worden als een gebruiksvriendelijke en efficiënte tool door beleidsmakers om een risicoanalyse uit te voeren voor (potentieel) invasieve soorten en om hun verdere verspreiding te voorspellen om zo het aantal introducties en de impact van invasieve soorten te beperken.

Naast het uitvoeren van een risicoanalyse zijn beheersmaatregelen zoals de behandeling van ballastwater, een goede wetgeving aangaande de handel in exotische aquatische soorten en verder inzicht in de ecologie van deze soorten en geinvadeerde ecosystemen noodzakelijk om de verdere verspreiding en impact van exotische soorten te verhinderen of te reduceren.

Curriculum vitae

Personalia

Naam	Boets
Voornaam	Pieter
Geboortedatum	17 september 1985
Geboorteplaats	Asse, België
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Studies

2008-heden	Doctoraatsopleiding in de Toegepaste Biologische Wetenschappen, Universiteit Gent, Faculteit Bio-ingenieurswetenschappen
2007-2008	Ma na Ma in de milieusanering en het milieubeheer, Universiteit Gent, Faculteit Bio-ingenieurswetenschappen, afgestudeerd met grote onderscheiding
	Thesis: Experimentele impactanalyse van dominante invasieve predatoren op macro-invertebratengemeenschappen in rivieren
2003-2007	Master/Licentiaat in de Biologie, Universiteit Gent, Faculteit Wetenschappen
	Thesis: Nestpredatie in een gefragmenteerd afromontaan nevelwoud, afgestudeerd met onderscheiding
1997-2003	Moderne-talen wetenschappen in het Sint-Maarteninstituut te Aalst

Loopbaanoverzicht - Werkervaring

2008-heden	Assistent en doctoraatsstudent aan de Vakgroep Toegepaste Ecologie en Milieubiologie (Faculteit Bio-ingenieurswetenschappen, Universiteit Gent)
	Doctoraat: Impactanalyse van niet-inheemse macroinvertebraten in Vlaanderen (België)

A1-peer reviewed, Science Citation Index

Boets P., Lock K., Cammaerts R., Plu D., Goethals P.L.M. (2009). Occurrence of the invasive crayfish *Procambarus clarkii* (Girard, 1852) in Belgium (Crustacea: Cambaridae). Belgian Journal of Zoology 139(2): 173-175.

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Boets P., Lock K., Adriaens T., Mouton A., Goethals P.L.M. (2012). Distribution of crayfish (Decapoda, Astacoidea) in Flanders (Belgium): an update. Belgian Journal of Zoology 142(1): 86-92.

Boets P., Lock K., Goethals P.L.M., Janssen C.R., De Schamphelaere K.A.C. (2012). A comparison of the short-term toxicity of cadmium to indigenous and alien gammarid species. Ecotoxicology 21(4): 1135-1144.

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Mereta S.T., Boets P., Ambelu A.B., Malu A., Ephrem Z., Sisay A., Endale H., Yitbarek M., Jemal A., De Meester L., Goethals P.L.M. (2012). Analysis of environmental factors determining the abundance and diversity of macroinvertebrate taxa in natural wetlands of Southwest Ethiopia. Ecological Informatics 7(1): 52-61.

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Holguin J.E., Boets P., Alvarado A., Cisneros F., Carrasco M.C.,Wyseure G., Nopens I., Goethals P.L.M. (2013). Integrating hydraulic, physical-chemical and ecological models for decision support in water management of the Cuenca river in Ecuador. Ecological Modelling 254: 1-14.

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Boets P., Holguin G.J.E., Lock, K., Goethals, P.L.M. (in press). Habitat analysis of the Ponto-Caspian amphipod *Dikerogammarus villosus* in two invaded regions in Europe based on data-driven approaches. Ecological Informatics. Doi: 10.1016/j.bbr.2011.03.031.

Everaert G., Pauwels I.S., Boets P., Buysschaert F., Goethals P.L.M. (in press). Development and assessment of ecological models in the context of the European Water Framework Directive: key issues for trainers in data-driven modelling approaches, Ecological Informatics doi: 10.1016/j.ecoinf.2012.10.007.

Everaert G., Pauwels I.S., Boets P., Verduin E., de la Haye M.A.A., Blom C., Goethals P.L.M. (in press). Model-based evaluation of ecological bank design and management in the scope of the European Water Framework Directive. Ecological Engineering. Doi: 10.1016/j.ecoleng.2012.12.034.

Heger T., Liebaug A.T., Botta-Dukát Z., Gherardi F., Hoppe C., Hoste I., Jax K., Lindström L., Boets P., Haider S., Kollmann J., Wittmann M., Jeschke J.M. (in press). Conceptual frameworks and methods for advancing invasion ecology. Ambio. Doi: 10.1007/s13280-012-0379-x.

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Boets P., Van De Vijver E., Lock K., Töpke K., Thas O., De Cooman W., Janssen C.R., Goethals P.L.M. (submitted). Relating taxonomy-based traits of macroinvertebrates with sediment pollution by means of basic and Zero-Inflated Poisson models. Ecological Informatics.

Boets P., Lock, K., Goethals, P.L.M. (submitted). Assessment of biocontamination in aquatic ecosystems: analysing key factors favouring the establishment and spread of alien macroinvertebrates. Biological invasions.

Holguin JE, Everaert G, Boets P., Goethals P.L.M., Alberto G. (submitted). Development and application of an integrated ecological modelling framework to analyze the impact of wastewater discharges on the ecological water quality of the Cauca river in Colombia. Environmental Modelling and Software.

Peer reviewed, not in Science Citation Index

MacNeil C., Boets P., Platvoet D. (2012). 'Killer shrimps', dangerous experiments and misguided introductions; how freshwater shrimp (Crustacea: Amphipoda) invasions threaten biological water quality monitoring and ecological assessment in the British Isles. Freshwater Reviews 5: 21-35.

Boets P., Lock K., Tempelman D., Van Haaren T., Platvoet D., Goethals P.L.M. (2012). First occurrence of the Ponto-Caspian amphipod *Echinogammarus trichiatus* (Martynov, 1932) (Crustacea: Gammaridae) in Belgium. BioInvasion Records 1(2): 115-120.

Peer reviewed, national journals

Boets P., Michels E., Meers E., Lock K., Tack F.M.G., Goethals P.L.M. (2012). Integratie van natuurontwikkeling in zuiveringsrietvelden voor behandeling van de vloeibare fractie van drijfmest. WT afvalwater 1: 61-73.

Abstracts of oral presentations

Goethals P., Colson L., Boets P., Mouton A., Lock K. (2008). Application of cellular automata support river restoration planning. 6th International Conference on Ecological Informatics. Cancun, Mexico.

Boets P., Lock K., Goethals P.L.M. (2008). Combinig datadriven methods and lab studies to analyse the ecology of *Dikerogammarus villosus*. 6th International Conference on Ecological Informatics. Cancun, Mexico.

Boets P., Lock K., Goethals P.L.M. (2009). Alien macro-crustaceans in freshwater ecosystems in Flanders. Science facing aliens conference Brussels, Belgium, 11 May 2009.

Boets P., Lock K., Goethals P.L.M. (2010). Using long-term monitoring to investigate the changes in species composition in the harbour of Ghent (Belgium). 17th ICAIS, San Diego, California.

Boets P., Lock K., Goethals P.L.M. (2010). Integrating field observations and lab studies to assess the impact of biological invasions. Workshop: tackling the emergent crisis of invasion biology, Benediktbeuren (Germany).

Everaert G., Boets P., Lock K., Goethals P.L.M. (2009). Application of decision trees to analyze the ecological impact of invasive species in Polder lakes in Belgium. International Society for Ecological Modelling (ISEM). Québec, QC, Canada.

Boets, P., Lock, K., Goethals, P.L.M. (2010). Predicting presence, species richness and abundance of alien macrocrustaceans in surface waters in Flanders (Belgium) using decision trees. 7th International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Everaert G., Pauwels I., Boets P., Goethals P.L.M. (2010). Development of ecological assessment models for the European Water Framework Directive: key issues for trainers in data-driven modeling approaches. 7th International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Goethals P., Zarkami R., Pauwels I., Boets P., Lock K., Mouton A. (2010). Habitat suitability modeling for Master of Science students: case of pike modeling in Flanders. 7th International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Holguin J., Boets P., Lock K., Goethals P.L.M. (2010). Habitat analysis of invasive crustaceans based on datadriven approaches applied on recently and long-term colonized habitats. 7th International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Mereta S., Ambelu Bayih A., Boets P., De Meester L., Goethals P.L.M. (2010). Classification and regression trees for habitat analysis of macroinvertebrate taxa in the natural wetlands of south-western Ethiopia. 7th International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Boets P., Lock K., Goethals P.L.M. (2010). Influence of alien macroinvertebrates on species assemblages and ecological water quality assessment in Flanders (Belgium)'. 7th International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Goethals P.L.M., Boets P., Lock K. (2010). Modelling approaches to analyse and predict invasive species behaviour in aquatic ecosystems. 7th International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Boets P., Lock K., Goethals P.L.M. (2010). Using long-term monitoring data to detect changes in macroinvertebrate species composition in the harbour of Ghent (Belgium). 16th PhD Symposium on Applied Biological Sciences, Ghent, Belgium, 20 December 2010.

Boets P., Lock K., Goethals P.L.M. (2011). Modelling habitat preference, species richness and abundance of alien macrocrustaceans in surface waters in Flanders (Belgium). Netherlands Annual Ecology Meeting 2011. Lunteren, The Netherlands, 8 to 9 February 2011.

Boets P., Lock K., Goethals P.L.M. (2011). Influence of alien macro-Crustacea (Malacostraca) on macroinvertebrate assemblages in Belgian coastal harbours. VLIZ Young Marine Scientists' Day. 25 February 2011.

Boets P., Lock K., Goethals P.L.M. (2011). Sensitivity of brackisch water invertebrates along a salinity gradient. Seminar:'Regulations meets science'. August 17, 2011, Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology.

Boets P., Lock K., Goethals P.L.M. (2011). Which factors favour the establishment and spread of alien macroinvertebrates in aquatic ecosystems? Biolief, Mar del Plata, Argentina. 21-24 November 2011.

Boets P., Lock K., Goethals P.L.M. (2012). Integrated ecological modelling to predict the distribution and impact of alien invasive species in rivers. 1st Annual World Congress of Biodiversity, Xi'an, China.

Boets P., Lock K., Goethals P.L.M. (2012). Invasieve macroinvertebraten in aquatische ecosystemen: hoe, waar en waarom? Themadag exoten, 13 december 2012, Nijmegen, The Netherlands.

Boets P., Pauwels I., Lock K., Goethals P.L.M. (2012). Using external model coupling for risk assessment of the 'killer shrimp' *Dikerogammarus villosus* in Flanders (Belgium). 8th ISEI conference, 3-6 December, Brazil.

Boets P., Lock K., Goethals P.L.M. (2013). Impact and spread of alien macroinvertebrates in surface waters in Flanders. Netherlands Annual Ecology Meeting 2013, Lunteren, The Netherlands, 5 and 6 February 2013.

Boets P., Lock K., Goethals P.L.M. (2013). Integrated ecological modelling as a tool for detection of potential hot spots for invasive macroinvertebrates. 18th ICAIS, Niagara Falls, Ontario, Canada, April 21-25, 2013.

Abstracts of poster presentations

Boets P., Lock K., Goethals P.L.M. (2010). What caused changes in gammarid fauna in brackish polder waters? Netherlands Annual Ecology Meeting 2010, Lunteren, The Netherlands, 9 to 10 February 2010.

Boets P., Lock K., Goethals P.L.M. (2012). Modelling habitat preference, abundance and species richness of alien macrocrustaceans in surface waters in Flanders (Belgium) using decision trees. Benelearn conference Ghent, Belgium.

Boets P., Lock K., Goethals P.L.M. (2012). Using an integrated modelling approach to assess the potential spread of the 'Killer shrimp' *Dikerogammarus villosus* in Flanders (Belgium). Freshwater Invasives – Networking for Strategy (FINS). 9-11 April, 2013. Galway, Ireland.

Organization of scientific meetings or conferences

Co-organiser of the 7th International Conference of the Ecological Informatics Society, Ghent, Belgium, 13 to 16 December 2010.

Educational activities

Practical exercises

- Aquatische en Terrestrische Ecologie
- Environmental Ecology
- Aquatic Ecology
- Ecology and Environmental Microbiology
- Ecological Engineering
- Natural systems for Wastewater Treatment
- Total Quality Management - Problem Based Learning
- Waterkwaliteitsbeheer
- Biological Monitoring of Aquatic Systems
- Ecotechniek en Natuurbouw

Tutor of master theses

- Everaert Gert (2008-2009). Ecologische impactanalyse van invasieve macroinvertebraten in Vlaamse kreken. Universiteit Gent, Master in de Mileusanering en het Milieubeheer.
- Regmi Anup Kumar (2008-2009). Constructed wetlands for the treatment of wastewater from prisons in Nepal. Universiteit Gent, Master of Sciences in environmental sanitation.
- Vande Walle Naïma (2009-2010). An integrated ecological assessment of wetlands in Ethiopia. Universiteit Gent, Master in de bio-ingenieurswetenschappen: milieutechnologie.
- Zewdie Sisay (2009-2010). Ecological assessment of wetlands based on macroinvertebrates and waterfowl in Ethiopia. Universiteit Gent, Master of Sciences in environmental sanitation.

- Hebbelinck Lot (2009-2010). Monitoring van exotische macro-invertebraten in de Vlaamse havens. Universiteit Gent, Master in de bio-ingenieurswetenschappen: milieutechnologie.
- Verstraeten Kristof (2009-2010). Modellering en vergelijkende ecotoxicologie van inheemse en invasieve macro-invertebraten. Universiteit Gent, Master in de bio-ingenieurswetenschappen: milieutechnologie.
- Fevery Davina (2011-2012). Gecombineerde stress: impact van metalen op inheemse en exotische Amphipoda bij verschillende saliniteiten. Universiteit Gent, Master in de bio-ingenieurswetenschappen: milieutechnologie.
- Scheers Kevin (2011-2012). Rode lijst en verspreidingsonderzoek van de waterroofkevers (Coleoptera: Dytiscidae) van Vlaanderen. Hogeschool Van Hall Larenstein, Master Natuur- en Bosbeheer.
- Mwanda Regina (2012-2013). Impact of mining on river water quality in Zimbabwe. Universiteit Gent, Master of Sciences in environmental sanitation.
- Abebe Yonas Alemnew (2012-2013). Impact of land use change and population growth on river water quality and macroinvertebrate diversity in South West Ethiopia. Universiteit Gent, Master of Sciences in Environmental Sanitation.
- De Lange Nicolas (2012-2013). Wetlands en impact op biodiversiteit/ontvangende waterlopen. Universiteit Gent, Master in de bio-ingenieurswetenschappen: milieutechnologie.
- Verdegem Koen (2012-2013). Impact analysis of introduced salmonids on ecosystem structure and functioning in the Cajas Natioanal Park (Ecuador). Universiteit Gent, Technology for Integrated Water Management.

Scientific awards

Sustainability award at the 16th PhD Symposium on Applied Biological Sciences, Ghent, Belgium, 20 December 2010.

International research stays

Assessment of wetlands and rivers in the framework of the IUC-JU. August and September 2009 and October and November 2011.