









## **Science Facing Aliens Proceedings of a Scientific Meeting on Invasive Alien Species**









# Science Facing Aliens

Proceedings of a scientific meeting on Invasive Alien Species held in Brussels, May 11<sup>th</sup>, 2009

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This conference is an initiative of the Belgian Biodiversity Platform's Forum on Invasive Alien Species

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## Welcome to the World? Non-native breeding birds in Flanders (Belgium)

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## Introduction

Non-native bird species with their natural breeding range in the Americas, Asia, Africa and Australia have become more common in large areas of Europe, and the situation in Flanders (Belgium) is no exception to this. Waterbirds constitute an important part of these non-native species. In Flanders, the keeping of waterbirds in captivity, in zoos and private collections is commonplace and the deliberate and accidental release of full-winged birds has led to the development of most of our present feral populations. It is widely accepted that non-native species could be a threat to biodiversity and several international legislative instruments, including the Convention on Biological Diversity (1992), the Bern Convention (1979), the AEWA Agreements (1999) under the Bonn Convention (1983) and the European Birds Directive (1979) strengthen the need to control invasive and non-native species that threaten that biodiversity. In this contribution, we give a short overview of the status of the most important non-native bird species in Flanders, with the exception of the Rose-ringed Parakeet, a species mostly concentrated in and around Brussels and for which we refer to Weiserbs (2010, present volume). We first present information on population numbers, distribution, trends, dispersion potential and known impact, to determine the Invasive Species Environmental Impact Assessment scores (ISEIA scores) of the various species, but to show also possible economic and public health hazards. We give a short summary of the existing legislation and management measures, and end with some guidelines for future actions and needs.

## Assessment of environmental and other hazards

#### Population numbers, trends and distribution

For most of the species good information is available on historical presence, long-time breeding population numbers (Vermeersch & Anselin, 2009), wintering numbers (Devos 2009, in prep) and distribution (Vermeersch et al, 2004). At present, in 70% of all 5×5 km UTM squares in Flanders at least one non-native species is breeding. Three species, Canada, Egyptian and Barnacle goose occupy 93% of all squares and count for 92% of the total breeding population of all non-native species. Wintering trends are in general very similar to breeding trends.

The Canada Goose, *Branta canadensis* is the most successful non-native waterbird species in Flanders. Numbers increased from 1 breeding pair in 1973 to the present population of 1600-2000 breeding pairs! The species is now widely distributed and is particularly abundant in the central part and along the Scheldt and Leie river valleys. Areas with high numbers coincide with the (former) presence of wildfowl collections. Numbers of the





Egyptian Goose, Alopochen aegyptiacus have increased markedly from a few pairs in 1973-1977 (Brussels) to the present population of 800-1500 pairs. The species is now widely distributed but is more abundant in the central and eastern part of Flanders. Squares with more than 10 breeding pairs are frequent and local densities can reach 35 pairs/square. Breeding birds in eastern Limburg most probably originate from adjacent Dutch populations. In certain areas growth rates are very high. Although the feral Barnacle Goose, Branta leucopsis has a smaller breeding population (120-150 bp) and a less extensive range than the two former species, numbers are increasing rapidly. The first free-living birds were observed in the 1960s, and the first free-breeding pair was only recorded in 1992 in the Campine region, but the species is now distributed in the whole of Flanders and occupies 10% of all atlas squares. The Bar-headed Goose, Anser indicus is a scarce breeding species (20-25 bp) over the whole of Flanders and shows a very slow increase. It has been observed in the wild since the 1960s and the first breeding record dates from 1989. The Magellan Goose, Cloephaga picta has 50-60 breeding pairs of which the majority is present in one area in the province of East-Flanders. These birds originate from a nearby waterfowl collection and started breeding in the wild in 1993. The population has slowly increased. Free-living birds have been reported since the seventies, but numbers have always remained low. The feral Black Swan, Cygnus atratus is a rather scarce breeding species with 40-45 pairs (5% of the atlas squares) but shows a steady increase. In most squares only one breeding pair is recorded. They have been reported since the 1970s. Observations increased in the 1990s and the first free-breeding pair was noted in 1998 north of Brussels. The population of the Mandarin Duck, Aix galericulata is estimated at 80-95 pairs. It occurs over the whole of Flanders but is scarce in the western part. Free-living birds have been reported since the 1950s, but observations have become more frequent since the 1980s. The first pair was noted near Brussels in 1987. The species seems to increase slowly. The Wood Duck, Aix sponsa occupies only 3.8 % of the atlas squares and has a population of 25-30 breeding pairs, which remains very low. The species occurs less in the western part. The first free-living birds were observed in 1957, and the first breeding record was noted in 1982. The Ruddy Shelduck, Tadorna ferruginea is a very scarce and irregular breeding species (5-10 bp). Breeding pairs are probably all escaped birds although wild birds could arrive by natural dispersion. The first breeding record dates from 1981 and since, only a few breeding attempts have been reported. In 2008 two pairs of the Ruddy Duck, Oxyura jamaicensis bred for the first time in Flanders and several young were observed. In 2009 at least 4 pairs (maybe 5) pairs are present and the chances are high that the population will quickly extend if no measures are taken (obs. Geert Spanoghe).

#### Dispersal

To gather more information on the spatial and seasonal distribution and dispersion of the Canada Goose in Flanders a neck-banding project was started in 1995 in cooperation with the Belgian ringing service (Anselin et al, 1996) and later also in the southern part of the country. Between 1994 and 2003 a 200 neck bands and 69 leg rings were fitted on adult and immature birds in 17 locations. Analysis of the 4600 controls show that there is a lot of exchange between locations (and regions) but birds move mostly no further than 20-25 km and usually a radius of 50 km is not exceeded. Movements over several hundreds of kilometres seem to be an exception. With a species distributed over large parts of Belgium and neighbouring zones and showing a high level of exchange this means that to control numbers in an efficient way, the actions need to be organized on a wide scale (Cooleman et al, 2005). Colour ring programs for the Barnacle Goose in the Netherlands (Van der Jeugd, 2005) and a similar program in Brussels for the Egyptian Goose (Vangeluwe & Roggeman, 2000) show that in





these geese species exchange between populations is also frequent, which demonstrates again the need for wide scale, and preferably, international actions. *Impact* 

Most likely environmental problems with non-native waterbirds arise from hybridisation with closely related species, previously separated by geographical barriers. Other environmental hazards are aggression towards other species, damage to vulnerable habitats by grazing and trampling and deposition of nutrients by roosting birds leading to eutrophication (geese). Apart from this, economic damage can be caused by large flocks of geese species to crops and grasslands by year-round grazing and trampling. Other impacts on man are damage to amenity areas, threats to public health in parks and water areas and threats to air safety (collisions with aircraft)(Owen et al, 2006). In Flanders, damage on vulnerable habitats in nature reserves by overgrazing, trampling and water eutrophication by the three most common non-native geese species is frequently reported and is most certainly a major problem, but no studies have been carried out on the effects. Damage in agricultural habitats occurs but has not been quantified. Aggressive behaviour of Canada and Egyptian geese towards other species has been reported, but again very few is known about magnitude and effects (Beck & Anselin, 2005). On the other hand, the problem of interbreeding of the North American Ruddy Duck and the rare European White-headed Duck, Oxyura leucocephala (an Annex I species of the EU Birds Directive) is well documented and guidelines for actions are presented in an international action plan (Hughes et al. 2006). Now that the Ruddy Duck has recently started breeding in Flanders, special attention must be paid to the species and actions undertaken to halt this development.

Species	Breeding	Wintering	Dispers	Impact	Impact	ISEIA score	Impact
	pop/trend	pop/trend		habitat	species	category	human
Branta canadensis					?	Black	
Alopochen aegyptiacus					?	Watch>Black?	
Branta leucopsis					?	Not>Black?	
Anser indicus					?	Watch	
Cloephaga picta					?		
Cygnus atratus					?		
Aix galericulata					?	Watch	
Aix sponsa					?		
Tadorna ferruginea					?		
Oxyura jamaicensis						Alert>Black	

Table 1: The various risk factors in three broad categories from light grey (low or medium/less important) to dark (high/more important), together with the proposed changes in ISEIA category for some species (in bold)

In Table 1 we can distinguish two main groups. First, the three most common geese, (now all proposed for Black) and the Ruddy Duck (Alert), now proposed for Black taken into account the recently changed status of the species in Flanders. The second group consist on two 'Watch' species, the Bar-headed Goose and the Mandarin Duck and several not categorised species, thus generally spoken, the species with (at present) a lower environmental impact.





## Legislation and Management

Apart from the international actions in the framework of the Ruddy Duck interbreeding problem, only the management of the Canada Goose is legally regulated. Ruddy Shelduck and Barnacle Goose are special cases. The first has wild breeding populations within the EU (in the Black Sea region) and is as such not considered as a 'non-native' species, although feral populations mostly origin from escapees. The Barnacle Goose figures on the Annex I of the EU Birds' Directive and the unclear mix of populations with birds from escaped and wild origin outside their traditional Scandinavian breeding grounds complicates actions to prevent further growth of these populations (in certain regions in major part originating from escapees). All other non-native species are 'outlawed' and can be 'destroyed' by all means. The management of Canada Goose populations consists of hunting, destroying of eggs and rounding up and killing of flightless birds during moult. Bag statistics are gradually better documented (Scheppers & Casaer, 2008). Statistics on other measures exist but are still less accessible.

## Future actions and needs

To be able to evaluate management actions in an efficient way, the existing monitoring systems have to be maintained and developed or adapted where necessary. Non-native species form part of a regional environmental indicator and therefore it is important to have sound data. There is a need to start thinking about developing structural and legally regulated management actions for the Egyptian Goose and to find a solution for the Barnacle Goose 'problem'. Intervention actions on these species should be coordinated and organised on a much wider (international) scale. Special attention must be paid to the Ruddy Duck and actions undertaken to halt its present population development. We strongly advice to support or start studies on damage impact (crops, vulnerable vegetations, species interactions) as sound quantitative information is largely lacking, and bring together all non- published knowledge in a comprehensive review. And last but not least we simply propose to apply more extensively the AEWA Conservation Guidelines on Avoidance of Introductions of Non-native Waterbird Species (Owen et al, 2006).





#### References

- Anselin A., Geers V. & Kuijken E., 1996. Population trends and ecology of the Canada Goose Branta canadensis in Flanders. In: Holmes J. & Simons J. The introduction and naturalisation of birds. HSMO, London 71-72.
- Beck O. & Anselin A., 2005. *Management of feral geese populations in Flanders* (in Dutch). Beheer van verwilderde ganzenpopulaties in Vlaanderen. Natuur.oriolus 71:166-169.
- Cooleman S., Anselin A., Beck O., Kuijken E. & Lens L., 2005. *Movements and mortality of Canada Goose Branta canadensis in Flanders* (in Dutch). Verplaatsingen en mortaliteit van de Canadese Gans *Branta canadensis* in Vlaanderen. Natuur.oriolus 71:152-160.
- Devos K., 2009. Wintering waterbirds in Flanders. Numbers, distribution and trends in the period 1991/92 2008/09 (in Dutch). Overwinterende watervogels in Vlaanderen. Aantallen, verspreiding en trends in de periode 1991/92 2008/09. Mededeling INBO, Brussel (in prep).
- Hughes B., Robinson J., Green A., Li D. & Munkur T., 2006. International Single Species Action Plan for the Conservation of the White-headed Duck *Oxyura leucocephala*. CMS Technical Series 13, AEWA Technical Series 8. Bonn.
- Owen M., Callaghan D. & Kirby J., 2006. Guidelines on Avoidance of Introductions of Nonnative Waterbird Species. AEWA Technical Series N° 12. Bonn.
- Scheppers T. & Casaer J., 2008. *Hunting Management Units: statistics, report and analysis of the period 1998-2007.* (in Dutch). Mededelingen INBO nr 9, Brussel.
- Van der Jeugd H., 2005. Barnacle Geese Branta leucopsis on the move (in Ditch). Brandganzen Branta leucopsis volop in beweging! Natuur.oriolus 71:161-164.
- Vangeluwe, D. & Roggeman W., 2000. Evolution, structure et gestion des rassemblements d'Ouettes d'Egypte Férales en Région Bruxelles-Capitale. Rapport IRSNB, Brussel.
- Vermeersch G. & Anselin A., 2009. *Breeding birds in Flanders 2006-2007* (in Dutch). Broedvogels in Vlaanderen 2006-2007. Mededelingen INBO nr. 3, Brussel).
- Vermeersch G., Anselin A., Devos K., Herremans M., Stevens J., Gabriëls J. & Van Der Krieken B., 2004. Atlas of the breeding birds in Flanders (in Dutch). Atlas van de Vlaamse Broedvogels 2000-2002. Mededelingen IN nr. 23, Brussel.
- Weiserbs, A., 2010. Brussels Psittacidae: impacts, risk assessment and action range. Present volume, 77-79.











## Alien macro-crustaceans in freshwater ecosystems in Flanders

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## Introduction

The introduction of invasive species has increased enormously during the past decades. Currently, eighteen alien macro-crustaceans have been found in freshwater ecosystems in Flanders. One of these invasive species, which is already widely distributed throughout Europe, is *Dikerogammarus villosus* (Sowinsky, 1894)(Bollache et al., 2004). Since 1997, *D. villosus* has been found in Flemish watercourses (Messiaen et al., unpublished data). *D. villosus* was first observed in the Albert canal, while nowadays, it also occurs in other canals and other stagnant and running watercourses in Flanders. Lab experiments have proven useful to determine the impact of *D. villosus* on a microcosm scale (Dick and Platvoet, 2000). A problem with these experiments is to translate the obtained results to field situations. Therefore, it can be useful to combine lab experiments with field observations and data-driven models. This study aims to identify the most important variables determining the habitat suitability of *D. villosus* using decision trees. In addition, lab experiments were conducted to gain insight in the behaviour of *D. villosus*. In this way, models based on field observations can be used in combination with lab experiments to make useful predictions about the impact of the invasive species *D. villosus* on native macroinvertebrate communities.

## Material and methods

Multiple as well as single prey experiments were conducted in glass aquaria filled with five litres of carbon filtered water. In the multiple prey experiments, five individuals of the predator *Dikerogammarus villosus* were released in the aquaria containing four individuals of five different prey (*Asellus aquaticus, Crangonyx pseudogracilis, Gammarus pulex, Cloeon dipterum* and *Chironomus species*). Single species experiments with *A. aquaticus, C. pseudogracilis, G. pulex, G. tigrinus* or *C. dipterum* as prey were also conducted to study the interaction between prey and predator in the absence of other macroinvertebrates. In these experiments, five individuals of one prey species were exposed to five individuals of *D. villosus*. All experiments lasted 24 h after which the survival of the macroinvertebrates was checked. To check the influence of the substrate on predation, all experiments were conducted three times: once on gravel, once on sand and once without substrate. All predator-prey experiments were replicated five times.

To determine the substrate preference of the invasive *D. villosus* and the native *G. pulex*, 10 individuals of each one species were released in an aquarium filled with 10 litres of carbon filtered water. To assess if substrate preference changed if prey and predator occurred together, an additional experiment was conducted, where 10 individuals of both species were put together in one aquarium. The substrate preference was checked after 24 hours.

The dataset used to model the habitat suitability is based on the samples collected by the Flemish Environment Agency, which monitors a large number of sampling points scattered over the different stagnant and running water systems in Flanders. Environmental





variables, hydro-morphological characteristics and data of other macroinvertebrates were available. In total, 232 presence or absence data were available with information on the hydro-morphological characteristics and physical-chemical variables. In 145 samples, *D. villosus* was absent, while in 87 other samples, the species was present. For the decision tree construction, the machine learning package WEKA – J 48 algorithm (Witten and Frank, 2000) was used.

## **Results**

The multiple prey experiment showed a strong predation on all macroinvertebrates in the presence of *D. villosus* (Figure 1), while all individuals of *D. villosus* survived. The highest survival of prey was usually found with gravel as substrate, except for *G. pulex*, which had the highest survival with sand as substrate. There was a significant difference in survival of the different prey (p<0.001) and a significant difference in survival when using different substrates (p=0.039). The single-prey experiments (Figure 1) showed a significant difference in survival of *A. aquaticus* (p<0.001), *C. pseudogracilis* (p=0.012) and *G. tigrinus* (p<0.001) when different substrates were used. As in the multiple prey experiment, survival was again highest on gravel.

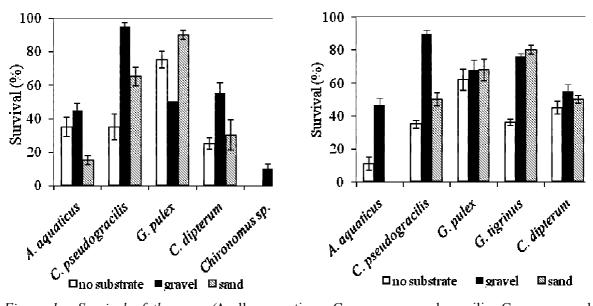


Figure 1 - Survival of the prey (Asellus aquaticus, Crangonyx pseudogracilis, Gammarus pulex, Cloeon dipterum, Chironomus species and G. tigrinus) after 24 h exposure to the predator Dikerogammarus villosus in the multiple (left) and single (right) prey experiments when exposed without substrate, on gravel and on sand.

The single species substrate preference experiments showed that *D. villosus* has a clear preference for gravel (p<0.001): after 24 h,  $63.3 \pm 1.9$  % of the individuals were found between the gravel. When both species were exposed together, there was a significant shift in substrate preference of *G. pulex* (p<0.001) and *D. villosus* (p=0.006): more individuals of *D. villosus* and less individuals of *G. pulex* were present between the gravel.

A decision tree with 77 % CCI and a K of 0.5 was constructed. The most important variables determining the presence or absence of *D. villosus* were bank structure, oxygen saturation and conductivity. The model showed that *D. villosus* preferred canals with a high oxygen concentration and a low conductivity.





#### Discussion

Results of the predator-prey experiments showed a similar predatory behaviour of *D. villosus* compared to the results of previous studies (Dick and Platvoet, 2000; Krisp and Maier, 2005). Not only native species, but also the exotic species *G. tigrinus* and *C. pseudogracilis* originating from North America were predated. In this way, *D. villosus* not only has an influence on native fauna, but also on exotic species, as was already observed in the river Rhine and the river Meuse (Josens et al., 2005; Van Riel et al., 2006). In the presence of *D. villosus*, a general decline in macroinvertebrate diversity and abundance in natural systems was observed (Van Riel et al., 2003). Despite its predatory behaviour, *D. villosus* should, however, not be seen as a strict carnivore: studies conducted by Platvoet et al. (2005), Maazouzi et al. (2007) and Mayer et al. (2008) showed that *D. villosus* is an omnivorous species able to eat plant as well as animal material. This diverse food spectrum probably contributes to the successful spread of this species.

The substrate preference experiment pointed out that *D. villosus* preferred gravel substrate, as was also found in previous laboratory studies (Van Riel et al., 2003; Kley and Maier, 2006). Studies conducted in the Moselle river (France) indicated that *D. villosus* was present on different types of substrates (Devin et al., 2003), however, there was a difference in preference based on the age and the size of the species. Juveniles were more often present between roots and macrophytes whereas adults had a preference for boulders and stones.

The developed habitat suitability model indicated that watercourses with an artificial bank structure, a high oxygen saturation and a low conductivity were preferred by *D. villosus*. *D. villosus* can thus invade artificial watercourses, however, the water quality regarding oxygen content and conductivity has to be good. The species avoided watercourses with a good biological water quality, which possibly means that natural systems with a high diversity of macroinvertebrates are more resistant to invasions than watercourses with a low diversity. However, according to Bollache et al. (2004), it is possible that in the near future, whole drainages of natural and semi-natural rivers, can be invaded by this species. Therefore, continuous monitoring of invasive species remains necessary.

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#### References

- Bollache, L., Devin, S., Wattier, R., Chovet, M., Beisel, J-N., Moreteau, J-C., Rigaud, T., 2004. Rapid range extension of the Ponto-Caspian amphipod *Dikerogammarus villosus* in France: potential consequences. *Arch. Hydrobiol.* 160, 57-66.
- Devin, S., Piscart, C., Beisel, J.N., Moreteau, J-C., 2003. Ecological traits of the amphipod invader *Dikerogammarus villosus* on a mesohabitat scale. *Arch. Hydrobiol.* 158, 43-56.
- Dick, J.T.A., Platvoet, D., 2000. Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proc. R. Soc. Lond.* B. 267, 977–983.
- Josens, G., Bij de Vaate, A., Usseglio-Polatera, P., Cammaerts, R., Cherot, F., Grisez, F., Verboonen, P., Vanden Bossche, J-P., 2005. Native and exotic Amphipoda and other Peracarida in the River Meuse: new assemblages emerge from a fast changing fauna. *Hydrobiologia* 542, 203-220.
- Kley, A., Maier, G., 2006. Reproductive characteristics of invasive gammarids in the Rhine-Maine-Danube catchment South Germany. *Limnologica* 36, 79-90.
- Krisp, H., Maier, G., 2005. Consumption of macroinvertebrates by invasive and native gammarids: a comparison. *J. Limnology* 64, 55-59.
- Van Riel, M.C., Van der Velde, G., Bij de Vaate, A., 2003. Alien amphipod invasions in the river Rhine due to river connectivity: a case of competition and mutual predation. In Douben, N., Van Os, A.G., (eds). Proceedings NCR-days (2003); Dealing With Floods Within Constraints. NCR publication 24. Netherlands Centre for River Studies, Delft, 51– 53.





## ISEIA, a Belgian non-native species assessment protocol

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## Introduction

Belgian land managers and policy makers have to face up to an increasing number of nonnative species with contrasted impacts on the environment. To help them in the identification of species of most concern for preventive or mitigation actions, *Harmonia*, an information system on invasive species in Belgium, has recently been developed at the initiative of scientists gathered within the Belgian Forum on Invasive Species (http://ias.biodiversity.be).

*Harmonia* is based on a standardised assessment protocol (ISEIA) which allows assessing, categorising and listing of non-native species from any taxonomic group according to their invasion stage in Belgium and to their impact on native species and ecosystem functions (Branquart 2007). The ISEIA protocol is one of the first national standardised risk assessment tools developed for non-native species in Europe (Essl et al., submitted).

Here we present the ISEIA protocol, the assessment procedure and the results of the first assessments performed on vascular plant and vertebrate species in Belgium. The way those results may be used to develop regulatory instruments and management guidelines are also briefly discussed.

## The ISEIA protocol and the Belgian list system

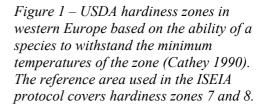
The ISEIA protocol aims at categorising non-native species on the basis of a standardised methodology designed to minimise the use of subjective opinions and to make the process of assessing and listing invasive species transparent and repeatable.

Contrary to predictive pest risk assessment protocols mainly based on species' intrinsic attributes for evaluating invasion likelihood and potential to cause adverse ecological effects, the ISEIA approach favours the use of invasion histories documented in peer-reviewed publications and in scientific reports from Belgium and neighbouring areas. It is considered that non-native species are likely to cause significant impacts on native species and ecosystems in Belgium if they have already done so in neighbouring countries. The reference area taken into consideration for the assessment includes the European regions with ecoclimatic conditions comparable to Belgium, i.e. hardiness zones 7 and 8 characterised by an average annual minimum temperature between -7 and  $-17^{\circ}$ C (Cathey 1990). It covers Denmark, the Netherlands and large parts of Germany, France, Ireland, Switzerland and the United Kingdom (figure 1).









The Belgian list system is based on three different list categories as proposed in the European strategy on Invasive Alien Species (Genovesi & Shine 2003). Those categories are defined according to the severity of impacts on the environment: no negative impact (white list), negative impact suspected (grey list) and negative impact confirmed (black list). The assignment of a non-native species to one of those categories is assessed by four main criteria matching the last steps of the invasion process, i.e. potential for spread, colonisation of natural habitats and adverse ecological impacts on native species and ecosystems. Consistent with other risk assessment standards, equal weight is assigned to each of the four criteria and a three-point scale is used for criteria scoring: low (or unlikely), medium (or likely) and high. The global ISEIA score is calculated as the sum of risk rating scores of the four criteria (see Branquart 2007 for additional explanation).

Non-native species are allocated to the different categories of the Belgian list system combining information from the ISEIA scoring and data on their invasion stage in the country (figure 2). Detrimental species not yet established in Belgium (A0) or occurring only in a few localities (A1) are to be considered as a high priority for prevention and eradication actions (Genovesi & Shine 2003, Genovesi et al. 2009).

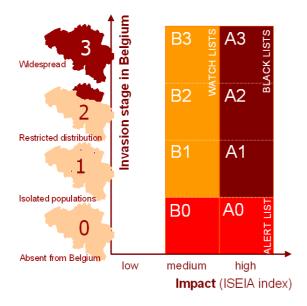


Figure 2 - List system proposed to identify non-native species of most concern for preventive and mitigation actions in Belgium (Branquart 2007).





#### The assessment procedure

Five different expert working groups were established to deal with vascular plants, fishes, amphibians, birds and mammals, each of them including three to six scientists from different research institutes and universities in Belgium. In a first step, the environmental impact of each species is assessed independently by the different experts, sometimes leading to diverging results. At last, these results are discussed during a working group meeting in order to make sure that experts share a common understanding of criteria and definitions and to search for a robust consensus for each species.

The ISEIA protocol has been improved several times based on those discussions. It has proven to be flexible enough to be used to assess the environmental impact of non-native species from very different taxonomic groups. The major difficulty encountered during the assessment process was that environmental impacts of non-native species in the reference area are often poorly documented in the literature. This is typically the case for most species included in the watch list (e.g. *Acer rufinerve, Alopochen aegyptiacus, Ameiurus* spp., *Cyperus eragrostis, Lepomis gibbosus* and *Tamias sibiricus*).

Up to now, assessments were focused on species considered as detrimental in at least one country of the reference area (e.g. according to Pascal et al. 2003, Muller 2004, Weber et al. 2005, Wittenberg et al. 2006, Copp et al. 2008).

#### **Results and trends**

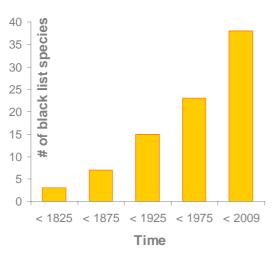
Nearly 400 non-native species of vascular plants and vertebrates can be considered as currently established in Belgium. So far, 57 non-native vascular plant (neophytes) and 32 vertebrate species have been evaluated by Belgian experts using the ISEIA protocol, comprising 72 species considered as naturalised in Belgium. Thirty-nine out of these 72 species were assessed as organisms with a strong detrimental impact on native biodiversity (black list species, A1-A3), for which preventive and mitigation actions are strongly recommended (see the list in appendix). Most of the remaining species were recorded on the watch list (B1-B3), which means either that their impact on native biodiversity is moderate or that their impact is still unclear due to a deficiency in scientific studies.

Compared to vascular plant species, a higher proportion of the vertebrate species has been shown to be detrimental to native species and ecosystem functions. Invasive neophytes typically affect biodiversity by growing in very dense populations in semi-natural habitats, outcompeting native plant species and modifying the vegetation structure. Invasive vertebrate species rather adversely impact biodiversity through a wide range of interspecific interactions (competition, predation, disease transmission and hybridisation) that may act separately or synergistically (*Harmonia* database 2009).

Besides the evaluation of 72 non-native species naturalised in Belgium, 17 taxa established in neighbouring countries but not (yet) in Belgium were assessed and included in the alert list (A0-B0) (see appendix). All of them are likely to become established in the coming years if no preventive action is undertaken.







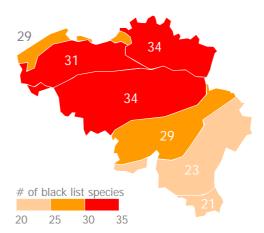


Figure 3A – Number of black list species established in the wild in Belgium at different periods of time. Data: Harmonia database 2009.

Figure 3B – Number of black list species within the main biogeographic regions of Belgium Data: Harmonia database 2009.

When examining the change in number of invasive species for 200 years (*Harmonia* database 2009), we can see that there is an increasing number of established black-list species in Belgium causing major ecological damages and growing management costs (figure 3A). Those species tend to concentrate in areas where human density and activities are at the highest (figure 3B) and where habitats incur frequent alteration, eutrophication and pollution, and therefore may be prone to invasion. Many of those black-list species thrive along river banks and in freshwater environments.

#### From science to management

Eighteen non-native species out of the group of organisms responsible for high environmental impacts are either not yet established in Belgium (A0) or only known from a limited number of localities (A1) (see appendix). Prevention actions and early eradication of these species deserve to be conducted in priority. Indeed, the ecological damages they may cause can still be restricted to a minimum at a low cost if actions are undertaken without delay. This is the reason why these 18 species have been proposed to be considered in a new Royal Decree (expected in early 2010) aiming at restricting their importation, exportation and rearing.

Another 30 detrimental species are already widely distributed in Belgium (A2 & A3) and cannot be eradicated anymore. However, it is still worthwhile avoiding further secondary releases to slow down the invasion process. Voluntary codes of conduct developed in partnership with key sectors of activity (horticulture, pet industry, etc.) may help to reduce the introduction of these species in the wild (Branquart & Halford 2009). In addition to preventive actions, the monitoring and the management of those species is strongly recommended in areas of high conservation value in order to preserve native red-listed species and threatened habitats (Tu 2009).

At last, it is now widely acknowledged that early warning and rapid response are crucial for mitigating the impacts caused by biological invasions in Europe. Early warning tools should ideally be developed through information exchange at a regional scale and need a strong international scientific collaboration, a common understanding of invasiveness issues and shared risk assessment schemes (Genovesi et al. 2009, Hulme et al. 2009). A standardised ISEIA-like protocol deserves to be developed at a European scale to reach that goal.





#### Acknowledgements

The following scientists (listed by taxonomic group and in alphabetical order) have participated to the impact assessment of non-native vascular plant and vertebrate species: Iris Stiers, Ludwig Triest, Sonia Vanderhoeven (FNRS), Wouter Van Landuyt, Fabienne Van Rossum, Filip Verloove (vascular plants); Dieter Anseeuw, François Lieffrig, Jean-Claude Micha, Denis Parkinson, Hugo Verreycken (fishes); Arnaud Laudelout, Gérald Louette, Youri Martin, Joachim Mergeay, Chistiane Percsy (amphibians); Anny Anselin, Diederik Strubbe, Anne Weiserbs (birds); Margo D'aes, Alain Licoppe, Grégory Motte, Vinciane Schockert, Jan Stuyck (mammals); Etienne Branquart (coordination).

#### References

- Branquart E. (Ed.), 2007. Guidelines for environmental impact assessment and list classification of non-native organisms in Belgium (available from <a href="http://ias.biodiversity.be">http://ias.biodiversity.be</a>).
- Branquart E. & Halford M., 2009. Increase awareness to curb horticultural introductions of invasive plants in Belgium (InvHorti). EPPO/Council of Europe Workshop: 'Code of conduct on horticulture and invasive alien plants', Ski, 4-5 June 2009.
- Cathey H.M., 1990. USDA plant hardiness zone map. USDA, Washington D.C.
- Copp G.H., Vilizzi L., Mumford J., Fenwick G.V., Godard M.J. & Gozlan R.E., 2008. Calibration of FISK, an invasiveness screening tool for non-native freshwater fishes. Risk Analysis 29: 457-467.
- Essl F., Klingenstein F., Milasowszky N., Nehring S., Otto C. & Rabitsch W., submitted. The German-Austrian black list information system (GABLIS) : a tool for assessing biodiversity risks of invasive alien species in Europe. Journal of Nature Conservation.
- Genovesi P. & Shine C., 2003. European strategy on invasive alien species. Convention on the Conservation of European Wildlife and Natural Habitats. Council of Europe Strasbourg, T-PVS, 60 pp.
- Genovesi P., Scalera R., Solarz W. & Roy D., 2009. Towards an early warning and information system for invasive alien species threatening biodiversity in Europe. Extensive executive summary, contract n° 3606/B2008/EEA.53386.
- Harmonia database, 2009. Belgian Forum on Invasive Species, last accessed on 05 May 2009 from: <u>http://ias.biodiversity.be</u>.
- Hulme P.E., Pysek P., Nentwig W. & Vila M., 2009. Will threat of biological invasions unite the European Union? Science 324: 40-41.
- Muller S., 2004. Plantes invasives en France: état des connaissances et propositions d'actions. Publication scientifique du Museum d'Histoire naturelle, Patrimoines naturels n°62, 168 p.
- Pascal M., Lorvelec O.L., Vigne J.D., Keith P. & Clergeau P., 2003. Evolution holocène de la faune de vertébrés de France: invasions et extinctions. INRA, CNRS, MNHN & MEDD.
- Tu M., 2009. Assessing and Managing Invasive Species within Protected Areas. Protected Area Quick Guide Series. Editor, J. Ervin. Arlington, VA. The Nature Conservancy, 40 pp.
- Weber E., Köhler B., Gelpke G., Perrenoud A. & Gigon A., 2005. Schlüssel zur Enteilung von Neophyten in der Schweiz in die Schwarze Liste oder die Watch-Liste. Botanica Helvetica 115: 169-194.
- Wittenberg R., 2005. An inventory of alien species and their threat to biodiversity and economy in Switzerland. CABI Bioscience Switzerland Centre report to the Swiss Agency for Environment, Forests and Landscape. The environment in practice no. 0629: 155 pp.





Scientific name	entific name English name		List category
Species not established in Be	lgium		
Callosciurus finlaysonii	Finlayson's squirrel	М	A0
Carpobrotus spp.	Hottentot fig	Р	A0
Cervus nippon	Sika deer	Μ	A0
Muntiacus reevesi	Reeves' muntjac	Μ	A0
Mustela vison	American mink	Μ	A0
Neogobius melanostomus	Round goby	F	A0
Nyctereutes procyonoides	Raccoon dog	Μ	A0
Perccottus glenii	Rotan, Amur sleeper	F	A0
Sciurus carolinensis	Grey squirrel	М	A0
Threskiornis aethiopica	Sacred ibis	В	A0
Species with isolated popula	tions in Belgium		
Callosciurus erythraeus	Pallas's squirrel	М	A1
Crassula helmsii	Australian swamp stonecrop	Р	A1
Egeria densa	Brazilian waterweed	Р	A1
Lagarosiphon major	Curly waterweed	Р	A1
Ludwigia peploides	Water primrose	Р	A1
Myocastor coypus	Coypu, Nutria	М	A1
Myriophyllum heterophyllum	Variable watermilfoil	Р	A1
Rana catesbeiana	American bullfrog	А	A1
Species with a restricted dist			
Acer negundo	Box-elder, Ash-leaved maple	Р	A2
Ailanthus altissima	Tree of heaven	Р	A2
Baccharis halimifolia	Eastern baccharis	Р	A2
Branta canadensis	Canada goose	В	A2
Cornus sericea	Red-osier dogwood	Р	A2
Cotoneaster horizontalis	Rockspray	Р	A2
Hydrocotyle ranunculoides	Water pennywort	Р	A2
Ludwigia grandiflora	Water primrose	Р	A2
Myriophyllum aquaticum	Parrotfeather	Р	A2
Pelophylax ridibundus	Marsh frog	А	A2
Persicaria wallichii	Himalayan knotweed	Р	A2
Procyon lotor	Raccoon	М	A2
Pseudorasbora parva	Topmouth gudgeon	F	A2
Rhododendron ponticum	Rhododendron	Р	A2
Rosa rugosa	Rugosa rose	Р	A2
Spiraea spp.	Meadowsweet	Р	A2

Appendix – List of non-native species with a high detrimental impact on the environment





Appendix (cont'd) – List of non-native species with a high detrimental impact on the environment

Widespread species in Belgium					
Aster americ.	North American asters	Р	A3		
Carassius gibelio	Prussian carp	F	A3		
Elodea canadensis	Canadian waterweed	Р	A3		
Elodea nuttallii	Nuttall's waterweed	Р	A3		
Fallopia japonica	Japanese knotweed	Р	A3		
Helianthus tuberosus	Jerusalem artichoke	Р	A3		
Heracleum mantegazzianum	Giant hogweed	Р	A3		
Impatiens glandulifera	Indian balsam	Р	A3		
Mahonia aquifolium	Oregon grape	Р	A3		
Ondatra zibethicus	Muskrat	Μ	A3		
Prunus serotina	Black cherry	Р	A3		
Rattus norvegicus	Brown rat	М	A3		
Solidago canadensis	Canada goldenrod	Р	A3		
Solidago gigantea	Giant goldenrod	Р	A3		

\* Taxonomic groups: amphibians (A), birds (B), fishes (F), mammals (M) and vascular plants (P).









## Impact of *Fallopia* spp. on ecosystem functioning: Nitrogen and organic matter cycling and implicated soil biota

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## Introduction

*Fallopia japonica* is one of the most invasive alien plant species in NW Europe. Its impact on indigenous vegetation is high and well documented, whereas its impact on ecosystem processes has been less studied. It considerably increases primary productivity in invaded ecosystems (Dassonville et al., 2008). It has also been shown to increase cations and P availability in the topsoil by a nutrient uplift mechanism thanks to its very deep rooting system (Dassonville et al., 2007). In the ALIEN IMPACT project, we evaluated the impact of the species on different steps of nitrogen and organic matter cycling and on implicated soil biota. Considering the thick permanent litter layer often observed under clones of *Fallopia*, it was hypothesized that *Fallopia* slows down litter decomposition and nitrogen cycling rate.

## **Results and Discussion**

The litter decomposition dynamic of *Fallopia* has been assessed using the litterbag technique (Dassonville et al., 2009). We measured the decomposition of *Fallopia* litter (stems and leaves) and of native vegetation of the study site (50-50 mixture of *Eupatorium cannabinum* and *Calamagrostis epigejos*). The litter of *Fallopia* decomposes much more slowly than that of native vegetation (Figure1), probably due to its very high C:N ratio (151 and 72 for *Fallopia* stems and leaves, respectively, against 33 for native litter). On the other hand, the decomposition of each litter type was slightly faster in invaded compared to uninvaded plots. This could be an effect of the moister microclimate under the dense canopy of *Fallopia* than on the soil surface of the uninvaded grassland.

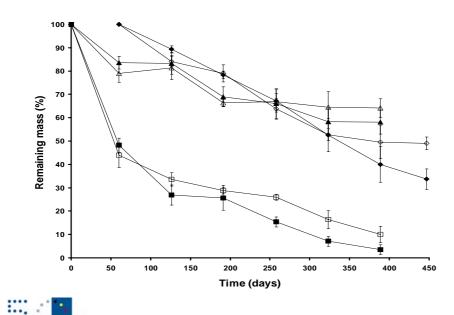
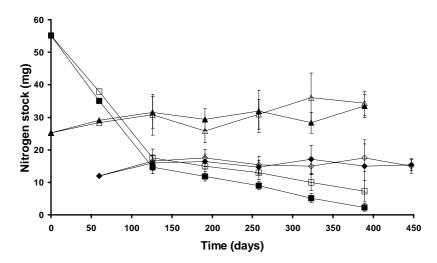


Figure 1: Decomposition kinetics of Fallopia leaves (triangles) and stems (diamonds) and native litter (squares) during one year. All litter types were incubated in invaded (black) and uninvaded (white) environment; Decomposition is expressed as the percentage of initial mass lost. Values are means  $\pm$ standard deviation.



The evolution of the nitrogen stock in the litterbags was followed (Figure2). The N stock decreases rapidly in litterbags with native litter. This means that this litter easily releases N to the soil. On the other hand, N tends to accumulate over time in the litterbags containing *Fallopia*. This suggests that microorganisms living on the decomposing litter have to use mineral N from the soil to compensate for the low N concentration of their substrate. This leads to fixation of mineral nitrogen into organic matter.





From N fluxes measurements in the invaded ecosystem, it has been found that the internal cycling of N in *Fallopia* is exceptionally efficient. Indeed, 80 % of the N present in aboveground biomass during summer is translocated to the rhizomes before the abscission of the leaves (This phenomenon explains the high C:N ratio of the litter). This process allows the plant to grow rapidly in spring, independently of soil N mineralization. This contributes to the high productivity of the species and to its competitive superiority.

*Fallopia* has also been found to decrease the intensity of nitrification and denitrification in sites with high nitrification potential. Molecular analyses show that these differences in activity were partially explained by a decreased number of nitrifying/denitrifying bacteria (assessed by quantitative PCR targeting nitrification/denitrification genes). On the other hand, the structure of the soil microbial communities does not seem to be altered (PCR-DGGE analyses). Our results suggest a potential allelopathic effect of *Fallopia* on soil microbes. The reduction of nitrification and denitrification intensity result in the reduction of N loss from the ecosystem by nitrate leaching and NOx emissions.

From the results mentioned above, it appears that *Fallopia* has a very economic N management, which tends to conserve N in the ecosystem (mineral N fixation on decomposing litter, efficient N retranslocation and reduced nitrification and denitrification intensity). This could be a key trait explaining the invasive success of the plant.

Finally, *Fallopia* also impacts soil fauna. The invertebrate density is 50% lower under *Fallopia* compared to uninvaded grassland. The major groups of the mesofauna (0.2 to 4 mm) are similar (springtails, gamasid and oribatid mites) when comparing invaded and uninvaded plots. On the other hand, some groups of macrofauna (4 to 80 mm) differed between invaded and uninvaded plots. Typical forest taxa like diplopods and isopods or the earthworm *Lumbricus terrestris* are more frequent under *Fallopia* than under the uninvaded grassland vegetation. These taxa are important for litter fragmentation and incorporation to the soil. This could explain the higher decomposition rate under *Fallopia*. On the other hand, ants, aphids and the earthworm *L. castaneus* were totally absent under *Fallopia*. These taxa are





thermophilous grassland species. Based on these differences, the invaded and uninvaded plots are very well separated in the PCA analyses (Figure 3). The first axis of the PCA could represent a light and moisture gradient. The changes in soil fauna are thus mainly explained by a reduction of food diversity and a change in soil microclimate.

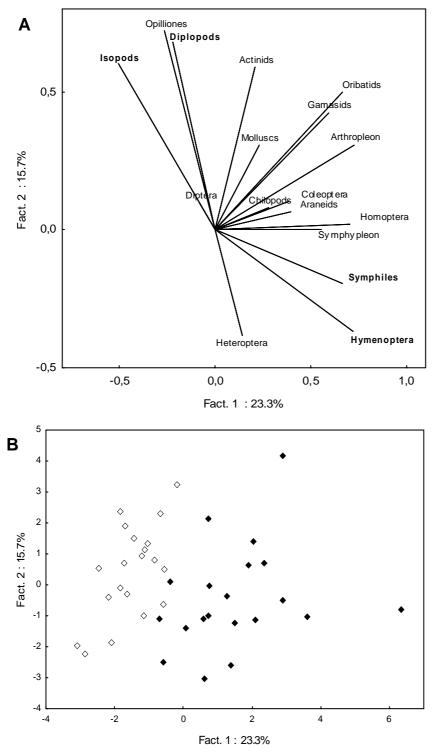


Figure 3: Principal Component Analysis (PCA). A: Projection of variables (taxonomic groups) on PC1 and PC2 for soil fauna but the earthworms. B: Projection of the sampling points from the invaded (white) and the uninvaded (black) plots.





#### References

- Dassonville N., Vanderhoeven S., Domken S., Meerts P., Chapuis-Lardy L., 2009. Impacts of Alien Invasive Plants on Soil and Ecosystem Processes in Belgium: Lessons from a Multispecies Approach. In Wilcox C.P. and Turpin R.D. (eds). Invasive Species: Detection, Impact and Control. Nova publishers. In press.
- Dassonville N., Vanderhoeven S., Vanparys V., Hayez M., Gruber W., Meerts P., 2008. Impacts of alien invasive plants on soil nutrients are correlated with initial site conditions in NW Europe. Oecologia 157:131-140.
- Dassonville N., Vanderhoeven S., Gruber W., Meerts P., 2007. Invasion by *Fallopia japonica* increases topsoil mineral nutrient concentrations. Ecoscience 14: 230-240.





## Soil-dependent growth strategy of invasive plants: empirical evidence and model predictions using *Carpobrotus edulis* as target species

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## Introduction

Plants experience different soil conditions that may influence several aspects of their biology such as nutrient uptake, root competition, growth and even floral display (Mal & Lovett-Doust 2005). For clonal plants, the relative investment in sexual reproduction or vegetative growth can vary according to the context in which they grow (e.g. invaded soil vs. noninvaded) since environmental constrains may result in contrasting dispersal strategies due to differential cost-benefit balances (Kot et al., 1996). Carpobrotus edulis (L.) N. E. Br., is considered a highly invasive species in coastal areas of Southern Europe because it forms dense fast-growing mats that displace the native dune vegetation (Vila et al., 2006). Carpobrotus edulis can change drastically the characteristics of the invaded soil and the longterm occurrence of the species has been associated with a decrease in pH and increase in organic content (Conser in press; D'Antonio & Mahall, 1991). Nevertheless, and in spite of the large dense patches of C. edulis formed in invaded areas, there is no information available about how these changes in soil may affect the posterior growth and colonization rate of the species. The objective of the present study was to evaluate whether the residual effects on soil after C. edulis invasion affect the growth plasticity of the species and to model the long-term consequences of such growth responses. Our working hypothesis was that soil modification introduced by C. edulis leads to plant growth responses oriented to maximize the colonization rate of invaded areas

## **Material and Methods**

Using a lab experiment we assessed whether the residual effects on soil caused by the invasion of *Carpobrotus edulis* would affect the vegetative and reproductive traits of the species and ultimately the dynamics of establishment. We compared *C. edulis* performance on rhizosphere soil collected under native vegetation in the Quiaios dunes (Portugal) that has never been occupied by *C. edulis* (virgin soil, VS), which was used as a reference situation, with the performance of plants on soil collected under monospecific patches in the same locality where the species grew vigorously (*Carpobrotus* rhizosphere soil, CRS) or was dying-back after a long period of establishment (CDS). After four months growing *C. edulis* in controlled conditions plants were harvested and different plant-growth related features were assessed (e.g. biomass, root length, production of flowers). To understand the long term consequences of the observed plant responses, we built up a Monte-Carlo simulation model in which we integrated clonal growth and seed dispersal under different soil scenarios (absence or presence of residual effects).We modeled the spreading of the plant in a grid of 300\*300





grid cells, with one grid equaling 0.3\*0.3 m<sup>2</sup>, being the species annual growth rate as assessed by Sintes et al. (2007). Each grid cell was characterized by soil type: either virgin (VS), occupied (CRS) or previously occupied soil (CDS). We consequently analyzed the rate of colonization (or coverage) in a hectare. The rate of colonization is followed for one seed entering the center of the landscape for three scenarios: colonization of virgin soil without residual effects, colonization of soil where the species is present and colonization of soil where C. edulis has been removed as a restoration measure (hence leaving behind formerly occupied soil). For each scenario we ran 100 replications and calculated average occupancy rates as the number of occupied grid cells/total number of grid cells. Soil status changes according to the emerging plant dynamics. Local plant spreading occurs by clonal growth and by seed dispersal (D'Antonio 1990). With exception of the initial seedling, we introduced a grid cell-extinction rate  $\varepsilon$  of 0.1 in all situations. Seed dispersal is modeled as a stochastic process where seeds are distributed according to a Gaussian distribution N(0, $\sigma^2$  from the mother plant in all directions. We chose  $\sigma^2 = 10$  (i.e. variance of the dispersal function approximates 3 meter in a thin-tailed dispersal kernel) in the simulation program because this accords with dispersal distances in similarly dispersed plant species (Cain et al. 1998). We did not model data on the effective number of produced seeds because earlier studies already indicated mass seed production per flower (>1000), with only moderate variance according to different environmental parameters (Suehs et al. 2004) and extremely low survival and germination rates (D'Antonio et al. 1993). Instead, we decided to emphasize on relative differences according to the differences in flower production (number of flowers per grid ~ number of flowers per plant in the experiment), which consequently would determine the number of seeds. Annual clonal growth in C. edulis follows fractal rules concentrically from a central branching node and is strongly related to biomass (Sintes et al. 2007). Because annual growth rates are estimated ~ 0.3 meter/vear (Sintes et al. 2007) we allowed C. edulis to spread clonally one grid cell a year in the four direct neighboring grid cells. This corresponds with the star-shaped growth at maturity (Wisura 1993). Clonal growth was modeled as a decreased probability of 0.2 to colonize each of the adjacent cells based on the results of biomass obtained in the lab experiment with different rhizosphere soils (data not shown).

## Results

## Experiment

The type of soil in which plants were grown affected the production of flowers (Figure 1A). In that sense, *C. edulis* plants growing on soil collected from the native plant community (VS) produced a greater number of flowers (P=0.001,  $F_{2,35}$ =4.026 Figure 1A) than plants growing on soil collected from the two *C. edulis* patches (i.e. CRS, CDS). The average number of flowers produced in plants growing on virgin soil was 1.8 whereas for the other two types of soil, CRS and CDS, plants presented only 0.4 and 0.6 flowers respectively.

## Model

The outcome of our simulations indicated that the local spreading of *C. edulis* is faster in the scenarios where there are no residual effects on soil (Figure 1B). Therefore, the fastest covering rate was observed in the model compatible with the responses observed experimentally in which the production of flowers resulted in a higher number of seeds in virgin soils. This is also reflected by the higher slopes of the fitted logistic function





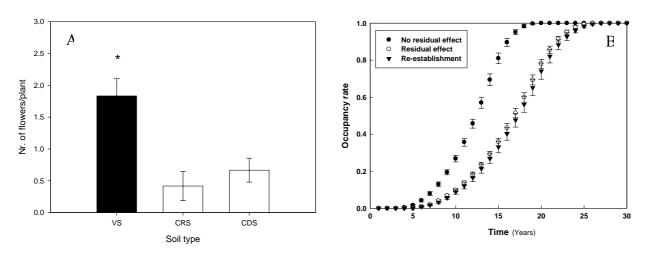


Figure 1. Mean number of flowers (A) for Carpobrotus edulis plants growing on Virgin soil (VS); C. edulis rhizosphere soil (CRS); C. edulis rhizosphere soil from dying mats (CDS). Error bars indicate  $\pm$ SE.\* indicate significant difference after One-Way ANOVA. (B) Occupancy rates of C. edulis in a virtual landscape of 1ha under three different soil scenarios: no residual effect on flower production and growth rates, residual effect and residual effect after C. edulis removal (re-establishment).

(respectively r= $5.85\pm0.29$ SE). By contrast, on already occupied sites (with residual effects) or after *C. edulis* removal the covering rate decreased (r= $5.19\pm0.23$ SE and r= $5.23\pm0.25$ SE).

#### Discussion

The phenotypic plasticity of the plant (individual plant responses in function of soil characteristics) in combination with the resulting contrasting soil environments has interesting consequences in C. edulis invasion. In native areas where the plant is not present, investing in the production of flowers is the fastest strategy to occupy a given area. Increasing the number of flowers allows for the formation of a seed-bank after one growing season. A percentage of those seeds would manage to establish (even assuming particularly low germination rates) (D'Antonio, 1990; Vila & D'Antonio, 1998), which would result in a fast short-term spread. On the other hand, in patches were C. edulis is already present, investment in vegetative growth would increase the competitive potential against the native plant community (Otfinowski & Kenkel, 2008). If in those already occupied areas the production of seeds were increased, this would result in high kin competition or, alternatively, in less chances of seed germination due to the dense mat formed by the mother plant. As our model supports, in such situations, it is more advantageous to rely on clonal growth as the main means of dispersal. Previous studies have modeled the growth pattern of C. edulis in invaded areas however; in those cases the combination of clonal vs. sexual reproduction was not integrated. Interestingly, based on field observations and also model predictions those authors could see that the growth decreased after approximate twenty years (Sintes et al., 2007). This type of pattern is perfectly compatible with the residual effects on formerly occupied soil presented in our model, and therefore, rather than dispute it completes previous observations. The key result of our paper is that the residual effect on soil produced by the species Carpobrotus edulis leads to changes in individual plant responses that are oriented to maximize the colonization of invaded areas. In this sense, soil context (invaded vs. virgin) is the factor determining the shift from an enhanced flower production in virgin soils, never exposed to the exotic species, towards the induction of vegetative growth in previously occupied soils. The





changes observed at individual level as a function of soil-context have a dramatic effect on the rates of colonization of invaded landscapes. Our findings reveal a key mechanism to understand the invasion dynamics of *C. edulis*, a species that is a serious threat in the Mediterranean region, and, more importantly, illustrate how some invasive species can quickly respond to soil heterogeneity to maximize the probability of establishing long-term plant populations.

#### References

- Cain M. L., Damman H. & A. Muir., 1998. Seed dispersal and the Holocene migration of woodland herbs. Ecological Monographs 68:325-347.
- Conser C. & Connor, E.F., in press. Assessing the residual effect of *Carpobrotus edulis* invasion, implication for restoration. Biological Invasions.
- D'Antonio C. 1990. Seed production and dispersal in the non-native, invasive succulent *Carpobrotus edulis* (Aizoaceae) in coastal strand communities of Central California. Journal of Applied Ecology 27:693-702.
- D'Antonio C. & Mahall. B. E., 1991. Root profiles and competition between the invasive, exotic perennial, *Carpobrotus edulis*, and two native shrub species in California coastal scrub. American Journal of Botany 78:885-894.
- D'Antonio C., Odion D. C. & Tyler C. M., 1993. Invasion of maritime chaparral by the introduced succulent *Carpobrotus edulis*. Oecologia 95:14-21.
- Kot M., Lewis M. A & van den Driessche P. 1996. Dispersal data and the spread of invading organisms. Ecology 77:2027-2042.
- Mal T. K. &. Lovett-Doust J., 2005. Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, *Lythrum salicaria* (Lythraceae) in response to soil moisture. American Journal of Botany 92:819-825.
- Otfinowski R. & Kenkel N. C., 2008. Clonal integration facilitates the proliferation of smooth brome clones invading northern fescue prairies. Plant Ecology 199:235-242.
- Sintes T., Moragues E., Traveset A. & Rita J., 2007. Clonal growth dynamics of the invasive *Carpobrotus* affine *acinaciformis* in Mediterranean coastal systems: A non-linear model. Ecological Modelling 206:110-118.
- Suehs C. M., Affre L. &. Medail F., 2004. Invasion dynamics of two alien *Carpobrotus* (Aizoaceae) taxa on a Mediterranean island: I. Genetic diversity and introgression. Heredity 92:31-40.
- Vila M. & D'Antonio C. M., 1998. Fruit choice and seed dispersal of invasive vs. noninvasive *Carpobrotus* (Aizoaceae) in coastal California. Ecology 79:1053-1060.
- Vila M., Tessier M., Suehs C. M., Brundu G., Carta L., Galanidis A., Lambdon P. et al., 2006. Local and regional assessments of the impacts of plant invaders on vegetation structure and soil properties of Mediterranean islands. Journal of Biogeography 33:853-861.
- Wisura W. & Glen H.F., 1993. The South African species of *Carpobrotus* (Mesembryanthema Aizoaceae). Contributions to Bolus Herbarium 15:76–107.





## Detection of intraguild predation by *Harmonia axyridis* on native cocccinellids by alkaloids

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## Introduction

In laboratory conditions, the invasive ladybird Harmonia axvridis Pallas is well known as an intraguild predator of ladybird species (Pell et al. 2008, Ware & Majerus 2008) but also of other aphidophages (Koch 2003). However, the real impact of H. axyridis intraguild predation (IGP) on native coccinellids is poorly known in natural conditions, where multiple prev species occur and where prey has the opportunity to escape. To follow predator - prey interactions, several techniques have been developed (Harwood & Obrycki, 2005): analysis of food remains by gut dissection (Triltsch, 1999), use of monoclonal antibodies (Hagler, 2006) and molecular analysis with detection of species-specific DNA sequences (Hoogendoorn & Heimpel, 2001). Molecular techniques have been used successfully to detect IGP in coccinellids, however, with some limitations regarding detection time as a result of the rapid digestion of prey DNA (Gagnon et al., 2005). A new method to monitor IGP of coccinellids in natural conditions is based on alkaloid detection by gas chromatography - mass spectrometry (Hautier et al., 2008). As many ladybird species use alkaloids as a chemical defence (Daloze et al., 1994), these compounds can be used as predation tracers. To apply this approach, we firstly developed a laboratory alkaloid detection technique for H. axyridis larvae. Next, the influence of several factors on the detection efficiency was studied under laboratory conditions: prey instar, risk of false positives and time of detection. Finally, the method was validated by testing it on *H. axyridis* larvae sampled in potato fields.

## **Alkaloid detection**

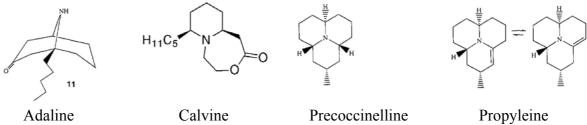
*H. axyridis* larvae were crushed in 200  $\mu$ l of methanol and soaked during 10 minutes. The extracts were subsequently filtered on cotton wool and the filtrate was concentrated under nitrogen. The residues were dissolved in methanol and an aliquot was analysed by gas chromatography - mass spectrometry. Analysis conditions are fully described in Hautier *et al.* (2008).





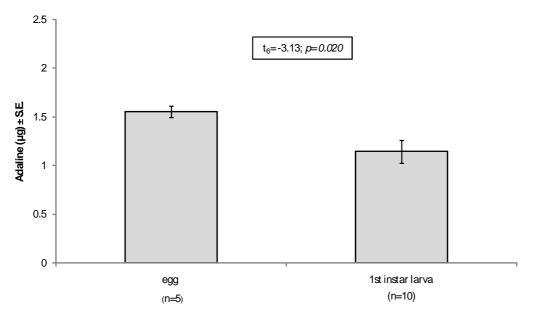
## Laboratory results

Several alkaloids from native ladybird species: adaline, calvine, precoccinelline, propyleine (Figure 1), were unambiguously detected in fourth instar larvae of *H. axyridis* that had ingested one first instar larva of *Adalia bipunctata* (L.), *Adalia decempunctata* (L.), *Calvia quatuordecimguttata* (L.), *Coccinella septempunctata* L. or *Propylea quatuordecimpunctata* (L.), respectively.



*Figure 1. Various alkaloids detected in H. axyridis according to the coccinellid prey consumed.* 

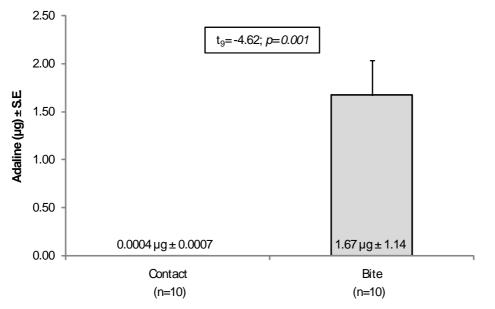
The alkaloid quantity of a single first instar larva, as a prey, is the smallest amount of exogenous alkaloid that can be fed and detected in *H. axyridis*; it is inferior to that of a single egg (Figure 2). The method is sensitive enough to detect all instars. In addition, when the predator larva has had a contact with a prey but without biting, the quantity of alkaloid detected is significantly lower (4000x) than when consumption has occurred (Figure 3). The risk of false positives is thus very limited. After feeding, the exogenous alkaloid concentration in the predator decreases over time. However, when a first instar *A. bipunctata* larva was consumed by fourth instar *H. axyridis* larvae, the alkaloid was detected in the predator larvae had become pupae and adults. Alkaloid traces were found in the exuviae as well (Figure 4).



*Figure 2. Adaline in H. axyridis larvae after consumption of one A. bipunctata egg or one 1st instar larva.* 







*Figure 3. Adaline in H. axyridis larvae after contact with, or after biting a A. bipunctata larva.* 

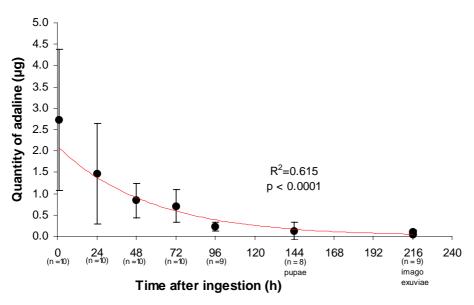


Figure 4. Temporal change of adaline measured in H. axyridis larvae after consumption of one first instar A. bipunctata.

## **Field results**

In 2005, 28 *H. axyridis* larvae were sampled in potato fields in Belgium and analyzed by GC-MS. Nine larvae out of 28 were positive. Three alkaloids were detected: precoccinelline (n=4 larvae), propyleine (n=4) or adaline and precoccinelline (n=1), indicating that the larvae had attacked and consumed native species such as *C. septempunctata*, *P. quatuordecimpunctata* and *A. bipunctata*.

Conclusion





In conclusion, using alkaloids as predation tracers allows to follow IGP interactions between *H. axyridis* and native coccinellids under field field conditions. This new detection technique is very sensitive and permits to detect the smallest prey that can be attacked by *H. axyridis*. The risk of detecting a contact instead of a predation event is very limited. In addition, exogenous alkaloids are persistent in the predators and can still be detected several days after an IGP event. Results from potato fields confirm that *H. axyridis* is an intraguild predator of native coccinelids (*A. bipunctata*, *C. septempunctata*, *P. quatuordecimpunctata*) under natural conditions.

#### Acknowledgements

We thank Evelyne Haulotte (Laboratory of Organic Chemistry, ULB) for the supply of synthetic adaline. Financial support was provided by the Belgian National Fund for Scientific Research, FNRS (grant FRFC 2.4615.06)

#### References

- Daloze D., Braekman J. & Pasteels J.M., 1994. Ladybird defence alkaloids: Structural, chemotaxonomic and biosynthetic aspects (Col.: Coccinellidae). Chemoecology 5:173-183. doi: 10.1007/BF01240602.
- Gagnon A.E., Heimpel G.E. & Brodeur J., 2005. Detection of intraguild predation between coccinellids using molecular analyses of gut-contents. International symposium on biological control of aphids and coccids (Tsuruoka, Japan).
- Hagler J.R., 2006. Development of an immunological technique for identifying multiple predator-prey interactions in a complex arthropod assemblage. Ann App Biol 149: 153-165
- Hautier L., Grégoire J., de Schauwers J., Martin G., Callier P., Jansen J. & de Biseau J., 2008. Intraguild predation by *Harmonia axyridis* on coccinellids revealed by exogenous alkaloid sequestration. Chemoecology 18:191-196. doi: 10.1007/s00049-008-0405-4.
- Harwood J.D. & Obrycki J.J., 2005. Quantifying aphid predation rates of generalist predators in the field. Eur. J. Entomol. 102: 335-350
- Hoogendoorn M. & Heimpel G.E., 2001. PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Mol Ecol 10: 2059-2067
- Koch R.L., 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non-target impacts. Journal of Insect Science 3:32.
- Pell J., Baverstock J., Roy H., Ware R. & Majerus M., 2008. Intraguild predation involving *Harmonia axyridis* : a review of current knowledge and future perspectives. BioControl 53:147-168. doi: 10.1007/s10526-007-9125-x.
- Triltsch H., 1999. Food remains in the guts of *Coccinella septempunctata* (Coleoptera: Coccinellidae) adults and larvae. Eur. J. Entomol. 96: 355-364
- Ware R., & Majerus M., 2008. Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. BioControl 53:169-188. doi: 10.1007/s10526-007-9135-8.





# Measuring the impact of *Harmonia axyridis* intraguild predation on native coccinellids in the field

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#### Introduction

The Multicoloured Asian Ladybird, Harmonia axyridis Pallas, was introduced in 1997 in Belgium for aphid biological control (Adriaens et al. 2003). In less than five years, this ladybird has invaded the whole of Belgium in urban, agricultural and semi-natural habitats and has an overlapping niche with that of several native species (Adriaens et al. 2008). In parallel, a decline of native ladybird species such as Adalia bipunctata (L.) and Adalia decempunctata (L.), was observed in tree habitats in Brussels (San Martin et al. in prep). The causes of this decline are not clearly identified and could be due to competition with or to intraguild predation by, *H. axyridis*. Intraguild predation (IGP) is defined as "killing and eating of species that use similar resources" (Polis et al. 1989); this interaction is very frequent in aphidophagous guilds because aphids are limited resources (Lucas 2005). Several laboratory studies have reported that H. axyridis acts as an intraguild predator of Cecidomyiidae, Coccinellidae and Chrysopidae (Koch 2003, Pell et al. 2008, Ware & Majerus 2008), except for interactions with the ladybird Anatis ocelata (L.) (Ware & Majerus 2008). However, laboratory conditions are extreme circumstances for interactions and are considered a worst case. Under field conditions, IGP can be influenced by many factors such as extraguild prey (e.g. aphids), possibility of intraguild prey escape, and importance of niche overlap.

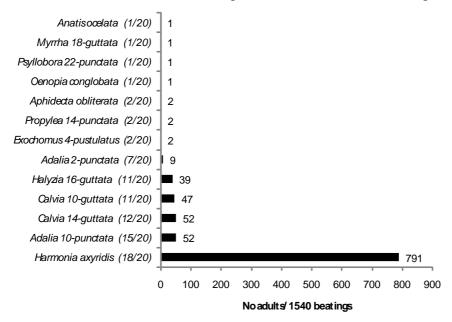
To follow IGP by *H. axyridis* on native coccinellids in field conditions, a new method for detecting IGP was developed based on the detection of exogenous alkaloids from native ladybirds in *H. axyridis* larvae, using gas chromatography - mass spectrometry (Hautier *et al.*, 2008). With this detection method, IGP by *H. axyridis* in lime trees (*Tilia spp.*) was studied in twenty sites in Brussels between June and July 2008. 40 to 110 branchs of lime tree, depending on site size, were beaten with a stick above a sweep net (65 cm in diameter and 130 cm in depth). *H. axyridis* larvae were isolated in microtubes and were kept in a freezer at -20°C until alkaloid analysis.





# Ladybirds sampled

From the 20 sites sampled, 13 species of adult ladybirds were collected (Figure 1). The most abundant species was H. axyridis: it was present in 18 out of the 20 sites sampled and was also the most abundant with 791 specimens collected. Next, but 15 times less abundant and each present on half of the sites, were 4 natives species: 3 aphidophagous tree species, *A. decempunctata* (L.), *Calvia quatuordecimguttata* (L.) and *Calvia decemguttata* (L.), and 1 mycetophagous species: *Halyzia sedecimguttata* (L.). Further, 2 generalist species and 2 tree species were caught: *A. bipunctata* (L.), *Propylea quatuordecimpunctata* (L.) and *Exochomus quadripustulatus* (L.), *Oenopia conglobata* (L.), respectively. *Anatis ocelata* (L.), *Myrrha octodecimguttata* (L.) and *Aphidecta obliterata* (L.) live in coniferous trees and are not associated with lime trees, which explains the low catch of these species.

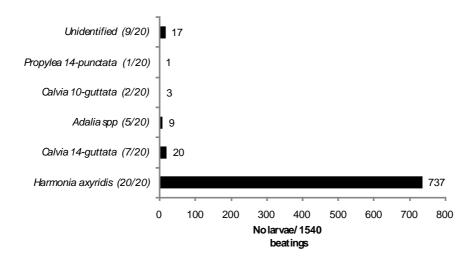


*Figure 2. Numbers of adult ladybirds collected. In brackets: number of sites in which they were caught.* 

An even lower number of species was present as larvae (Figure 2). Again, *H. axyridis* was the dominant species, present in all sites with 737 larvae. To the contrary, only 33 native coccinellids larvae (*C. quatuordecimguttata*, *Adalia* spp., *C. decemguttata* and *P. quatuordecimpunctata*) were collected in 7, 5, 2 and 1 out of 20 sites, respectively. All these aphidophagous species are in competition for food and are potential intraguild prey for *H. axyridis*.





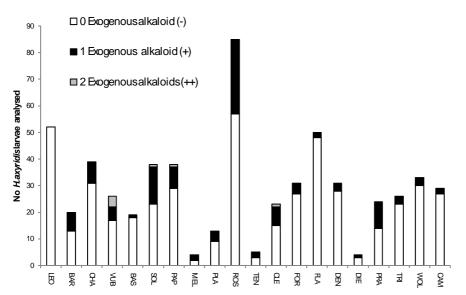


*Figure 2. Numbers of ladybird larvae collected. In brackets: number of sites in which they were caught.* 

# **Alkaloid contents**

The analysis of 590 *H. axyridis* larvae collected on lime trees revealed exogenous alkaloids in 21% larvae coming from all of sampled sites, except for one site (LEO) (Figure 3). Positive larvae contained mainly one alkaloid but in 6% of the positive larvae, two alkaloids were detected in each individual, resulting from double predation on two different coccinellid genera.

Three exogenous alkaloids were identified in the *H. axyridis* larvae analysed: adaline, propyleine and calvine. They are naturally present in *Adalia* spp., in *P. quatuordecimpunctata*, and in *Calvia* spp. (Laurent *et al.* 2005). The detection of these exogenous alkaloids in *H. axyridis* larvae confirms the existence of intraguild predation on these native species in the field.



*Figure 3. Numbers of H. axyridis larvae with (black and grey) and without (white) exogenous alkaloids in each site.* 





#### Conclusion

In conclusion, we can state that the invasive ladybird *H. axyridis* is becoming the dominant coccinellid species on lime trees in Brussels, both in terms of presence in the sites and abundance. The analysis of exogenous alkaloid content of *H. axyridis* larvae reveals the existence of intraguild predation on native coccinellids in 21% of the individuals collected, in 19 out of the 20 sites sampled. This intraguild predation concerns mainly *Adalia* spp. and, to a lesser extent, *Calvia* spp. and *P. quatuordecimpunctata*. These results indicate that intraguild predation is not a fortuitous event and support the hypothesis that *H. axyridis* intraguild predation on *Adalia* spp. could explain the observed decline of the latter species in trees.

#### References

- Adriaens T., Branquart E. & Maes D., 2003. The multicoloured Asian ladybird *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), a threat for native aphid predators in Belgium? Belgian Journal of Zoology 133:195-196.
- Adriaens, T., San Martin y Gomez G. & Maes D., 2008. Invasion history, habitat preferences and phenology of the invasive ladybird *Harmonia axyridis* in Belgium. BioControl 53:69-88. doi: 10.1007/s10526-007-9137-6.
- Hautier L., Grégoire J., de Schauwers J., Martin G., Callier P., Jansen J. & de Biseau J., 2008. Intraguild predation by *Harmonia axyridis* on coccinellids revealed by exogenous alkaloid sequestration. Chemoecology 18:191-196. doi: 10.1007/s00049-008-0405-4.
- Koch R.L., 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. Journal of Insect Science 3.
- Laurent P., Braekman J. C. & Daloze D., 2005. Insect chemical defense. Topics in Current Chemistry 240:167-230.
- Lucas E. 2005. Intraguild predation among aphidophagous predators. European Journal of Entomology 102:351-363.
- Pell J., Baverstock J., Roy H., Ware R. & Majerus M., 2008. Intraguild predation involving *Harmonia axyridis*: a review of current knowledge and future perspectives. BioControl 53:147-168. doi: 10.1007/s10526-007-9125-x.
- Polis G.A., Myers C.A. & Holt R.D., 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297-330.
- Ware R. & Majerus M., 2008. Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. BioControl 53:169-188. doi: 10.1007/s10526-007-9135-8.





# Comparing Fallopia japonica, F. sachalinensis and their hybrid F. xbohemica in Belgium: population ecology, functional traits and invasiveness

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# Introduction

In the course of the nineteenth century two Fallopia (knotweed) species were introduced to Europe from Asia. F. japonica (2n=88) has become one of the most proliferous invasive plants in Europe (Beerling et al, 1994). F. sachalinensis (2n=44) is much less invasive and still rare in Western Europe. Though F. japonica is clonal and male sterile in Europe, it can be pollinated by F. sachalinensis. This cross produces F. ×bohemica, a hybrid that is said to spread even more rapidly than its parents (Bailey & Wisskirchen, 2006; Gammon et al, 2007; Mandak et al, 2004). The biological reasons for this high invasiveness are unknown. The physiology of the hybrid has been less studied than the physiology of its parents (Adachi et al, 1996; Marigo & Pautou, 1998; Price et al, 2001), but the cause could be genetic. Recent data (Tiébré et al, 2007) show that the hybrid is represented in Belgium (and other European countries: Mandak et al, 2003) by at least three cytotypes (2n = 44, 66, 88) and that it could be more variable than its parents. It is possible that the hybridisation in the *japonica*sachalinensis complex is a factor promoting quick evolution. Hybridisation and polyploidisation are well-known evolutionary mechanisms and their importance in the evolution of invasiveness has been shown in other polyploidy complexes (Lee, 2002; Ainouche et al, 2003).

Our objective is to test if the three taxa have contrasting values of key functional traits that might explain their contrasting invasiveness. We also examine if the hybrid is intermediate between its parents or, alternatively, if it shows transgressive variation in some traits.

#### Study outline and preliminary results

In the course of 2008, we monitored, from April to August, the following traits in populations from six sites where two or three taxa coexist in sympatry: shoot height, number of leaves and ramifications (secondary and tertiary), number and length of internodes, leaf area. That the taxa coexist allows us to assume that phenotypic variation originates solely from genetic differences and is not environmentally induced. The first results reveal interesting differences among the three taxa. *F.* ×*bohemica* is intermediate between its parents for height (fig 1), number of internodes and ramifications, length of the internodes, and leaf area. It shows transgressive variation for number of leaves (Figure 2), outperforming both parental taxa.

Other functional traits were measured once or twice during the year, on populations of the three taxa in a site called "Verrewinkel graveyard" (Uccle, Brussels). Specific leaf area (SLA) was measured during biomass peak (July). Leaf and shoot water, nitrogen and carbon content were measured during biomass peak and leaf senescence (October). Results show that





*F. japonica* has lower SLA (Figure 3) but higher N resorption efficiency (up to 70% N is resorbed from senescing leaves, vs. 40% in *F. sachalinensis*) than the two other taxa (Figure 4). *F. sachalinensis* has higher N content than the two other taxa. *F. ×bohemica* is intermediate between its parents for most traits.

This monitoring of functional traits will be carried on from April to July 2009. SLA, leaf water, nitrogen and carbon content will also be measured again. Field observations will be complemented by a "semi-controlled conditions" experiment to test if the three taxa show contrasting phenotypic plasticity of functional traits in response to different soil fertility conditions. For this, rhizomes were taken from seven knotweed populations (two F. japonica, two F. sachalinensis and three F. ×bohemica). They were planted in twenty litre pots, with different doses of NPK fertilizer. Their functional traits will be monitored from 2009 to the end of 2010.

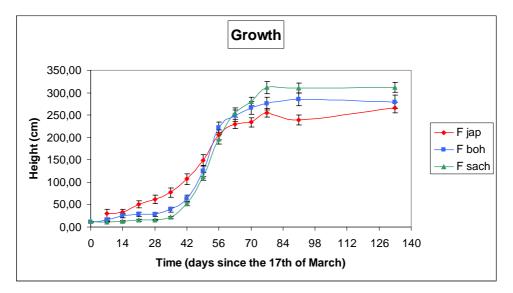


Figure 1: Monitoring of the main axis height of the three Fallopia taxa. Bars indicate the standard error.

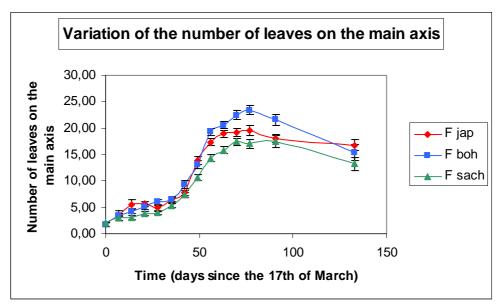


Figure 2: Monitoring of the number of leaves on the main axis of the three Fallopia taxa. Bars indicate the standard error.





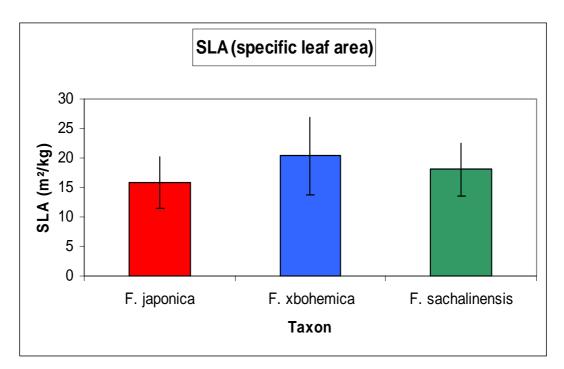


Figure 3: Specific leaf area  $(m^2/kg)$  of the three Fallopia taxa. Bars indicate the standard deviation.

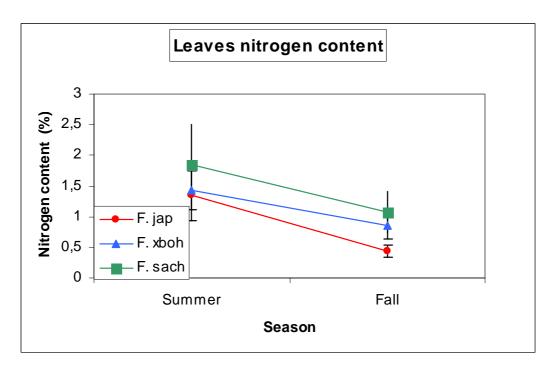


Figure 4: leaves nitrogen content (%) of the three Fallopia taxa, measured at biomass peak (summer) and during leaf senescence (fall). Bars indicate the standard deviation.





#### References

- Adachi N., Terashima I. & Takahashi M., 1996. Nitrogen translocation via rhizome systems in monoclonal stands of *Reynoutria japonica* in an oligotrophic desert on Mt Fuji: Field experiments. Ecological research 11: 175-186.
- Ainouche M.L., Baumel A., Salmon A. & Yannic G., 2003. Hybridization, polyploidy and speciation in *Spartina* (Poaceae) New Phytologist 161: 165-172.
- Bailey J., & Wisskirchen R., 2006. The distribution and origins of *Fallopia x bohemica* (Polygonaceae) in Europe. Nordic Journal of Botany 24: 173-200.
- Beerling D.J., Bailey J.P. & Conolly A.P., 1994. *Fallopia japonica* (Houtt.) Ronse Decraene. J Ecol 82: 959–979.
- Gammon M.A., Grimsby J.L., Tsirelson D. & Kesseli, R., 2007. Molecular and morphological evidence reveals introgression in swarms of the invasive taxa *Fallopia japonica*, *F. sachalinensis*, and *F. xbohemica* (Polygonaceae) in the United States. American Journal of Botany 94: 948-956.
- Lee C.E., 2002. Evolutionary genetics of invasive species. Trends in Ecology and Evolution 17: 386-391.
- Mandák B., Pyšek P., Lysak M., Suda J., Krahulcova A. & Bimova K., 2003. Variation in DNA-ploidy levels of *Reynoutria* taxa in the Czech Republic. Annals of Botany 92: 265-272.
- Mandák B., Pyšek P. & Bímová K., 2004. History of the invasion and distribution of *Reynoutria* taxa in the Czech Republic: a hybrid spreading faster than its parents. Preslia 76:15-64.
- Marigo G. & Pautou G., 1998. Phenology, growth and ecophysiological characteristics of *Fallopia sachalinensis*. Journal of Vegetation Science 9: 379-386.
- Price E.A.C., Gamble R., Williams C.G. & Marshall, C., 2001. Seasonal patterns of partitioning and remobilization of C-14 in the invasive rhizomatous perennial Japanese knotweed (*Fallopia japonica* (Houtt.) Ronse Decraene). Evolutionary Ecology 15: 347-362.
- Tiébré M.S., Bizoux J.-P., Hardy O.J., Bailey J.P. & Mahy, G., 2007. Hybridization and morphogenetic variation in the invasive alien *Fallopia* (Polygonaceae) complex in Belgium. American Journal of botany 94: 1900-1910.





# Mediterranean container plants and their stowaways: A potential source of invasive plant species

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# Introduction: An expanding catalogue of neophytes

A recently published catalogue of neophytes (Verloove 2006) lists 1969 non-native vascular plants recorded from Belgium between 1800 and 2005. By the end of 2008, only a few years later, 144 new taxa (+7.3 %) had been added to the catalogue, not including some 20 new taxa resulting from the current study. The composition of the group of 144 new additions differs markedly from the one of the catalogue 1800-2005. Species originally introduced as ornamentals make up 60 % of the additions, against 33 % in 1800-2005. Together the two most important families in the catalogue (Poaceae and Asteraceae) make up only 5 % of additions, as compared to 29 % in the catalogue.

The clear differences between the catalogue and the additional recent dataset are linked with real changes and inevitable bias. The area of origin of diaspores of alien plant species and the routes and vectors involved have changed as a result of historical trends and events, which often reflect worldwide economic change. Certain categories have disappeared, whereas others are new or at least more important today than before. For instance, 14 % of the species in the 1800-2005 catalogue have only been recorded as wool aliens from the Vesdre valley. Yet, the historical bias in the cumulative dataset explains only part of the differences. The list of post-2005 additions also reflects an important bias. The increased number of introduced ornamentals in the list, recorded as garden escapes or locally naturalizing species, undoubtedly reflects more intensive fieldwork in urban areas in the past few years. The partial shift from grain terminal aliens to urban aliens has several different causes, including the attraction of formerly unexplored fields for the fieldworker, a possible real reduction in the number of imported grain aliens in Belgian port areas, a strongly diminished potential for finding novelties in the well-studied group of grain aliens in Belgium, and a long-standing neglect of certain groups of escapes from cultivation (e.g. shrubs and trees).

*Cardamine corymbosa*, from New Zealand, is a rapidly spreading plant species. In 2008, while doing fieldwork on this weed in nurseries and garden centres, we chanced upon a seemingly important and largely overlooked category of introduced aliens. We therefore decided to study this alien weed flora more in detail.

#### Mediterranean container aliens in Belgium

Propagule pressure has recently been described as "the new frontier in invasion ecology" (Richardson & Pyšek, 2008). However, our knowledge about the precise pathways followed by incoming aliens is often very incomplete. The information we gathered during a single year of prospection in garden centres in Belgium amply illustrates this.

Between late spring and autumn a large number of garden centres were visited, with the aim of preparing a list of alien weeds growing in containers with Mediterranean plants, especially palms, olives and figs. Occasionally we also recorded plants that had obviously





escaped from such containers and that thrived on the ground in the direct vicinity of the containers.

In Belgium, the highly increased popularity of Mediterranean container plants is a very recent, early 21<sup>st</sup> century phenomenon. This popularity can be seen as the end result of a cascade of events and trends, linking the increased level of prosperity of the 1960s with tourism around the Mediterranean, a heightened esteem for gardening, and finally the desire to evoke in the home garden a tinge of the Mediterranean flavour and memories from summer holidays in the South.

In Western Europe, most Mediterranean container plants are imported from Spain or Italy. Together with the ornamentals, large numbers of weeds (seeds as well as young plants), and frequently also other organisms such as snails, are unintentionally introduced in garden centres, situated all over the country. Once sold, the containers and their stowaway weeds find their way into hundreds and thousands of private gardens, parks, etc. While a lot of these weed species have also been recorded as grain aliens in port areas, this recently discovered pathway offers excellent opportunities for widespread dispersal. Furthermore, seeds can germinate in a microhabitat that is literally the same as the one in which the mother plant once grew.

An overview of the results of our prospections is given in table 1. Of 122 identified species, 27 are indigenous to Belgium, and these are also indigenous to Spain and/or Italy. The remaining 95 species are naturalized in Belgium (28 species), casuals (44), or are recorded for the first time (23). A remarkably high number among these 95 species (33 = 35 %) entered Belgium from a secondary distribution range in Spain and/or Italy, not from their natural range. (It should be kept in mind, though, that in the past some of these, e.g. *Coronopus didymus*, might have entered Belgium directly from their natural range too.) See for more details on the species list Hoste *et al.* (2009).

	Number of container aliens (records 2008)			
Status in Belgium (*)	Indigenous to Spain and/or Italy	Naturalized, casual or not yet recorded from Spain and/or Italy	Total	
Indigenous s.l.	27	0	27	
Not indigenous, but rather widespread and/or more or less naturalized	16	12	28	
Casual	30	14	44	
Not previously recorded	16	7	23	
Total	89	33	<b>122</b> (**)	

*Table 1. An overview of records of Mediterranean container aliens from garden centres in Belgium in 2008.* 

(\*) Based on Lambinon et al. (2004) and Verloove (2006).

(\*\*) Not including a number of crypto-aliens (that is, species that are common in both Belgium and at least part of the western Mediterranean), probable ornamental escapes, and taxa that could only be identified to genus level.





All species from our survey have in common that they followed more or less the same trajectory and used the same vector to travel from the Mediterranean to Western Europe. As such, they illustrate a new episode in the worldwide exchange of biota that has been going on for centuries. Apart from that, many species often previously had a rather different species history, as suggested by a few individual examples.

In the mid-19<sup>th</sup> century *Bowlesia incana*, indigenous to America, persisted for some years at a single location in southern France. It was first recorded from Spain in 1986, where it is now well established but rare. From Spain it reached Belgium in 2008 (figure 1). In contrast with other *Bowlesia*, this weedy species has little apparent morphological adaptation for seed dissemination. It is therefore remarkable that this plant, which has fruits devoid of glochids, has become a much more widespread alien in several parts of the world than all seven glochidiate-fruited *Bowlesia* combined (Mathias & Constance 1965).

Today three American *Chamaesyce* frequently enter Belgium from Spain, but their pre-2008 history is different. *Chamaesyce maculata* (first recorded <1950) is a well-established weed in nurseries. Before 2008, *C. serpens* (first recorded in 1992) was only known as a rare casual grain alien from America. Finally, it seems rather likely that *C. prostrata*, first recorded in 2002 and known as a rare urban weed, entered Belgium exclusively as a Mediterranean container alien.

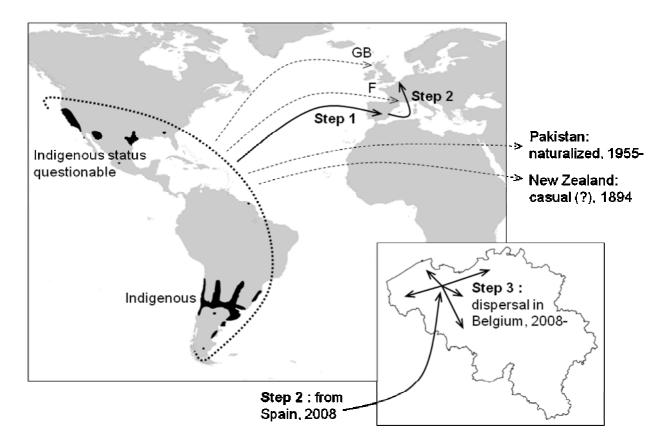


Figure 1. Bowlesia incana, indigenous to America, has been recorded from several different parts of the world. Introduced in the mid-19<sup>th</sup> century, it persisted for some years in southern France ('F' on the map), and was recorded as a casual from Great Britain before 1930 ('GB' on the map). In theory, three steps are sufficient to link its native range with any garden in Belgium, using nurseries in Spain and Belgian garden centres as stepping stones. (Map based on Mathias & Constance [1965], with additions.)





The new link between the Mediterranean and Western Europe has brought us some new species, along with a much larger group of species that previously followed other trajectories and used alternative vectors to enter Belgium. The active importation of a relatively homogeneous group of Mediterranean plants creates a propagule pressure bias toward species of warmer climates, and we should therefore not interpret this upsurge of Mediterranean aliens as a clear-cut illustration of global warming. Several species in the list apparently have not yet been mentioned in the Spanish or Italian botanical literature, indicating that the study of propagule pressure is indeed still hampered by insufficient data on the precise area of origin – primary or secondary – of introduced aliens.

Given the large number of arrivals (both range of species and amount of diaspores), we can expect that at least some species will naturalize in Belgium as a result of the importation of Mediterranean container plants. The tens rule, a useful rule of thumb, states that 10 % of imported species become casuals, and 10 % of those casuals become naturalized (Williamson 1996). There are indications that in the early 21<sup>st</sup> century some candidates for naturalization are increasing in urban areas, including several species from our container aliens list: *Piptatherum miliaceum, Polycarpon tetraphyllum, Sisymbrium irio, S. orientale.* Species from our list have recently been recorded from similar habitats in England, e.g. *Urtica membranacea* (Boucher & Partridge 2006, anon. 2008) and *Galium murale* (Nicolle 2008), and in France (e.g. *Chamaesyce prostrata*; Bedouet 2008).

#### Where do we go from here?

The present study on Mediterranean container aliens clearly shows that there is still an urgent need for new data on propagule pressure. Again and again new data remind us of the complexity of the naturalization and invasion process. In our urge to find the laws and mechanisms that drive these processes, we should never forget that invasions are context specific (Richardson & Pyšek 2008).

The early stages of invasion include long-distance transport and successful introduction of plants and animals outside their natural area of distribution. Especially in these early stages human activities largely determine what happens, where, when, and how. Among invasion ecologists these activities are often perceived as annoying *interference with* or *disturbance of* ecological processes. A more fitting approach accepts humans for what they really are: A primary agent in the bewilderingly rich and complicated succession of events that constitutes the essence of history.

Both deficiencies in the available data and the fads and fancies of human history often make it difficult to interpret cumulative datasets on individual species. Before analysing results we should always carefully check whether the data is uniform. A single cumulative curve, based on historical data that span decades or more than a century, is often based on two or more subsets of records. This may result from changing global trade routes, from dwindling or increased trade volumes, etc.

An example is the small dataset for *Setaria adhaerens* in Belgium: in the first half of the 20<sup>th</sup> century it was exclusively recorded as a rare wool alien, whereas in 2008 it was frequently recorded from garden centres. A quite different example is the invasion of *Senecio inaequidens* in Western and Central Europe. In a recent study, Bossdorf *et al.* (2008) argue that *S. inaequidens* could only start to spread after new frost-resistant and competitive genotypes had been introduced from mountainous regions in southern Africa, decades after the species had first been introduced in Central Europe. Such studies on the introduction and invasive history of an individual plant species, based on molecular research, illustrate the





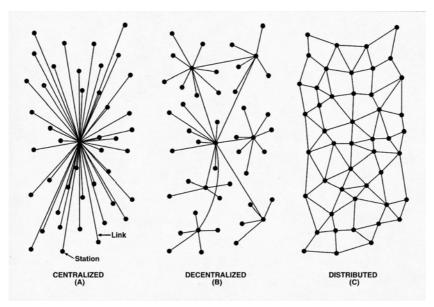
dangers of uncritically using the term 'lag time'. Close scrutiny of data that span long periods of time can prevent us from turning lag time into a black box.

Barabási (2002) observed that we live in a small world. Our world is small because society is a very dense web. It is indeed humans who have created this small world in which plants and animals continue to disperse and propagate, basically following the same ecological rules as before. The science of networks described by Barabási offers opportunities for a new framework for the study of biological invasions. On all levels, from the local to the intercontinental, it is important to better understand how propagules are actively or passively being dispersed. How and in what numbers are seeds dispersed within and between plant nurseries, garden centres, private gardens, and their immediate vicinity? And how have pathways for the introduction of aliens changed over time?

What explains the arrival of over 100 *Bowlesia incana* seedlings in a Belgian garden centre in the spring of 2009, thousands of kilometres away from its natural area of origin in America? Chances are small indeed for this to happen if dispersal were only possible through a distributed network (figure 2c). But that is not what networks look like in the real world. Real networks don't have a homogeneous, mesh-like architecture. Ideas and goods, including ornamentals and weeds, spread across heterogeneous networks with numerous tiny nodes and a few large hubs characterized by an extraordinary number of links (figure 2b). For *Bowlesia* to reach Belgium, chances rise dramatically once it can travel through large decentralized networks.

In theory, *Bowlesia* in its native range could be just three steps away from your home garden (figure 1). First it travels incognito from America to southeast Spain, where it settles as a persistent weed in a nursery that operates as a major node in an international horticultural trade network. The second step takes *Bowlesia*, still incognito, from Spain into a Belgian garden centre. There *Bowlesia* and its companion *Trachycarpus* palm abide their time, until one day they are sold and together transported into your private garden.

"Networks are only the skeleton of complexity, the highways for the various processes that make our world hum." (Barabási, 2002) *Our* world. And the container aliens' world as well.



*Figure 2. Centralized, decentralized and distributed networks. (Source: A.-L. Barabási, Linked)* 





#### Acknowledgements

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#### References

- Anonymous, 2008. Climate change could spread plants. *Horticulture Week* 17 October 2008: 5.
- Barabási A.-L., 2002. Linked. The new science of networks. Cambridge (MA), Perseus Publishing.
- Bedouet F., 2008. Euphorbia prostrata Aiton (Euphorbe prostrée). Le jouet du vent 20: 3.
- Bossdorf O., Lipowsky A. & Prati D., 2008. Selection of preadapted populations allowed *Senecio inaequidens* to invade Central Europe. *Diversity and Distributions* 14: 676-685.
- Boucher A. & Partridge J., 2006. *Urtica membranacea*, an annual nettle, in Warwick: a first British record? *BSBI News* 103: 29-30.
- Hoste I., Verloove F., Nagels C., Andriessen L. & Lambinon J., 2009. De adventievenflora van in België ingevoerde mediterrane containerplanten. *Dumortiera* 97: 1-16.
- Lambinon J., Delvosalle L. & Duvigneaud J., 2004. Nouvelle Flore de la Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des Régions voisines. 5<sup>ème</sup> édition. Meise, Jardin botanique national de Belgique.
- Mathias M.E. & Constance L., 1965. A revision of the genus *Bowlesia* Ruiz & Pav. (Umbelliferae–Hydrocotyloideae) and its relatives. *Univ. of California Publications in Botany* 38. Berkeley & Los Angeles, Univ. of California Press.

Nicolle D. J., 2008. Galium murale - a foothold in Eastbourne? BSBI News 109: 57-58.

- Richardson D. M. & Pyšek P., 2008. Fifty years of invasion ecology the legacy of Charles Elton. *Diversity and Distribution* 14: 161-168.
- Verloove F., 2006. Catalogue of neophytes in Belgium (1800-2005). *Scripta Botanica Belgica* 39. Meise, National Botanic Garden of Belgium.
- Williamson M., 1996. Biological invasions. London etc., Chapman & Hall.





# Water frogs in Wallonia: genetic identification of the introduced taxa (*Pelophylax* ssp.) and impact on indigenous water frogs (*Pelophylax lessonae* and *P*. kl. *esculentus*).

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#### Introduction

Two taxa of water frogs are native in Wallonia: *Pelophylax lessonae* and *P*. kl. *esculentus*. But water frogs, from different origins, have been introduced in Wallonia during the last 25 years, mainly as a consequence of aquatic horticulture: aquatic plants are imported from central Europe, in containers, with eggs, tadpoles or adults of water frogs; these frogs reproduce successfully in the horticulture ponds and are sold (or given) to people creating an ornamental pond in their garden. So, the frogs are introduced in many places in the country; then they spread in the neighbourhood and colonize (semi-)natural habitats. As a consequence, *P. ridibundus* (Figure 1) has become the most frequent green frog in Brabant wallon (Percsy & Percsy, 2002a and 2002b). It is also abundant in the neighbourhood of large cities (Brussels, Liège, Namur, Verviers) (Percsy & Percsy, 2007).

Alien water frogs – i.a. *P. ridibundus* – occupy sites where *P.* kl. *esculentus* and *P. lessonae* are present, as well as other amphibians (mainly *R. temporaria, Bufo bufo, Triturus* sp.); it is a predator of these species. Since *P. ridibundus* is bigger than the two native green frogs, they outcompete the latter for territory, feeding and breeding Furthermore, because of the very particular genetic relationship between the three green frogs (hybridogenesis : see, e.g., Berger 1988), the introduction of *P. ridibundus* in *P.* kl. *esculentus* and *P. lessonae* populations may lead to genetic pollution of the latter species. Finally, foreign frogs may carry diseases, which is a threat for indigenous amphibians (Kok 2001).



*Figure 1. Lake Frog (*Pelophylax ridibundus), *Lasne, Wallonia.* © *C. & N. Percy* 





*P. ridibundus* has been introduced in other countries of western Europe and is known to have outcompeted other amphibians sharing the same habitat (see, e.g., Günther *in* Gasc et al. 1997, Grossenbacher 1988). Consequently, it is important to evaluate the impact of the introduced water frogs in Wallonia. Unfortunately, recognizing the different taxa of water frogs present in Wallonia is not easy. To insure the identifications we have made, we collected, in 2002, samples from 47 frogs from 8 different populations and submitted these for enzymatic and genetic analyzis. The « Laboratoire d'Ecologie des Hydrosystème fluviaux » (Prof. Joly) at the University of Lyon performed protein electrophoresis and the « Museum für Naturkunde » (Prof. Plötner) in Berlin investigated mitochondrial DNA. The results of these analyses allow to obtain:

- a validation of the identification method of the taxa on the field and, thus, a reliable assessment of the evolution of the populations;
- the determination of the geographic origin of the introduced frogs;
- evidence of hybridization and/or introgression between *Pelophylax ridibundus* and the indigenous frogs *P. lessonae* or *P.* kl. *esculentus;* similar introgressions between taxons of water frogs have been observed in other European countries (e.g., Vorburger *et al.* 2003, Plötner *et al.* 2008, Holsbeek *et al.* 2008).

# Validation of the identification method

We wanted to test a method of field identification that can be performed without capturing the frogs, hence relying on morphological characters (possibly by observing using field-glasses) and mating calls. We select, for Wallonia, the following easy criteria.

	Pelophylax lessonae	P. kl. esculentus	P. ridibundus
✓ Length of the hind	short	intermediate	long
leg vs body length			
$\checkmark$ Tibia length vs	shorter or equal	equal	longer
femur length			
✓ Colour of the back	often with a vivid yellow	with or without a vivid	no vivid yellow tint
of the thigh and/or	tint	yellow tint	
of the groin	1		11
✓ Skin coarseness of	weak	weak	generally strong
the back			
✓ Vocal bags	white, sometimes tinged with pink	greyish or medium-grey	dark grey
✓ Call	continuous, long and	shorter, modulated	jerky
	uniform		

Note that the evaluation of these criteria is somewhat subjective and may depend on the observer's experience. This is why identification is validated only if most of the criteria are coherently satisfied. By comparing our field identifications with the enzymatic- and DNA-analysis, we obtain the following results concerning the reliability of the above identification method of the taxa in the field (see Percsy & Percsy 2009):





- the distinction between the native green frogs, on the one hand, and the introduced water frogs, on the other hand, is valid in 100% of the cases;
- the distinction between *P*. kl. *esculentus* and *P*. *lessonae* is less valid : two of the seven presumed « *lessonae* » are actually « *esculentus* »;
- determination of the geographical origin of the alien water frogs is not reliable;
- the distinction between native and non native male green frogs may be done using only the mating call. (This result has been confirmed by further bioacoustic analyses, using oscillograms and sonograms of the calls).

Moreover, analyses of various photographs of the captured frogs allows to evaluate different other classical criteria for the identification of the water frogs (Percsy & Percsy 2009).

# Geographic origin of the alien water frogs

All alien water frogs are determined as *P. ridibundus* or *P.* cf. *ridibundus* (from Anatolia). The mitochondrial analysis of our samples shows that introduced water frogs in Wallonia have at least two different geographic origins, corresponding to three different haplotypes:

- haplotype C, present in Central Europe;
- haplotype E1 and
- haplotype E2, both typical for Anatolian, northern Greek or Bulgarian water frogs).

Three of the eight populations we studied contain both indigenous and exotic water frogs (the latter having the same haplotype); two other populations contain only alien water frogs, all with the same haplotype; both haplotypes C and E1 are present on a same site; finally, two sites do not have exotic water frogs.

#### Introgression

Two samples corresponding to frogs that we have identified in the field as *P*. kl. *esculentus* and whose enzymec analyses concurs with *P*. kl. *esculentus*, have mitochondrial DNA of type E2. This shows that hybridization between native frogs and *P*. *ridibundus* has occurred. Concerning the *« lessonae »* haplotype of our samples, note that this type is different from all other *« lessonae »* haplotypes known (T. Ohst, pers. comm.).

# Conclusion and management strategy

Alien water frogs have spread in certain regions of Wallonia: they come from at least two different origins (central Europe or Balkan and Anatolia). Such frogs have a negative impact on our native water frogs and, probably, on other amphibian species.

Our work validates a method to separate, on the field, the native water frogs (*P. lessonae* and *P.* kl. *esculentus*) from the introduced frogs *P. ridibundus* and *P.* cf. *ridibundus*. It also shows the existence of hybridation between alien and native frogs in Wallonia.

Alien water frogs are already abundant in certain regions of Wallonia and it is impossible to eradicate them there. On the contrary, exotic frogs seem absent from a large part of the country, in particular in oligotrophic ecosystems where *P. lessonae* is dominant. Such





populations of water frogs have to be protected from invasion, because they are less frequent (Günther *in* Gasc *et al.* 1997); furthermore, the results above show that the *« lessonae »* haplotype of Wallonia is original, . Since, probably, *P. ridibundus* will not spontaneously colonize such ecosystems (Pagano *et al.* 2001), it is urgent to avoid introductions in these areas. Consequently, the following measures should be implemented:

- control of trade of water frogs in Belgium and in Europe (laws about trade should be enacted);
- control of introduced frogs in target regions;
- public awareness, to avoid the transfer of frogs from one place to another.

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#### References

- Berger, L., 1988. On the origin of genetic systems in European water frogs hybrids. *Zoologica Poloniae* 36 (1-4): 5-32.
- Gasc, J.P. *et al.* (eds), 1997. *Atlas of Amphibians and Reptiles in Europe*. Societas Europaea Herpetologica & Muséum national d'Histoire naturelle, Paris, 186 pages.
- Grossenbacher, K., 1988. *Atlas de distribution des Amphibiens de Suisse*. Documenta Faunistica Helvetiae n°8, Schweizerischer Bund für Naturschutz, Ligue Suisse pour la Protection de la Nature et Centre Suisse de Cartographie de la Faune, Bâle, 208 pages.
- Holsbeek, G., Mergeay, J., Hotz, H., Plötner, J., Volckaert, F. A. M. & De Meester, L., 2008. A cryptic invasion within an invasion and widespread introgression in the European water frog complex: consequences of uncontrolled commercial trade and weak international legislation. *Molecular Ecology* 17: 5023-5035.
- Kok, P., 2001. Note sur l'introduction de *Rana bedriagae* Camerano, 1882 (Anura: Ranidae) en Belgique et ses possibles implications sur la batrachofaune indigène. *Les Naturalistes belges* 82 (1) : 25-30.
- Pagano, A., Joly, P., Plénet, S., Lehman, A. & Grolet, O., 2001. Breeding habitat partitioning in the *Rana esculenta complex*: the intermediate niche hypothesis supported. *Ecoscience* 8 (3): 294-300.
- Percsy, C. & Percsy, N., 2002a. Dix ans de suivi des populations indigènes et introduites de grenouilles « vertes » (*Rana (Pelophylax*) ssp., Anura, Ranidae) dans le bassin de la Lasne (Brabant wallon, Belgique). *Bulletin de la Société Herpétologique de France* n° 103: 59 72.
- Percsy, C. & Percsy, N., 2002b. Evolution des populations indigènes et introduites de grenouilles « vertes » en Brabant wallon. Pages 213 – 218 in Peeters M. & J.L. Van Goethem (Eds), 2002. Belgian fauna and alien species. Actes du symposium: Faune belge: statut et tendances observées avec une attention particulière pour les espèces exotiques. Bulletin de l'I.R.S.N.B., Biologie 72, supplément, 297 pages.
- Percsy, C. & Percsy, N., 2007. Quatre chapitres sur les grenouilles vertes *in* Jacob J.P. *et al., Amphibiens et reptiles de Wallonie.* Aves-Raînne et CRNFB, série « Faune-Flore-Habitats » n°2. Namur, 384 pages.
- Percsy, C. & Percsy, N., 2009. Identification des grenouilles « vertes » (*Pelophylax*) en Wallonie : résultats de la confrontation de critères morphologiques et acoustiques avec des analyses enzymatiques et d'ADN (in press).
- Plötner, J., Uzzell, T., Beerli, P., Spolsky, C., Ohst, T., Litvinchuk, S. N., Guex, G. D., Reyer, H. U. & Hotz, H., 2008. Widespread unidirectional transfer of mitochondrial DNA: a case in werstern water frogs. *J. Evol. Biol.* 21: 668-681.
- Vorburger, C. & Reyer, H. U., 2003. A genetic mechanism of species replacement in European water frogs? *Conservation Genetics* 4: 141-155.











# Trends in the distribution of the Chinese mitten crab in the Scheldt estuary

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# Introduction

The Chinese mitten crab (*Eriocheir sinensis*) is a catadromous species that spends most of its life in freshwater. *E. sinensis* is native to rivers and estuaries of central Asia, from North Korea to China. Since its arrival in Germany in the beginning of the 20<sup>th</sup> century, the Chinese mitten crab has rapidly invaded coastal and inland waters throughout Europe. The species was first observed in Belgium in 1933 in the Sea Scheldt near Antwerp and is found nowadays in the main rivers and canals of the Scheldt basin.

#### **Results and discussion**

Macrocrustaceans were caught as bycatch during fish surveys using fyke nets in the Sea Scheldt in 1995, 1997 and 2008. During the surveys in the nineties, only a few mitten crabs were found. Ten years later, however, *E. sinensis* had spread throughout the estuary and more than 50 crabs can now be caught per fyke net per day. The expansion of the distribution and the increase of the population size of the Chinese mitten crab coincided with the improvement of the water quality in the estuary during the last decade. It is suggested that pollution may decrease mitten crab densities, by reducing the abundance of prey (Gollasch 1999).

The abundance of mitten crabs in the estuary shows two distinct seasonal peeks (Figure 1). The first peek in spring coincides with the upstream migration of juveniles towards the freshwater reaches of rivers, where they burrow in the banks (Figure 2). The second peek in autumn coincides with the seaward spawning migration of adults. The highest densities are observed in the oligohaline and freshwater zone of the estuary. Their distribution is probably related to habitat availability and their ability to burrow (soft sediment banks).

High population densities of this crab may have a significant adverse impact on the natural balance of the Scheldt ecosystem. Because of the crab's flexible, omnivorous feeding habits, it may have a competitive edge over other bottom dwelling species. Their burrowing nature may also accelerate bank erosion and instability (Rudnick *et al.*, 2005).

#### References

Gollasch S., 1999. Current Status on the increasing abundance of the Chinese mitten crab Eriocheir sinensis H. Milne Edwards, 1854 in German rivers. Proceedings of the First Annual Meeting on the Chinese mitten crab in California. 23 March. Sacramento, CA

Rudnick D.A., Chan V. & Resh V.H., 2005. Morphology and impacts of the burrows of the Chinese mitten crab, Eriocheir sinensis H. Milne Edwards (Decapoda, Grapsoidea), in South San Francisco Bay, California, USA. Crustaceana 78(7): 787-807.





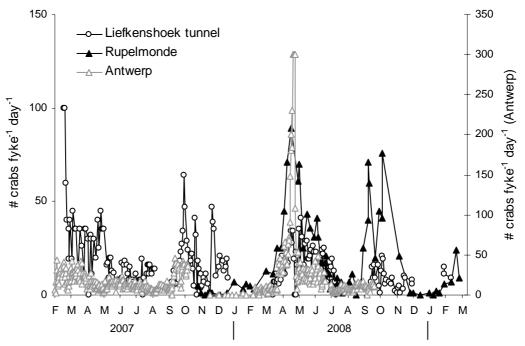


Figure 1. Seasonal abundance of the Chinese mitten crab in the Scheldt estuary.



*Figure 2. Burrows of the Chinese mitten crab in the left side bank of a freshwater marsh creek in the Scheldt estuary. Inset: mitten crab in the opening of its burrow.* 





# The invasion of ring-necked parakeet (*Psittacula krameri*) in Europe and Belgium: mechanisms and consequences for native biota.

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#### Establishment of ring-necked parakeets in Europe

In Europe, at least 75 nonnative bird species established feral populations (Chiron et al. 2009) and, due in large part to their popularity as cage birds, parrots (*Psittacidae*) are well represented as invaders, accounting for about 18 % of Europe's established exotic avifauna (DAISIE 2009). Among parrots, the ring-necked parakeet is undoubtedly the most successful invader. Although this parakeet originates from mostly subtropical regions in Africa and Asia, it has formed at least 65 populations in Europe (Lever 2005, Strubbe & Matthysen 2009a), with population sizes varying from several tens to several thousands of birds (Strubbe & Matthysen 2007). In order to identify the mechanisms that allow this parakeet to invade Europe, we gathered data on parakeet releases and correlated the outcome of an introduction event with human population density and climatic factors, thereby testing two of the major hypotheses on the establishment success of non-native species, i.e. the 'human activity' (Taylor & Irwin 2004) and the 'climate matching' hypothesis (Williamson 1996). The former hypothesis states that human activity facilitates the establishment of alien species while the latter postulates that species have a higher probability to establish if they are introduced to regions with a climate similar to that in their native area. We found that parakeet establishment correlated positively with measures of human activity such as human population density, but negatively with the number of frost days, providing support for both hypotheses (Strubbe & Matthysen 2009a). Human activity is a root cause of species introductions (Westphal et al. 2008), but human-dominated habitats are often characterized by abundant food and we argue that this increased food supply could well explain the link between the parakeets' establishment success and human population density, as food availability is one of the most important factors limiting bird populations and supplementary feeding may enhance breeding success (Robb et al. 2008), thus increasing population persistence probability. The negative relationship between parakeet establishment and the number of frost days indicates that parakeets may suffer from climate mismatch, although population crashes during harsh winter are rare, and establishment failure could also be caused by a reduced breeding performance, e.g. due to a lower body condition. Low temperatures can also impact on avian embryonic development, and further support for the climate matching hypothesis comes from Shwartz et al. (2009), who found that European parakeet populations have a much higher rate of egg infertility than parakeets in the native range (India), or populations introduced to warmer regions such as Israel. Predation pressure, however, is much lower in the introduced regions compared to the native range, and this 'predator release' (Liu & Stiling 2006) partly offsets the climate mismatch, allowing the parakeet to flourish throughout much of temperate and Mediterranean Europe (Shwartz et al. 2009).





# **Ring-necked parakeets in Belgium**

#### Parakeet habitat selection and competition with native species

Belgium, which is one of Europe's exotic bird hotspots (Chiron et al. 2009), harbors one of the continent's largest ring-necked parakeet populations. Originating from a deliberate release of about 40 parakeets in Brussels in 1974, the population numbered 8 000 to 8 500 birds in 2006 (Weiserbs & Jacob 2007). The population doubles approximately every 4 year, and this growing population raises concerns for the loss of biodiversity of the native avifauna. Ring-necked parakeets are secondary cavity-nesters and as secondary cavity nesting communities depend on existing cavities and are hierarchically structured based on the production of and the competition for suitable nesting cavities (Martin & Eadie 1999), parakeets could pose a threat to native hole-nesting species. Based on preferred nesting cavity characteristics, parakeets might come into conflict with native nuthatches (Sitta europaea), starlings (Sturnus vulgaris) and great and middle spotted and green woodpeckers (Dendrocopus major, D. medius and Picus viridis). In order to assess the effects of competition for nesting cavities, we determined the abundance of parakeets and common native hole-nesters (nuthatches, starlings, great spotted and green woodpecker, jackdaw (Corvus monedula) and stock dove (Columba oenas)) in 44 study sites in the Brussels Capital Region from 2004 to 2006 using point counts. Results show that parakeet abundance was highest in forests or parks surrounded by built-up areas and parakeet numbers were strongly associated with cavity availability, suggesting that this may be a limiting factor (Strubbe & Matthysen 2007). After accounting for habitat and landscape variables influencing the abundance of native hole-nesters, we found that parakeet abundance was a significant predictor of nuthatch density, suggesting competition for nesting cavities (Strubbe & Matthysen 2007). In order to verify these findings, we set up an experimental manipulation of cavity availability in two city parks in the Brussels metropolitan area. In these sites, we blocked all cavities in which parakeets were found breeding in 2006, thereby severely reducing the cavity availability. Nuthatch breeding cavities were not blocked, but in the next breeding season we observed a significant decline in nuthatch breeding densities, largely due to cavity takeover by parakeets (Figure 1, Strubbe & Matthysen 2009b).

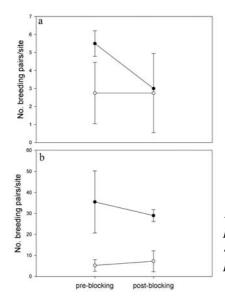


Figure 1. Number of nests (mean  $\pm$  SE) of (a) nuthatches and (b) parakeets at two experimental sites (black dots) and four control sites (white dots) before and after blocking of ring-necked parakeet breeding cavities.





Nuthatches defend their cavities by adjusting the entrance size of cavities to their own size by plastering up the entrance with mud. However, this does not protect the nuthatches from ringnecked parakeets, as parakeets start laying eggs already at the end of February (pers. obs.) while in western Europe, nuthatches start breeding only in the second half of April (Matthysen 1998). When breeding cavities were blocked, parakeets were forced to look for new breeding sites and the difference in timing of breeding enabled the parakeets to take the best nesting sites first.

#### Parakeet range expansion in Belgium and consequences for native avifauna.

Although the bulk of the parakeet population still occurs in the Brussels metropolitan area, parakeets can now be found breeding in a range of about 40 km around their release site and they are steadily increasing their range (Vermeersch et al. 2004, Vermeersch et al. 2006). To assess the potential distribution of parakeets in Belgium, we used a species distribution model (SDM) to identify areas suitable for parakeets. SDM use information on species occurrence data and environmental variables to generate statistical functions characterizing a species' ecological requirements, and these functions are then projected onto the geographical area of interest to obtain the species (potential) distribution (Guisan & Zimmermann 2000). Using a dataset of  $\pm$  400 point locations of breeding parakeets and a number of habitat variables derived from region-wide land-use and forestry maps, we modeled parakeet distribution using an Ecological Niche Factor Analysis (ENFA, Hirzel et al. 2002). ENFA is a presence-only method and searches for an environmental 'envelope' characterizing the areas in which the species is present. Results show that parakeet distribution is mainly governed by variables representing cavity availability (i.e. the presence of parks and old forests) and parakeets are again strongly associated with urban habitats (Strubbe & Matthysen 2009c). The resulting distribution map (not shown here) shows that parakeets have ample room to spread into. Most highly suitable habitats are found along the urbanized north-south axis from Brussels to Antwerp, indicating that known nuthatch strongholds such as the regions south and east of Antwerp are highly likely to be invaded (Strubbe & Matthysen 2009c).

However, as the real threat this parakeet poses depends on a combination of its potential distribution and abundance and its per capita impact on native birds, we not only need an assessment of its potential geographic distribution but also an estimate of its abundance (Parker et al. 1999). In order to obtain an estimate of the parakeets' expected abundance, we took advantage of recent improvements in the SDM field, and reanalyzed our data of parakeet abundance (see above, but point counts now converted to breeding densities, pairs/ha) using Boosted Regression Trees (Elith et al. 2008), a new technique capable of modeling abundance. BRT, coupled with the availability of exceptionally detailed geographic data layers containing information on land-use and vegetation types (the Biological Valuation Map and the Forest Reference Layer), allowed us to model parakeet abundance across the country at the level of individual forest patches. Region-wide predictions of nuthatch abundance are obtained using patch-level data obtained from a breeding bird atlas project. In order to quantify the parakeets' impact on nuthatches, we applied a regression model on our Brussels dataset of parakeet and nuthatch abundances, and we use the correlation coefficient between parakeet and nuthatch abundance as an indicator of competitive strength. The competition between parakeets and nuthatches was then quantified by superimposing their abundance maps and applying the competition coefficient, resulting in an estimate of the number of nuthatches that will be lost when parakeets have occupied all suitable sites.





Our models predict a mean number of about 22 000 ring-necked parakeet breeding pairs (90 % confidence limits from 9 000 to 39 800 pairs) indicating that these parakeets could become one of the most numerous cavity-nesting birds in the region. BRT results confirm that parakeet abundance is highest in older forests in urban environments, while there is also evidence that they prefer smaller, more fragmented forests (Strubbe, Graham & Matthysen in prep.). The resulting abundance map (Figure2) indicates that parakeets are expected to reach their highest densities along the strongly urbanized south-north axis from Brussels to Antwerp, while eastwards, there also is ample habitat for the parakeet to spread into, with high predicted abundances around the cities of Leuven and south of Hasselt. Westwards, there is less suitable habit and moderately high abundances are only found around the cities of Ghent and Bruges. Our nuthatch model estimates the number of nuthatches to be 4 646 (2 929 - 6 776) breeding pairs, which agrees very well with the 4 740 to 5 750 pairs estimated in the Flemish and Brussels Breeding Bird Atlas (Vermeersch et al. 2006, Weiserbs & Jacob 2007). As expected from previous results, nuthatch numbers were negatively associated with parakeet abundance and we were able to extract a competition coefficient, quantifying the effect of parakeets on nuthatch densities. As our predictions of parakeet and nuthatch abundance and our estimate of the competition coefficient all have associated uncertainties (i.e. confidence intervals), this leads to a number of possible scenarios.

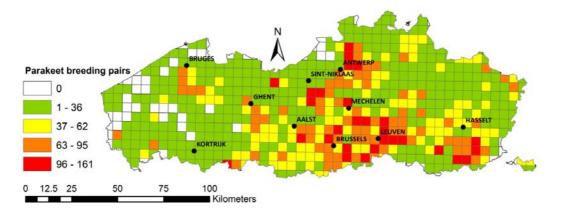


Figure 2. Region-wide predicted parakeet abundance. Number of breeding pairs per 5x5 km UTM grid were obtained by summing the predicted parakeet abundances for each forest fragment within each grid cell.

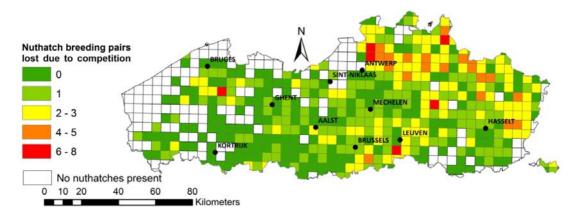


Figure 3. Region-wide, long-term impact of parakeets on nuthatches according to a moderate scenario, i.e. taking the mean values for both parakeet and nuthatch abundance and competition strength. Number of breeding pairs lost per 5x5 km UTM grid were obtained by summing the predicted nuthatch losses for each forest fragment within each grid cell.





Thus, we calculated the region-wide, long-term parakeet impact using the mean estimates and the upper and lower confidence limits of our predictions and these calculations show that despite the high predicted parakeet abundances, total impact on nuthatches will probably only be small, and even in a worst case scenario, i.e. the maximum estimate for parakeet abundance and competition strength, only one third of the nuthatch population would be at risk. A more moderate scenario, i.e. taking the mean values for both parakeet and nuthatch abundance and competition strength, indicates a loss of 11 % of the nuthatches (i.e. 551 pairs, Strubbe, Graham & Matthysen, *in prep.*). In Figure 3, we visualized the predicted parakeet impact according to this moderate scenario. To conclude, we argue that the establishment of ring-necked parakeets should be prevented, but that in areas where they are currently present, there is no imminent ecological threat that calls for an eradication campaign.

#### References

- Chiron F., Shirley S. & Kark S., 2009. Human-related processes drive the richness of exotic birds in Europe. Proceedings of the Royal Society B-Biological Sciences, 276: 47-53.
- DAISIE 2009. Handbook of Alien Species in Europe, Dordrecht: Springer Netherlands.
- Elith J., Leathwick J. R. & Hastie T. 2008. A working guide to boosted regression trees. Journal of Animal Ecology, 77: 802-813.
- Guisan A. & Zimmermann N. E., 2000. Predictive habitat distribution models in ecology. Ecological Modelling, 135: 147-186.
- Hirzel A., Hausser J., Chessel D. & Perrin N., 2002. Ecological Niche Factor Analysis : How to compute habitat suitability maps without absence data? Ecology, 83: 2027–2036.
- Lever C., 2005. Rose-ringed Parakeet (Ring-necked Parakeet) *Psittacula krameri*, p.: 124-130 Naturalized Birds of the World, London: T & AD Poyser.
- Liu H. & Stiling P., 2006. Testing the enemy release hypothesis: a review and meta-analysis. Biological Invasions, 8: 1535-1545.
- Martin K. & Eadie J. M., 1999. Nest webs: A community-wide approach to the management and conservation of cavity-nesting forest birds. Forest Ecology and Management, 115: 243-257.
- Matthysen E., 1998. The Nuthatches, London: T & A. D. Poyser.
- Parker I. M., Simberloff D., Lonsdale W. M., Goodell K., Wonham M., Kareiva P. M., Williamson B., Von Holle B., Moyle P. B., Byers J. E. & Goldwasser L., 1999. Impact: Toward a Framework for Understanding the Ecological Effects of Invaders. Biological Invasions, 1: 3 - 19.
- Robb G. N., McDonald R. A., Chamberlain D. E., Reynolds S. J., Harrison T. J. E. & Bearhop S., 2008. Winter feeding of birds increases productivity in the subsequent breeding season. Biology Letters, 4: 220-223.
- Shwartz A., Strubbe D., Butler C., Matthysen E. & Kark S., 2009. The effect of enemyrelease and climate conditions on invasive birds: a regional test using the rose-ringed parakeet Psittacula krameri as a case study. Diversity and Distributions, 15: 310-318.
- Strubbe D. & Matthysen, E., 2007. Invasive ring-necked parakeets *Psittacula krameri* in Belgium: habitat selection and impact on native birds. Ecography, 30: 578-588.
- Strubbe D. & Matthysen E., 2009a. Establishment success of invasive ring-necked and monk parakeets in Europe. Journal of Biogeography: Accepted manuscript.
- Strubbe D. & Matthysen E., 2009b. Experimental evidence for nest-site competition between invasive ring-necked parakeets (*Psittacula krameri*) and native nuthatches (*Sitta europaea*). doi:10.1016/j.biocon.2009.02.026 Biological Conservation, In Press





- Strubbe D. & Matthysen E., 2009c. Predicting the potential distribution of invasive ringnecked parakeets *Psittacula krameri* in northern Belgium using an ecological niche modelling approach. Biological Invasions, 11: 497-513.
- Taylor B. W. & Irwin R. E. 2004. Linking economic activities to the distribution of exotic plants. Proceedings of the National Academy of Sciences of the United States of America, 101: 17725-17730.
- Vermeersch G., Anselin A. & Devos K., 2006. Bijzondere Broedvogels in Vlaanderen in de periode 1994-2005. Populatietrends en recente status van zeldzame, kolonievormende en exotische broedvogels in Vlaanderen. Mededeling van het instituut voor natuur- en bosonderzoek, 2006(2). Instituut voor Natuur- en Bosonderzoek: Brussel : Belgium. 64 pp.
- Vermeersch G., Anselin A., Devos K., Herremans M., Stevens J., Gabriëls J. & Van Der Krieken B., 2004. Atlas van de Vlaamse broedvogels 2000-2002, Brussel.
- Weiserbs A. & Jacob J.-P., 2007. Oiseaux nicheurs de Bruxelles, 2000-2004: répartition, effectifs, évolution. AVES: pp 288.
- Westphal M. I., Browne M., MacKinnon K. & Noble I., 2008. The link between international trade and the global distribution of invasive alien species. Biological Invasions, 10: 391-398.
- Williamson M., 1996. Biological Invasions, London: Chapman & Hall.





# Patterns of Prunus serotina invasion in two contrasting forests

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#### Introduction

*Prunus serotina* Ehrh., a North-American tree species, is considered an invasive species in Western Europe. Most studies in its introduced range focused on areas heavily invaded by *P. serotina*. Nonetheless, the presence and abundance of *P. serotina* in these areas still reflects the massive plantings of the past (Starfinger et al. 2003). The large-scale plantings resulted in a high propagule pressure of *P. serotina*. Consequently, *P. serotina* exhibited a considerable invasion rate in these areas, which lead to problems in silviculture and nature conservation (Starfinger et al. 2003). Since actual rates of invasion appear to be largely determined by propagule pressure (Von Holle & Simberloff 2005), we wanted to study the spread of *P. serotina* in an area characterized by a far lower propagule pressure where the species has not been introduced deliberately. Would we still label *P. serotina* an aggressive invader in these circumstances? Besides, *P. serotina* has not yet fully occupied its potential range in Europe, and the spread of the species is thought to be limited by dispersal (Zerbe & Wirth 2006, Verheyen et al. 2007). To develop appropriate management strategies, we should gain insight into the factors that affect the colonization rate of *P. serotina* in new sites.

In this abstract, we compare the results of two studies on 70 years of forest development in areas with a low propagule pressure of *P. serotina*. The Liedekerke forest reserve (Belgium) and the Ossenbos forest reserve (the Netherlands) were particularly appropriate for our research because they have not been managed for over sixty years and *P. serotina* established spontaneously during the forest development. Based on the observed patterns of *P. serotina* colonization in these forests, we wanted to answer the following questions: which factors influenced the spread of *P. serotina*, and did *P. serotina* act as an invasive species in the studied forests?

#### **Materials & Methods**

A detailed description of the materials and methods can be found in Vanhellemont et al. (2009) and Vanhellemont et al. (in press) for the studies in the Liedekerke forest reserve and the Ossenbos forest reserve, respectively. Table 1 shows the main characteristics of the studied forest reserves. For the two forest reserves, we reconstructed the *P. serotina* invasion based on cadastral maps and aerial photographs, tree ring analysis, forest inventories and regeneration data.





#### Study area & data collection

Liedekerke: The Liedekerke forest reserve has been forested until 1926, when all the trees in the study area were cut. The subsequent management resulted in heathland and coppice. After WWII, management ceased, and the vegetation developed into a mixed deciduous forest with a herb layer dominated by Rubus fruticosus agg. In 1986, a 12.9 ha study area was defined and 65 circular plots (radius 15 m) were installed at the intersections of a 40 m x 50 m grid. These plots were used to study the changes in structure and species composition of the forest, based on ten-year interval data. The 65 plots were inventoried in 1986; 31of the plots were sampled in 1996; and all the 65 plots were re-inventoried in 2006. Data were collected for the tree, shrub and herb layer. In addition, aerial photographs of the period 1944–1986 were used to gain insight into the vegetation development after WWII. The colonization of P. serotina within the study area was analyzed by (1) identifying and locating by GPS the initial points of colonization within the entire forest reserve, (2) setting up an age distribution for the P. serotina in the inventory plots, and (3) predicting the presence of *P. serotina* in the 65 plots based on the plot history, the connectivity to seed trees, the basal area, the percentage of basal area made up by shade-casting woody species, the change in basal area between 1986 and 2006, and the percentage of cover by Rubus spp. in the herb layer. For 54 subcanopy P. serotina, diameter growth and age were determined based on stem cross sections or tree cores.

<u>Ossenbos</u>: The Ossenbos forest reserve is situated in a landscape matrix with forest patches, heathlands, and bare sand. The forest developed on the heathlands and drift sands around a mound that had been planted with pine (*Pinus sylvestris* L.) and oak (*Quercus robur* L.) around 1832. The game densities are extremely high in the forest reserve: ca. 1 ha<sup>-1</sup> of red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*). Data were collected in 40 circular plots (radius 12.6 m) located randomly at the intersections of a 50 m x 50 m grid in the entire forest and in a 70 m x 140 m study area (the core area) in the eldest part of the forest reserve. Position, diameter, and height were measured for the living trees. The height and number of saplings were recorded in the circular plots. In the core area, regeneration was enumerated in four classes: seedlings-of-the-year, seedlings < 20 cm, 20 cm < seedlings < 120 cm, and seedlings > 120 cm. Besides, in the core area, the spatial position of the seed-bearing *P. serotina* trees was noted, and we counted seeds in litter samples. In addition, diameter growth was studied for *P. serotina* growing below *P. sylvestris* or *Q. robur* (13 samples), below *P. serotina* (11), or in a canopy gap (9) in the core area.

Site	Area	Location		Soil type	Т	Precip.	BA	Tree species *
	(ha)	Ν	E		(°C)	(mm)	$(m^2 ha^{-1})$	
Liedekerke	21	50°52'	4°07'	sandy loam	2.5-17.2	821	31.1	Betula, Quercus
Ossenbos	54	52°08'	5°48'	sand	2-17	850	26.6	Pinus

Table 1 Characteristics of the two studied forest reserves: area (ha), geographic location, soil type, minimum and maximum monthly mean temperature (T,  $^{\circ}C$ ), mean annual precipitation (Precip., mm), mean basal area (BA,  $m^2$  ha<sup>-1</sup>), and the main tree species

\* Betula pendula Roth & Betula pubescens Ehrh.; Quercus petraea (Matt.) Liebl., Quercus robur L. & Quercus rubra L.; Pinus sylvestris L.





#### Data analysis

For both forest reserves, we first reconstructed the invasion process. Next, we tried to identify factors affecting the establishment and growth of *P. serotina*.

<u>Liedekerke</u>: Forest development was analyzed based on the comparison of basal area and stem density for 1986–2006 (paired samples t-tests) and 1986–1996–2006 (repeated-measures GLM). We compared the characteristics of plots with and without *P. serotina* (t-tests) and predicted the presence/absence of *P. serotina* seedlings/saplings, shrubs, and trees in the plots (logistic regression). Apart from *P. serotina*, we also looked at *Sorbus aucuparia* L., a species that frequently co-occurs with *P. serotina* (Verheyen et al. 2007). In addition, diameter growth (multiple linear regressions) and allometric relationships between age and height or diameter at breast height (curve estimation procedure) were studied for *P. serotina*.

<u>Ossenbos</u>: We focused on the core area, located in the oldest part of the forest, and used the data on the circular plots to check whether the patterns observed in the core area held for the younger parts of the forest. First, we analyzed the spatial patterns of trees and shrubs in the core area to determine the past establishment of *P. serotina* (bivariate Ripley's L). Second, inverse modelling was used to calculate dispersal kernels for *P. serotina* seed and seedlings in the core area. Third, presence/absence of *P. serotina* was modelled with binary logistic regressions based on the plot characteristics such as basal area of the tree and shrub layers (m<sup>2</sup> ha<sup>-1</sup>), stem density (ha<sup>-1</sup>), and canopy openness (%). Fifth, to explain the abundance of the seedlings/saplings of a species, we performed a data reduction (PCA) on the plot characteristics, and calculated Pearson correlations between the seedling/sapling densities and the principal components. Last, we studied allometric relationships for *P. serotina* age (curve estimation procedure) and diameter growth (multiple linear regressions). Interactive effects between the canopy tree neighbourhood and the age of the studied *P. serotina* on the achieved diameter or height were investigated with ANCOVA analysis.

#### Results

For the two forest reserves, we present the results on *P. serotina* spread, on the factors affecting its presence/abundance, and on *P. serotina* growth.

*Liedekerke*: The first *P. serotina* established around 1970–1975 on sites with high light availability. Further *P. serotina* colonization started 10–15 years later, when these trees presumably started producing seeds. *Prunus serotina* has spread through the study area. Of the 65 plots, 14 were colonized in 1986, 33 in 2006. For *S. aucuparia*, the number of plots was 38 in 1986 and 60 in 2006. The largest increase in number of plots occupied occurred between 1986 and 1996. For the 30 plots which were inventoried thrice, *P. serotina* was present in 6 (1986), 15 (1996), and 17 (2006) plots and *S. aucuparia* in 17 (1986), 28 (1996), and 29 (2006) plots. In 2006, *P. serotina* seedlings and saplings (age < 12 yr) occurred in only 10 % of the plots.

Plots with *P. serotina* were characterized by a higher connectivity to seed trees, a higher overall basal area, and a higher basal area of shade-casting trees. Plots without *P. serotina* had a higher basal area of light-demanding trees and a high cover of *Rubus*. A plot was more likely to be colonized by *P. serotina* if its connectivity to the seed trees was high.

The mean diameter growth of the subcanopy *P. serotina* in 2001–2006 was  $2.8 \pm 0.2$  mm yr<sup>-1</sup>. Diameter growth (mm yr<sup>-1</sup>) was determined by the diameter at breast height (dbh, in





cm) and age of the tree and competition with neighbouring trees (CI): diameter growth = 4.911 + 0.487dbh - 0.028dbh<sup>2</sup> - 0.077age - 0.071CI (R<sup>2</sup> = 0.80). The relationship between age (yr) and dbh (cm) was described most accurately by: age = 7.254dbh<sup>0.402</sup> (R<sup>2</sup> = 0.79).

<u>Ossenbos</u>: Prunus serotina first became established in the Ossenbos around 1940. Successful recruitment of *P. serotina* into the tree layer occurred mainly in gaps of the *P. sylvestris* - *Q. robur* canopy layer. Prunus serotina shrubs occurred more often below the light-demanding *P. sylvestris* and *Q. robur* than below the shade-casting *P. serotina*. In 2006, *P. serotina* was by far the most abundantly regenerating species and the only species with seedlings taller than 120 cm. Prunus serotina was found in all circular plots, and the high densities of seedlings smaller than 20 cm point towards the build-up of a persistent seedling bank.

The dispersal kernels showed that seeds and small seedlings of *P. serotina* mostly occurred close to a source tree while large seedlings showed the highest densities between 10–15 m from the source tree. Accordingly, the abundance of *P. serotina* regeneration was correlated significantly with seed density for small seedlings, and with basal area/canopy openness for larger seedlings. Ln-transformed abundances of *P. serotina* saplings were significantly correlated with the principal component that combined maximum tree height, basal area of tree and shrub layers, and stem density.

Radial growth of *P. serotina* was related to dbh: ln growth = 0.083 + 0.491dbh (R<sup>2</sup> = 0.66). *Prunus serotina* growing in gaps and below *P. serotina* showed a clear relationship between age and dbh (R<sup>2</sup> = 0.96 and 0.86) and between age and height (R<sup>2</sup> = 0.94 and 0.83). The increases of dbh and height with age were higher in gaps than below *P. serotina* (interaction: p = 0.013 and p = 0.034).

#### Conclusion

The initial *P. serotina* status was comparable in the two forest reserves: *P. serotina* had not been planted and the initial propagule pressure was low. Nonetheless, the outcome of the *P. serotina* invasion process contrasted sharply between the two studied forests: *P. serotina* was omnipresent and very abundant in the Ossenbos while the species did not act as an aggressive invader in the Liedekerke forest reserve. Consequently, it appears to be important to study an invasive species and the recipient ecosystem jointly and to gear the control measures to the characteristics of the recipient ecosystem.

Long-distance dispersal events and windows of opportunity triggered the invasion of *P. serotina*. Further colonization was directed by connectivity to seed sources and light availability. In the Liedekerke forest reserve, the presence of native shrub species, the quick canopy closure, and the recalcitrant herb layer seemed to hamper further *P. serotina* establishment. Conversely, in the Ossenbos forest reserve, the high herbivore pressure favoured *P. serotina* above native species, which resulted in *P. serotina* dominance.

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#### References

- Starfinger U., Kowarik I., Rode M. & Schepker H., 2003. From desirable ornamental plant to pest to accepted addition to the flora? the perception of an alien tree species through the centuries. Biological Invasions 5:323–335.
- Vanhellemont M., Wauters L., Baeten L., Bijlsma R.-J., De Frenne P., Hermy M. & Verheyen K., in press. *Prunus serotina* unleashed: invader dominance after 70 years of forest development under high herbivore pressure. Biological Invasions DOI 10.1007/s10530-009-9529-x.
- Vanhellemont M., Verheyen K., De Keersmaeker L., Vandekerkhove K. & Hermy M., 2009. Does *Prunus serotina* act as an aggressive invader in areas with a low propagule pressure? Biological Invasions Biological Invasions 11:1451–1462.
- Verheyen K., Vanhellemont M., Stock T. & Hermy M. 2007. Predicting patterns of invasion by black cherry (*Prunus serotina* Ehrh.) in Flanders (Belgium) and its impact on the forest understorey community. Diversity and Distributions 13:487–497.
- Von Holle B. & Simberloff D., 2005. Ecological resistance to biological invasion overwhelmed by propagule pressure. Ecology 86:3212–3218.
- Zerbe S. & Wirth P., 2006. Non-indigenous plant species and their ecological range in Central European pine (*Pinus sylvestris* L.) forests. Annals of Forest Science 63:189–203.











# Soil arthropods associated to the invasive *Senecio inaequidens* compared to the native *S. jacobaea* (Asteraceae)

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#### Introduction

Soil arthropods play a significant role in soil processes (e.g. nutrient cycling). Diversity of soil invertebrates has been shown to positively influence plant diversity (De Deyn et al. 2003). In turn, plant diversity, and even plant species identity, can influence soil communities (Armbrecht et al. 2004, Ayres et al. 2006, Wardle 2006). In the particular case of plant invasions, several authors have shown that some animal groups in the soil can be affected by the change in plant community. For instance, Belnap & Philips (2001) showed that invasion by the annual grass *Bromus tectorum* (western US) decreased the overall abundance of soil invertebrates as well as the species richness of soil arthropods. These changes in soil communities during invasion have been proposed to facilitate the development of invasive plants, creating positive feedbacks (Callaway et al. 2004, Wolfe & Klironomos 2005).

In the present study, the general hypothesis is that *Senecio inaequidens* (Asteraceae), invasive in Europe, and the native *S. jacobaea* (synonym: *Jacobaea vulgaris*) are not associated with the same community of soil arthropods, in terms of abundance, taxonomic assemblage and diversity.

# Material and methods

*Senecio inaequidens* is a perennial chamaephyte native to South Africa, whereas *Senecio jacobaea* is a biennial to perennial hemicryptophyte native to Europe, spending the first year as a rosette (Harper & Wood 1957).

This study was performed in a 25-ha wildland site, situated in Antwerp (51° 14' 36.40" N, 4° 23' 15.03" E), northern Belgium. Vegetation was dominated by *S. inaequidens* (present for at least 5 years), *S. jacobaea, Festuca rubra, Geranium molle* and *Tanacetum vulgare*. The soil was described as a sandy brown soil. A previous study revealed no difference in soil chemical properties nor in granulometry between invaded and uninvaded patches (Dassonville et al. 2008). There was, however, a higher phytomass and K concentrations in invasive plots.

Soil fauna was sampled in the Antwerp site in 2006 on October 13<sup>th</sup>. Three distinct zones were chosen, separated by approximately 10 m. The first zone was dominated by *S. inaequidens* (hereafter referred to as I), the second one by *S. jacobaea* (J) and in the third one the density was similar for both species and the soil was more gravelly (M). In each zone, four pairs of plants *S. inaequidens* - *S. jacobaea* were chosen, among the oldest and largest ones. Within a pair, plants were separated by 1.5 m at most. One soil core of 8 cm diameter and 5 cm depth (volume 251 cm<sup>3</sup>) was extracted, as close as possible to the root crown. A total of 24 samples were taken and placed on a Berlese-Tullgren extractor.





The abundance of soil fauna, i.e. numbers of individuals per sample, was compared between *S. inaequidens* and *S. jacobaea* by an ANOVA for effects of species, zone and their interaction. For the main taxa, the numbers of individuals were compared between the two plant species by Mann-Whitney U tests. A principal components analysis (PCA) was used to extract the axes summarising the community structure of soil samples. These analyses were performed with STATISTICA 7 (Statsoft 2006). In addition, Shannon-Wiener's index (H') was calculated for soil arthropod communities associated to *S. inaequidens* and *S. jacobaea*. A t-test was applied on these data to test for a difference between the two *Senecio* species.

#### **Results**

The overall abundance of soil fauna was significantly lower (F= 7.46, p= 0.014) under *S. inaequidens* in comparison with *S. jacobaea*, with respectively  $76 \pm 62$  (or 15,300 individuals m<sup>-2</sup>) and  $186 \pm 153$  individuals per sample (or 37,000 individuals m<sup>-2</sup>). This difference was essentially due to the Collembola Arthropleona (Table 1), which were very abundant in *S. jacobaea* samples (19,000 individuals m<sup>-2</sup>), while they were six fold less abundant in *S. inaequidens* samples (3,500 individuals m<sup>-2</sup>). Gamasid mites were twice more abundant in *S. jacobaea* samples compared to *S. inaequidens*, but this difference was only marginally significant. None of the other taxa differed significantly in their abundance under the two plant species. Notably, no significant difference was detected in the number of homopteran and heteropteran herbivores (Table 1). The effect of the zone was marginally significant on the abundance of soil fauna, with a tendency of higher densities in the mixed zone (Figure 1). No significant interaction zone\*species was detected.

Taxonomic richness was similar between *S. inaequidens* and *S. jacobaea* (Table 1). However, the Shannon diversity index was lower for *S. jacobaea* (H=1.54 and evenness = 0.52) than for *S. inaequidens* (H=1.96 and evenness = 0.71) and this difference was highly significant (T-test: t=-10.19, p<0.001). The PCA revealed no distinct pattern between *S. inaequidens* and *S. jacobaea* in the soil arthropod communities (Figure 2).

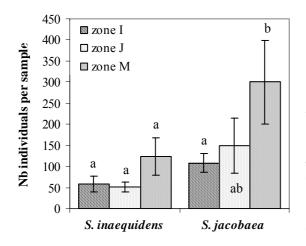


Figure 1. Abundance of soil fauna expressed as the number of individuals per soil sample collected under S. inaequidens and S. jacobaea in the three sampled zones in the site of Antwerp: vegetation dominated by S. inaequidens (I), S. jacobaea (J) and mixed zone with similar density of the two species (M). Error bars denote standard errors and different letters indicate significant difference.





Table 1. Abundance of soil fauna expressed as the total number of individuals (N) for each identified taxon in soil samples collected under S. inaequidens and S. jacobaea (12 samples per species) and percentage of the total number of individuals (%). Feeding groups: herbivore (H), microbivore-mycophagous (M), predator (P), honeydew (\*), saprophagous (S), undetermined or diverse (?). Numbers in brackets are the minima and maxima per sample. Mann-Whitney U tests for difference between the two plant species (for taxa present in more than half the samples): \* and <sup>+</sup> indicate respectively significant (p<0.05) and marginally significant (p<0.07) differences between S. inaequidens and S. jacobaea.

		<u>S. inaequidens</u>		S. jacobaed	S. jacobaea		
Taxon	Food	N	%	Ň	%	<u>Species effect</u> Z	
Diplopoda	S	2 (0-1)	0.22	3 (0-1)	0.13		
Chilopoda	Р	6 (0-3)	0.65	9 (0-4)	0.40		
Symphyla	S	0	0	1	0.04		
Aranea	Р	4 (0-2)	0.43	3 (0-1)	0.13		
Acari Gamasida	Р	289 (2-64)	31.1	564 (11-158)	25.1	$1,85^{+}$	
Acari Oribatida	M, S	94 (0-18)	10.1	91(0-19)	4.06	-0,03	
Acari non determined		13 (0-8)	1.40	18 (0-10)	0.80	0,00	
Isopoda	M, S	52 (0-9)	5.59	64 (0-14)	2.85	0,84	
Protura	M, S	0	0	1	0.04		
Collembola Arthrop.	М	210 (2-94)	22.6	1121 (0-429)	50.0	2,22*	
Collembola Symphy.	М	130 (1-25)	14.0	147 (1-35)	6.55	0,32	
Thysanoptera (thrips)	?	6 (0-2)	0.65	12 (0-4)	0.53		
Hymenoptera (ant)	P*	4 (0-2)	0.43	2 (0-1)	0.09		
Heteroptera (Tingidae)	Н	3 (0-1)	0.32	5 (0-2)	0.22		
Homoptera	Н	66 (0-38)	7.10	118 (0-42)	5.26	0,53	
Coleoptera adult	?	11 (0-3)	1.18	9 (0-2)	0.40	0,73	
larva	?	11 (0-3)	1.18	34 (0-10)	1.52	0,19	
Diptera adult	?	5 (0-4)	0.54	4 (0-2)	0.18	0.(0	
larva	?	6 (0-2)	0.65	8 (0-3)	0.36	0,69	
Lepidoptera (larva)	Н	0	0	1	0.04		
Non determined		13 (0-6)	1.40	20 (0-13)	0.89		
Total		925 (25-245)		2235 (42-551)			
Number of taxa		16		19			

#### Discussion

Although there was an overall tendency for smaller populations of soil arthropods under *S. inaequidens* compared to *S. jacobaea*, only one taxon was significantly affected by *S. inaequidens*, Collembola Arthropleona (and to a lesser extent, acari Gamasid). Since collembolans mostly feed on fungi (Briones et al. 1999), their lower abundance under *S. inaequidens* might be due to antifungal properties of this species (Loizzo et al. 2004). Differences between *S. inaequidens* and *S. jacobaea* in plant productivity, root biomass and architecture (De Jong & van der Meijden 2000, Garcia-Serrano et al. 2005) might also explain these results.

Surprisingly, diversity was higher under *S. inaequidens*, as a result of greater evenness. The difference in Collembola Arthropleona abundance is responsible for this result. Few authors have measured the effect of invasive plants on soil diversity but both positive (on bacteria: Duda et al. 2004) and negative effects are documented (on arthropods: Gratton & Denno 2005).





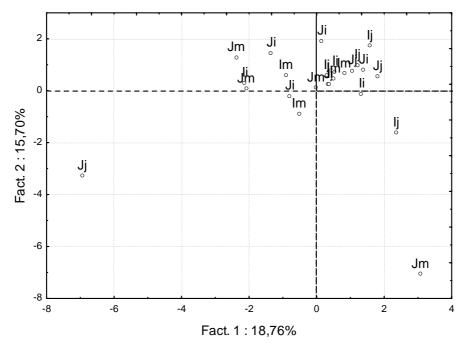


Figure 2. Results of the PCA for the abundance of the different taxa in soil samples for S. inaequidens and S. jacobaea: projection of taxa (A) and samples (B). Labels of samples mention the plant species (J for S. jacobaea or I for S. inaequidens), the sample No (1 to 12) and the zone (i, j or m).

As lower abundance and higher diversity of arthropods were found under *S. inaequidens*, the possible impacts of the invasion by *S. inaequidens* are difficult to assess. Our study needs to be repeated in other sites, as the response of soil arthropods may differ between sites (Yeates & Williams 2001).

In conclusion, this study showed that *S. inaequidens* and *S. jacobaea* were associated with similar soil arthropod assemblages, but that arthropod abundance was lower under *S. inaequidens*, whereas the diversity was higher, essentially due to collembolans Arthropleona. Further studies should focus on verifying this result in other sites with contrasting soil characteristics. Finally, more thorough identifications may help to understand our results.





#### References

- Armbrecht I., Perfecto I. & Vandermeer J., 2004. Enigmatic biodiversity correlations: Ant diversity responds to diverse resources. Science 304(5668): 284-286.
- Ayres E., Dromph K.M. & Bardgett R.D., 2006. Do plant species encourage soil biota that specialise in the rapid decomposition of their litter? Soil Biology & Biochemistry 38(1): 183-186.
- Belnap J. & Phillips S.L., 2001. Soil biota in an ungrazed grassland: response to annual grass (Bromus tectorum) invasion. Ecological Applications 11:1261-1275.
- Briones M.J.I., Ineson P. & Sleep D., 1999. Use of δ13C to determine food selection in collembolan species. Soil Biology and Biochemistry 31(6): 937-940.
- Callaway R.M., Thelen G.C., Rodrigez A. & Holben W.E., 2004. Soil biota and exotic plant invasion. Nature 427 731-733.
- Dassonville N., Vanderhoeven S., Vanparys V., Hayez M., Gruber W. & Meerts P., 2008. Impacts of alien invasive plants on soil nutrients are correlated with initial site conditions in NW Europe. Oecologia 157(1): 131-140.
- De Deyn G.B., Raaijmakers C.E., Zoomer H.R., Berg M.P., de Ruiter P.C., Verhoef H.A., Bezemer T.M. & van der Putten W.H., 2003. Soil invertebrate fauna enhances grassland succession and diversity. Nature 422, no6933: 711-713.
- de Jong T.J. & van der Meijden E., 2000. On the correlation between allocation to defence and regrowth in plants. Oikos 88(3): 503-508.
- Duda J.J., Freeman D.C., Emlen J.M., Belnap J., Kitchen S.G., Zak J.C., Sobek E., Tracy M. & Montante J., 2004. Differences in native soil ecology associated with invasion of the exotic annual chenopod, Halogeton glomeratus. Biology and Fertility of Soils 38(2): 77-77.
- Garcia-Serrano H., Escarré J., Garnier E. & Sans X.F., 2005. A comparative growth analysis between alien invader and native Senecio species with distinct distribution ranges. Ecoscience 12(1): 35-43.
- Gratton C. & Denno R.F., 2005. Restoration of arthropod assemblages in a Spartina salt marsh following removal of the invasive plant Phragmites australis. Restoration Ecology 13(2): 358-372.
- Harper J.L. & Wood W.A., 1957. Biological flora of the British Isles: Senecio jacobaea L. Journal of Ecology 45: 617-637.
- Loizzo M.R., Statti G.A., Tundis R., Conforti F., Bonesi M., Autelitano G., Houghton P.J., Miljkovic-Brake A. & Menichini F., 2004. Antibacterial and antifungal activity of Senecio inaequidens DC. and Senecio vulgaris L. Phytotherapy Research 18(9): 777-779.
- Wardle D.A., 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters 9(7): 870-886.
- Wolfe B.E. & Klironomos J.N., 2005. Breaking new ground: Soil communities and exotic plant invasion. BioScience 55(6): 477-487.
- Yeates G.W. & Williams P.A., 2001. Influence of three invasive weeds and site factors on soil microfauna in New Zealand. Pedobiologia 45(4): 367-383.











# Non-indigenous freshwater fishes in Flanders: status, trends and risk assessment

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# Abstract

At least eighteen non-indigenous freshwater fish species were reported to occur in the wild within the territory of Flanders. Nine are considered naturalized while the others are acclimatized and do not form self-sustaining populations. Nine of the introductions occurred prior to 1950, with the other nine species introduced since then. This contribution reviews the available information on these introductions, and evaluates a decade of data from fisheries surveys to assess the recent development of these non-indigenous populations. Gibel carp Carassius gibelio and topmouth gudgeon Pseudorasbora parva are the most widespread of the non-indigenous species in Flemish waters, and both continue to expand their ranges. A reduction in range has been observed in brown bullhead Ameiurus nebulosus only. Only four species occur in all eleven river basins while eight species are restricted to one or two basins and often only one specimen was found during fish stock assessments. We also discuss nonindigenous fish species that are likely to colonize Flanders inland waters in the near future. For all non-indigenous freshwater fish species present and expected to appear soon, different risk analysis tools (FISK and ISEIA) were used to screen these species for their possible invasiveness. Although scores from FISK and ISEIA differ for some species, gibel carp and topmouth gudgeon were in both assessments classified as 'highest risk' species in relation to their potential invasiveness.

# Introduction

In 1995, the Research Institute for Nature and Forest (INBO) (then Institute for Forestry and Game Management) started a monitoring network on freshwater fishes. Fish stock assessments were performed on a regular basis at more than 800 locations all over Flanders. The data collected during these fish stock assessments were brought together in an online database VIS or Vis Informatie Systeem (Fish Information System) which can be consulted at <a href="http://vis.milieuinfo.be">http://vis.milieuinfo.be</a>. At present, data of more than 200 000 native and non-native fishes with their individual lengths and weights are in this database. All these data are georeferenced.

This paper discusses the presence and status of the non-indigenous fish species in Flanders (Belgium). Data of non-native fishes from the VIS-database were analysed for trends in numbers and biomass over the period 1996 - 2005. Potential invasiveness of all non-indigenous freshwater fish species present and expected to appear soon was assessed using the FISK tool by Copp *et al.* (2005a) and the ISEIA protocol by the Belgian Forum on Invasive Species (Branquart, 2007). Both tools classify the invasiveness of the species into low, medium or high, and results from both methods were compared.





## Status of non-indigenous fish species in Flanders

Louette *et al.* (2001) found evidence in the literature of 47 considered, attempted or successful introductions of fishes in Belgium since 1800; of these, 23 were partially successful (i.e. recorded in public waters after introduction or known to be reproducing). Since 1990 at least 18 non-native freshwater fish species were reported during fish stock assessments by INBO, Agency for Nature and Forest (ANB) and several universities in Flanders. Nine species are naturalised and maintain self-sustaining populations while nine are acclimatised only. Two of these acclimatised species however occur exclusively near cooling water discharges of power plants. These non-native species constitute more than 35 % of the total number of freshwater fish species in Flanders. Most species originate from Asia and N. America, with seven and six species respectively, three species come from Eastern Europe and two from Africa. Nine species were introduced pre-1950s and nine species since then. These 18 species are listed in table 1.

To illustrate that non-indigenous fish species are not a localised phenomenon, table 2 shows the distribution over the 11 Flemish river basins. Four species occur in all river basins, and one in all but one. Seven species occur in one basin only and the other six were found in

Table 1: Species and common names of the 18 non-indigenous freshwater fishes occurring in Flanders, with their continent of origin (AS, Asia; EE, Eastern Europe; AFR, Africa; NA, North America), suspected pathways (AQ, aquaculture; OR, ornamental; AN, angling or bait fish; BC, biological control; UN, unintentional), date of introduction (year or period; c., century) and current status (Copp et al., 2005b; A, acclimatized; N, naturalized; A\*, acclimatized only in restricted areas at cooling water discharges of power plants).

I atin name	Common nomo	Orrigia	Intro du ation	Detherrory(a)	Status
Latin name	Common name	Origin	Introduction	Pathway(s)	Status
Acipenser baeri	Siberian sturgeon	AS	1990s	AQ, OR	А
Ameiurus nebulosus	Brown bullhead	NA	1871	AQ, OR	Ν
Aspius aspius	Asp	EE	1984	AN	Ν
Carassius auratus	Goldfish	AS	17th c.	OR	А
Carassius gibelio	Gibel carp	AS or EE	17th c.	UN	Ν
Clarias gariepinus	African catfish	AFR	1980s	AQ	A*
Ctenopharyngodon idella	Grass carp	AS	1967	ВС	А
Cyprinus carpio	Common carp	EE	13th c.	AQ	Ν
Hypophthalmichthys molitrix	Silver carp	AS	1975	BC	А
Hypophthalmichthys nobilis	Bighead carp	AS	1975	BC	А
Ictalurus punctatus	Channel catfish	NA	1884	AQ	А
Lepomis gibbosus	Pumpkinseed	NA	1885	OR	Ν
Oncorhynchus mykiss	Rainbow trout	NA	1884	AQ, AN	А
Oreochromis niloticus	Nile tilapia	AFR	1990	AQ	A*
Pimephales promelas	Fathead minnow	NA	1984	AN	Ν
Pseudorasbora parva	Topmouth gudgeon	AS	1992	UN, AN	Ν
Sander lucioperca	Pikeperch	EE	1890	AN	Ν
Umbra pygmaea	Eastern mudminnow	NA	1920	OR, AQ	Ν





two to five river basins. The species with a wide distribution over Flanders often also are widespread within the individual river basins. Brown bullhead, eastern mud minnow and pumpkinseed occur at highest densities in the northeast of Flanders. This region is characterised by a high concentration of pond fish farms, which were created in abandoned peat diggings. All of these three North American species are common or widespread there.

As Flanders is surrounded by countries with similar habitats and climates and in which the catchments of the rivers Danube, Rhine, Meuse and Scheldt are connected by canals, it is to be expected that new species as white-finned gudgeon *Romanogobio belingi* (present in Germany and the Netherlands), vimba *Vimba vimba* (reported from the Netherlands), round goby *Neogobius melanostomus* (also reported from the Netherlands), tubenose goby *Proterorhinus semilunaris* (the Netherlands, France and Germany) and bighead goby *Neogobius kessleri* (found in Germany) may enter Flanders in the very near future. Also the highly invasive Amur or Chinese sleeper *Perccottus glenii* was already observed in the Danube and may be a new invader in the years to come.

Table 2: Occurrence of non-indigenous fishes in river basins of Flanders [n = 11; Lower Scheldt (LS), Upper Scheldt (US), Bruges Polders (BP), Demer (Dm), Dender (Dn), Dijle (Di), Ghent Canals (GC), Leie (Le), Meuse (Me), Nete (Ne) and Yser (Ys)] expressed as percentage of sites where a non-indigenous species is present compared to the total number of sample sites per river basin [VR, very rare ( $\leq 2.0$  %); R, rare (2.1 - 10.0 %); C, common (10.1 - 25.0 %); W, widespread (> 25 %)]

	LS	US	BP	Dm	Dn	Di	GC	Le	Me	Ne	Ys	Ν
Gibel carp	R	С	W	W	R	С	W	С	С	С	W	11
Topmouth gudgeon	R	R	С	W	С	С	С	С	R	R	С	11
Pikeperch	С	VR	R	VR	VR	R	R	R	R	С	С	11
Common carp	R	R	W	С	R	С	W	R	R	С	W	11
Pumpkinseed	С	VR	VR	W	R	R	VR	R	С	W		10
Brown bullhead	VR			С		VR			R	С		5
Rainbow trout		VR		R		R			R			4
Goldfish		VR		VR					VR	VR		4
Fathead minnow		VR		R						VR		3
Eastern mudminnow				С					С	С		3
Grass carp				VR							VR	2
Asp									VR			1
Silver carp								VR				1
Bighead carp	VR											1
Siberian sturgeon	VR											1
Channel catfish	VR											1
African catfish				VR								1
Nile tilapia				VR								1
Nb	9	8	5	13	5	7	5	6	10	9	5	

Total number of basins where a species is present (N); Total number of non-indigenous pecies in a basin (Nb).





## Trends

At 487 site-specific surveys between 1996 and 2005, trends in frequency of occurrence and abundance were investigated. These sites were fished at least twice in this period, once between 1996 and 2000, and again between 2001 and 2005 with a minimum of 3 years in between. On 20 % of the sites no fish were caught. Standardised fishing techniques like electrofishing and fyke nets or a combination of both were used during these fish stock assessments. To evaluate the trends, a logistic regression was used to model the changes in frequency of occurrence and a linear mixed model for trends in abundance. Most non-indigenous species show an increasing trend in number of sites they occupy; in particular topmouth gudgeon (p<0.001) and gibel carp (p<0.01) but also pikeperch (p<0.001) are still expanding their ranges. Only three species displayed a decreasing trend with a significant decrease for brown bullhead (p<0.05) only.

Trends in abundance could be modelled for seven species only and solely gibel carp (p<0.05) and common carp (p<0.05) show a significant trend of a decreasing abundance. Remarkably, not only topmouth gudgeon, which is a recently introduced species (first record in Flanders in 1992 (Louette *et al.*, 2001)), is still in full expansion but also populations of species which are part of the Flemish fish fauna for over one hundred years (e.g. gibel carp) are still increasing their range. Some of the trends can be explained by changed stocking policies since it is no longer allowed to stock non-indigenous fishes in public waters, or by the recent overall improvement of the water quality from very bad to moderate, but most trends are influenced by many, interacting factors and are therefore very difficult to explain.

#### **Risk assessments**

The risk analysis tools FISK (Copp *et al.*, 2005a) and ISEIA (Branquart, 2007) were applied to assess the potential invasiveness of the present and expected non-native fishes in Flanders. FISK or the Freshwater Fish Invasiveness Scoring Kit is an adaptation of the Weed Risk Assessment by Pheloung *et al.* (1999). It uses 49 questions in 8 categories (Domestication, Climate and Distribution, Invasive elsewhere, Undesirable traits, Feeding guild, Reproduction, Dispersal mechanisms, Persistence attributes) and takes into account the confidence (certainty/uncertainty) ranking of the assessors. FISK scores range from -11 to 54 and it classifies non-native species into low-, medium-, and high-risk categories (High risk & Invasive  $\geq 19$ , High risk & Non-invasive  $\geq 6$  but < 19,  $1 \leq$  Medium risk  $\leq 6$ , Low risk < 1). The ISEIA protocol (Branquart, 2007) assesses the potential of non-native species for spreading and colonising natural habitats as well as the adverse impacts on native species and ecosystems and assigns invasive alien species to an alert, watch or black list. High impact species present in Belgium are on the black list while those with a moderate or unknown impact are included in the watch list. The alert list consists of high impact species present in neighbouring countries but not yet recorded in Belgium.

When using FISK, mean UK scores were consistently higher (i.e. higher risk to invasiveness) than the Belgian ones (Verreycken *et al.*, in prep.). For species with little published information, scores between assessors can differ substantially. However many species received similar scores from both assessors in their respective region, with differences in scores between assessors exceeding 10 units in 18 out of 67 species (27 %) in the UK and 2 out of 21 in Flanders only (9.5 %).

Of the nine fish species assessed by the ISEIA protocol, four are placed on the black list; these species have correspondingly high scores in FISK. All nine species however score





 $\geq$ 19 by the UK assessors and are considered high risk invasive species in FISK (Copp *et al*, 2008).

Two non-native fish species to be expected in Flanders, Amur sleeper and round goby, were categorized as potentially invasive and are thus on the Harmonia alert list (A<sub>0</sub>-category)(Harmonia database, 2009).

Despite the fact that both tools use different scoring systems, they manage to categorize the fishes more or less in the same 'invasiveness classes'. FISK and ISEIA therefore both represent useful and viable tools to aid decision- and policymakers in assessing and classifying freshwater fishes according to their potential invasiveness.

#### References

- Branquart E. (Ed.), 2007. Guidelines for environmental impact assessment and list classification of non-native organisms in Belgium, <u>http://ias.biodiversity.be/ias/</u>, last assessed 5 May 2009.
- Copp G.H., Garthwaite R. & Gozlan R.E., 2005a. Risk identification and assessment of nonnative freshwater fishes: a summary of concepts and perspectives on protocols for the UK. Journal of Applied Ichthyology 21, 371–373.
- Copp G.H., Bianco P.G., Bogutskaya N., Erős T., Falka I., Ferreira M.T., Fox M.G., Freyhof J., Gozlan R.E., Grabowska J., Kováč V., Moreno-Amich R., Naseka A.M., Peňáz M., Povž M., Przybylski M., Robillard M., Russell I.C., Stakenas S., Šumer S., Vila-Gispert A. & Wiesner C., 2005b. To be, or not to be, a non-native freshwater fish? Journal of Applied Ichthyology 21 (4), 242-262. doi: 10.1111/j.1439-0426.2005.00690.x
- Copp G.H., Vilizzi L., Mumford J., Fenwick G.V., Godard M.J. & Gozlan R.E., 2008. Calibration of FISK, an invasiveness screening tool for non-native freshwater fishes. Risk Analysis (online: DOI: 10.1111/j.1539-6924.2008.01159.x).
- Harmonia database 2009, Belgian Forum on Invasive Species, accessed on 5 May 2009 from: <u>http://ias.biodiversity.be</u>
- Louette G., Anseeuw D., Gaethofs T., Hellemans B., Volckaert F.A.M., Verreycken H., Van Thuyne G., De Charleroy D., Belpaire C., Declerck S., Teugels G.G., De Meester L. & Ollevier F. (2001). Ontwikkeling van een gedocumenteerde gegevensbank over uitheemse vissoorten in Vlaanderen met bijkomend onderzoek naar blauwbandgrondel. Eindverslag van project VLINA 00/11. Studie uitgevoerd voor rekening van de Vlaamse Gemeenschap binnen het kader van het Vlaams Impulsprogramma Natuurontwikkeling in opdracht van de Vlaamse Minister bevoegd voor Natuurbehoud. D/2002/3241/136. [In Dutch]
- Pheloung P.C., Williams P.A. & Halloy S.R., 1999. A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. Journal of Environmental Management, 57, 239-251.
- Verreycken H., Anseeuw D., Van Thuyne G., Quataert P. & Belpaire C., 2007. The nonindigenous freshwater fishes of Flanders (Belgium): review, status and trends over the last decade. Journal of Fish Biology 71 (Supplement D), 160–172.
- Verreycken H., Vandenbergh K., Vilizzi L. & Copp G.H., (in prep). Initial application of the Fish Invasiveness Screening Kit (FISK) to freshwater fishes in Flanders.











# Non-indigenous species of the Belgian part of the North Sea and adjacent estuaries

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Contact: Leen Vandepitte & Ann-Katrien Lescrauwaet

### Introduction

Colonization of marine, coastal and estuarine environments by non-endemic or nonindigenous species is not a recent phenomenon in the Belgian context. Some of our history books contain references to early human introductions of non-endemic species, related to aquaculture and other economic purposes. For those aquatic species, it is relatively easy to define the year of introduction or 'first observation' for Belgian waters. Non-endemic species are also often discovered by coincidence. However, for a number of species in the marine and estuarine environment, it is difficult to say whether they are endemic or not. For some taxa it is difficult to distinguish between local and non-endemic species, which may lead to erroneous determinations. Our knowledge and research techniques have evolved enormously over the last centuries, and we can not always say with certainty whether a species may have been present before. This is particularly true for smaller and inconspicuous species such as, e.g., bacteria, microscopic algae or species with an elusive behavior. We refer to those species as 'cryptogenic'.

Once non-endemic or 'alien' species have settled as reproductive populations, it is difficult to turn back the clock. Aliens may cause impact of differing type and degree in their new environments. This impact can cause damage to economy, public health and biodiversity. In this case, the alien invaders are catalogued as 'invasive' species. Directed management efforts may mitigate or reduce the damage caused by non-endemic species, or even anticipate them. It is therefore important to collect data on these particular species, their location and changes in their distribution, and the type of impact they may cause. These factual data - together with knowledge on the species' ecology - provides relevant information to policy and management.

Flanders Marine Institute and its consortium of experts on non-indigenous species ('VLIZ Alien Species Consortium') conduct an ongoing effort to collect and maintain a list of aliens with documented established populations in the Belgian part of the North Sea and its adjacent estuaries.





#### The non-indigenous species list

The discovery of America (1492) is set as the historical baseline for this assessment. This year marked the beginning of a strong increase in trans-Atlantic shipping. Associated with shipping, an increase in commerce between continents, their cultures and species evolved. Shipping and commerce are two important vectors for the 'transport' of non-endemic species. Species that arrived after 1492 in our waters are classified as non-endemic or alien. Those for which information indicates that resident populations existed before this reference year are considered as endemic.

The list strives to include all currently known non-indigenous and cryptogenic species registered in salty and brackish environments in the Belgian part of the North Sea, the Belgian coastal zone and adjacent estuaries of the rivers Yser and Scheldt (see figure 1). The Ostend Sluice dock, an artificial water body connected to the port docks of Ostend, is also part of the study area. The initiative of the VLIZ Alien Species Consortium scrutinizes intentional and unintentional introductions by man or other vectors. Alien species for which there is no evidence of resident populations are not included in the list, nor are species that are limited to the fresh water environment. Vagrant species or occasional observations therefore are not part of the scope. New arrivals as a consequence of naturally induced migrations are also excluded. To this purpose the possible effects of global warming are not accounted for in the list.

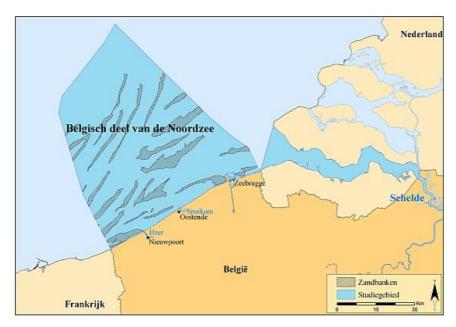


Figure 3: map of the study area

The trend in the number of observed non-endemic species in the Belgian part of the North Sea and adjacent estuaries is on the rise (figure 2). The steep increase from the 1980's onwards is largely due to an increased and improved observation effort. Therefore data before 1980 can not be compared to data after 1980.

Currently (May 2009), the list contains 64 resident non-endemic species. Figure 3 shows the distribution of these resident non-endemic species by larger groups. The arthropods count the highest number of non-endemic species (23). In this group, the share of Cirripedia (barnacles) is significant (5). Molluscs (i.a. bivalves and gastropods) and algae contribute with respectively 11 and 10 species.





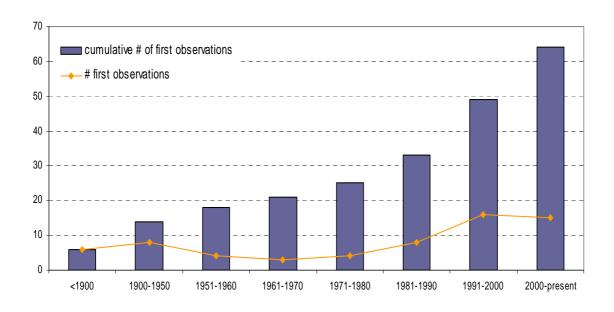
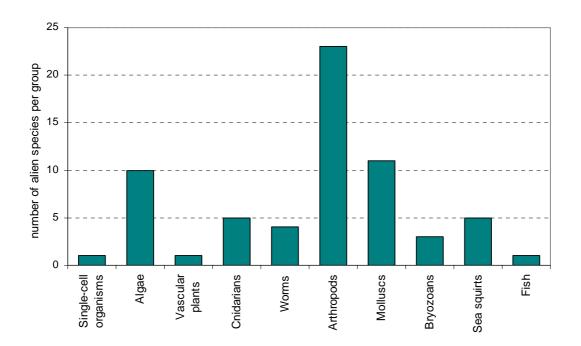


Figure 2: Cumulative number of 'first observations' of non-endemic species in the Belgian part of the North Sea and adjacent estuaries



*Figure 3: Distribution of non-endemic species in the Belgian part of the North Sea and adjacent estuaries, by larger groups* 

The list is accessible via the Internet. For each listed species, interactive links lead the user to a freely accessible on-line source of information on non-indigenous species and the associated network of experts for this study area. It includes definitions, information sheets and background literature by species, and further links to European and international initiatives. Each information sheet describes the life cycle and ecology of the species, the introduction pathways and distribution, the possible effects or impacts of the species on its





environment and potential mitigation measures. Pictures, schemes and diagrams add qualitative information. The information sheets are the result of a thorough literature study and the direct contribution of expert knowledge. They provide scientifically robust and reliable information. All sources (publications and documents) are included in a fully documented reference list - all digitally available at the VLIZ library. Contact information of the experts of the consortium is also available from the web pages.

Web: <u>http://www.vliz.be/NL/Cijfers\_Beleid/Niet\_inheemse</u> (Dutch)





# Brussels Psittacidae: impacts, risk assessment and action range

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### Introduction

Invasive species raise many questions regarding their environmental impacts. Literature provides ample advice to help field management choices, sometimes contradicting each other. For example, measures should be applied at low levels of abundance; but at the same time, it is advised to adapt action to impacts, which are often unknown at the moment of settlement. The necessity to define present and potential impacts of introduced species is increasingly obvious. In the case of the Psittacidae, there are only a few examples of feral population management. This could be linked to the fact that these species scarcely induce major economic damages. Furthermore, many people living in town welcome those birds which are to a certain extent a substitute to the contact between man and nature.

The Brussels avifauna has been studied for many years by the birding society Aves, in collaboration with Brussels Capital-Region Environment Institute. Information is partly obtained through common birds monitoring (by point counts) within the framework of the Brussels Environment Survey, but also thanks to research conducted in 2002 to assess the impact of the Ring-necked Parakeet *Psittacula krameri* in Brussels (Weiserbs *et al.*, 2002). Finally, a recent study carried out in 2008 reviewed the current status of Psittacidae populations in the Brussels Region and analysed their present and potential impacts in order to inform policy-makers about the best management practices to limit these impacts. This contribution stems from those different researches.

#### **Brussels Psittacidae**

Three Psittacidae species breed in Brussels: the Alexandrine Parakeet (*Psittacula eupatria*), the Ring-necked Parakeet (*Psittacula krameri*) and the Monk Parakeet (*Myiopsitta monachus*). The case of the Monk Parakeet will not be further developed here, the Brussels feral population being easier to manage (Weiserbs, in press). If Ring-necked Parakeet is known to be introduced in at least 35 countries, feral populations of Alexandrine Parakeet are much scarcer. In Brussels, both Ring-necked Parakeet and Alexandrine Parakeet are strongly increasing, although the second, having settled only recently, is much less numerous. Both populations are mixed in the field, sharing, for example, roost and feeding sites. Moreover, most of the Alexandrine Parakeet population is located in the North-West of Brussels, where the Ring-necked Parakeet is the most abundant. Feeding by man is supposed to have an important impact on demography, reducing winter mortality and increasing breeding success.

Present and potential impacts of the Ring-necked Parakeet in Brussels could be summarized as followed:

• Competition with indigenous fauna is at present the main threat of the species. In Brussels, a negative impact on Nuthatch has been suggested (Strubbe & Matthysen, 2007) and is observed when competition is experimentally forced (Strubbe & Matthysen, 2009).





- Point count survey between 1992 and 2008 indicates a favourable status of cavity nesting birds in Brussels (Weiserbs, 2008). Moreover, if no effect is observed for seven cavity nesting species, the Ring-necked Parakeet density has a significant positive effect on the trends of four other hole nesters: Green Woodpecker, Blue Tit, Great Tit (less significant) and Short-toed Tree creeper. This could be explained by the advanced age of most tree settlements in Brussels parks and the excavating behaviour of Ring-necked Parakeet, using any starting wounds on the trees to create new cavities. Besides, research conducted in 2002 showed extremely high cavity densities in parks inhabited by dense populations of Ring-necked Parakeet (Weiserbs *et al.*, 2002). Nevertheless, a negative impact is feared in the short-term, linked to the regeneration of tree settlements and resultant shortage of cavity supplies. Moreover, impact on other groups, like bats, is unknown, but could be real.
- On the fringe of the previous main threats, a local impact is possible on some fruit crops (as observed in Great Britain).
- Finally, very localised impacts are linked to noise disturbance and dirt under the roosts.

Present impacts of the Alexandrine Parakeet in Brussels are weak as the population is not very large, but are adding to those of Ring-necked Parakeet to which the Alexandrine is associated in the field. Moreover, a strong increase has to be expected in the future, which may result in growing impacts.

The risk assessment is based on two schemes. The "UK non-native organism risk assessment scheme" (Anonymous, 2005), concerning risks for environment and socioeconomy, leads, for both species, to the conclusion of a weak to moderate impact. The "Guidelines for environmental impact assessment and list classification of non-native organisms in Belgium" (Branquart, 2007), assessing risks for Belgian biodiversity, leads to classify both species between categories B (Watch list) and C (low environmental risk).

Since the two species are closely mixed (co-occur?) in the field, measures will have to consider both species in concert. The actions range reviews the possible management measures, from the weakest to the strongest:

- Reduce and modify feeding by man to try to slow down demography.
- Act at the cavity supply level to lower potential competition with native cavity-nester (nest boxes setting, old trees preservation,...).
- Sterilization using a chemical substance (as Diazacon) could be possible; this requires catching the birds at roosts, for example by cannon netting
- Eradication is difficult to plan in an urban context and discouraged, as the current impacts are assessed as low and as the public reaction could prevent future action against more problematic species.

#### Acknowledgments

We would like to thanks the Brussels Capital-Region Environment Institute who financed theses researches and whose collaboration is fruitful. We also are warmly grateful to all the volunteers contributing to the Brussels point counts monitoring.





#### References

- Anonymous 2005. UK non-native organism risk assessment scheme Version 3.3 (28.2.2005). Prepared by CABI Bioscience (CABI), Centre for Environment, Fisheries and Aquaculture Science (CEFAS), Centre for Ecology and Hydrology (CEH), Central Science Laboratory (CSL), Imperial College London (IC) and the University of Greenwich (UoG) under Defra
- Branquart E., 2007 (Ed). Guidelines for environmental impact assessment and list classification of non-native organisms in Belgium. ISEIA <u>http://ias.biodiversity.be</u>
- Strubbe D. & Matthysen E., 2007. Invasive ring-necked parakeets *Psittacula krameri* in Belgium: habitat selection and impact on native birds. *Ecography* 30: 578-588.
- Strubbe D. & Matthysen E., 2009. Experimental evidence for nest-site competition between invasive ring-necked parakeets (*Psittacula krameri*) and native nuthatches (*Sitta europaea*). *Biological Conservation* (in press)
- Weiserbs A., 2008. Surveillance de l'état de l'environnement bruxellois. Groupe de travail Aves – Rapport pour Bruxelles Environnement – IBGE 2008.
- Weiserbs A., 2009. Espèces invasives : le cas des Psittacidés en Belgique. Incidences, évaluation des risques et éventail de mesures. *Aves* (in press).
- Weiserbs A., Jacob J. P. & Rotsaert G., 2002. Evaluation de l'incidence du développement des populations de perruches sur les habitats et les espèces indigènes en Région bruxelloise. Aves - Rapport pour l'Institut Bruxellois pour la Gestion de l'Environnement.

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# Research on biological invasions: a Belgian perspective

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## Introduction

Three years after the SOS invasion milestone meeting, the Science facing Aliens conference was a new opportunity to produce an overview on Belgian research dedicated to biological invasions. In preparation to the conference, we inventoried the Belgian research linked to the conference theme and assessed its performance relative to European research, the results of which we present here.

# Methodology

#### Inventory of the Belgian research

Research projects dealing with biological invasions were surveyed through the BioBel database (Belgian Biodiversity Platform, http://biobel.biodiversity.be, accessed April 30, 2009). Projects extracted from the database were sorted in two different categories. The first includes projects that focus on biological invasions and involve at least 1 full- time scientist. The second includes projects that deal with invasion ecology incidentally.

Several attributes were assigned to the projects. This includes starting and ending date of the project, taxonomic affiliation, habitat type, research topic, and funding source. Five main research topics were considered based on the session themes of the last Neobiota conference (Prague, September 2008) : invasion and dispersion patterns, mechanisms and evolution of invasions, impacts of invasions, prediction and risk assessment and management practices. These topics are supposed to encompass the full spectrum of research activities linked to biological invasions.

For all the analyses presented below, individual projects were weighted based on the number of research teams involved with a least 1 full-time scientist. This implies that more weight was attributed to large networks than to individual PhD theses.

Although we made a great effort to include all invasion-related research projects in BioBel, some may have escaped our attention. The following results need to be interpreted with this caveat in mind.

#### Performance of Belgian research compared to European research

Bibliometric analyses were performed to compare Belgian with European research dedicated to invasion ecology. We used the terms "biological invasion\*" or "invasive species" or "alien species" or "non-indigenous species" or "non-native species" or "exotic species" or "invader\*" to search papers published between 1990 and 2008 included in Web of Science ® (WoS). Then, we extracted those papers to which at least one European author contributed. This yielded 2796 papers, among which 88 were produced by Belgian authors, alone or in collaboration with international authors.

Two different performance indicators were calculated using this information. Our first indicator intends to reflect the research attention dedicated to invasion-related issues in a





country, by calculating the number of publications divided by population size in each country; residuals of the linear regression of the number of publications on country population size were used to this purpose. The second indicator reflects the impact of the publications, and is based on the "times cited" count, or the number of times a published paper is cited by other papers indexed in WoS (figure 5).

## The Belgian research on biological invasions

We identified 56 research projects dedicated to biological invasions being conducted by Belgian scientists, from 1990 to 2009. As shown in figure 1, a rising interest in invasion ecology is manifest in the exponential growth of research projects related to invasive species since 1999.

In addition to these 56 projects, an additional 22 projects involve invasive species in a more incidental way. They are related either to biodiversity monitoring activities or to pest control studies. Such projects are not considered in further analyses.

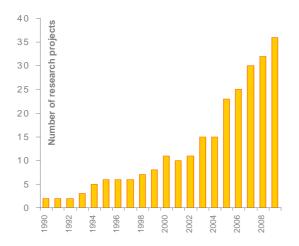
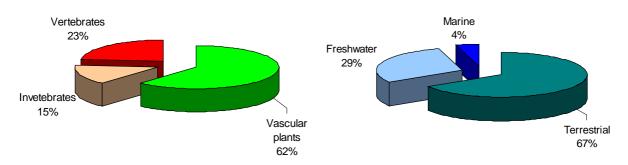


Figure 1: Number of ongoing Belgian research projects dedicated to biological invasions since 1990.

A majority of research projects (62 %) focus on invasive plants; vertebrates are considered in 23 % of the projects and invertebrates in only 15 % (see figure 2). There is no project in our BioBel database that deals explicitly with invasive micro-organisms, fungi or algae.

Although non-native species are known to invade most ecosystem types in Belgium, research dedicated to biological invasions is mainly conducted on terrestrial ecosystems (67%). Twenty-nine percent of projects target freshwater systems whereas only 4% deal with marine areas.



*Figure 2:* Share of Belgian research projects dedicated to biological invasions between main taxonomic groups (left) and major ecosystems (right)





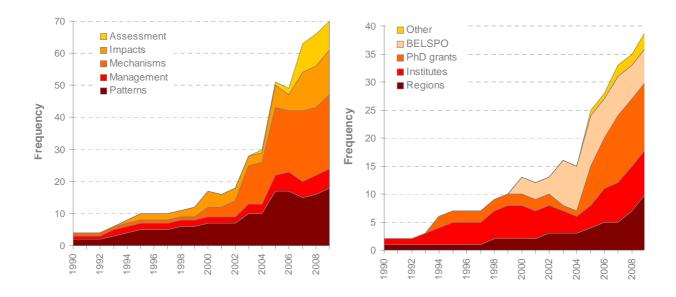


Figure 3 – Evolution of ongoing project frequency from 1990 to 2009, by research topics (left) and research funding sources (right).

Two out of the five main research avenues (invasion patterns and management) are investigated since the early nineties and constitute the baseline of Belgian invasion ecology research. The three other research topics were developed progressively later on. Prediction and risk assessment studies are the most recent and least developed subjects (see figure 3).

Different funding sources supported the Belgian research effort on biological invasions (1990-2009) to different degrees 25% of projects received funding from BelSPO, 25% concern PhD grants, 23% are funded by regional administrations, 18% rely on core institute budgets, and the remaining 9% draw from from various other sources (see figure 3). International funding of the Belgian invasion ecology research was nearly absent during that period.

Structural funding was available from regional administrations in charge of environment management and from biological research institutes (e.g. National Botanic Garden of Belgium, Research Institute for Nature and Forests, Royal Belgian Institute for Natural Sciences), allowing the development of long-term monitoring and research programmes. On top of that, more focused initiatives were developed from 1999 onwards based on the work of research teams involved in BelSPO projects and of PhD students. Those studies often focused on invasion mechanisms and impact of biological invasions; they often produced innovative results to be published in international journals (see further). The implementation of the BelSPO "Science for a Sustainable Development" programme, which included invasion ecology as a priority topic, resulted in a significant increase in research efforts and acted as a strong catalyst for the development of invasion ecology science in Belgium.

#### Belgian research in a European context

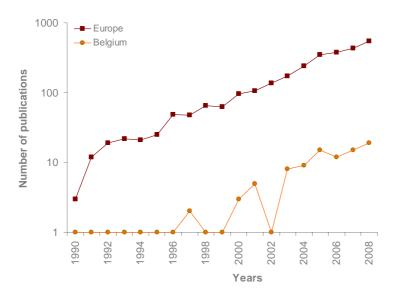
Fifty years ago, the publication of Charles Elton's book *The Ecology of invasions by animals and plants* (1958) launched the systematic study of biological invasions. However, biological invasion- related scientific papers only started to be readily produced some 30 years later, mostly emanating from the SCOPE programme on biological invasions. This programme

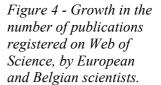




raised awareness on the importance of the phenomenon at a world-wide scale (Richardson & Pysek 2008).

In Europe, the number of publications dealing explicitly with invasion ecology is increasing exponentially since the early nineties (Figure 4). The top 10 international journals publishing papers on biological invasions is as follows (number of papers published between brackets): Biological Invasions (107), Diversity and distribution (90), Hydrobiologia (72), Journal of Applied Ecology (63), Molecular Ecology (59), Biological Conservation (58), Biodiversity and Conservation (41), Marine Ecology (38), Oecologia (38) and Journal of Biogeography (34).





Publications by Belgian scientists on biological invasions started to appear with a time lag of 6 to 9 years compared to the overall production in Europe (figure 4). With a total of 88 international publications, Belgium rates 7<sup>th</sup> amongst European countries, after correcting the number of publication for population size of the country (figure 5A). The impact of publications by Belgian scientists, as measured by the average number of times they are cited, appears to be relatively low when compared to those by their European colleagues (figure 5B).

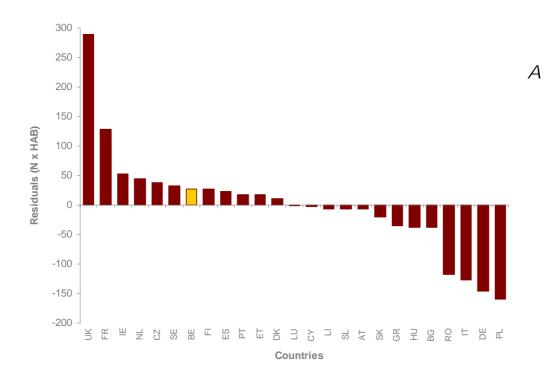
On top of international publications, Belgian scientists produce significant amounts of valuable "grey" literature. Notwithstanding the sometimes unjustified poor perception of such publications, they are particularly useful for risk assessment and management purposes. An example is the "Catalogue of the Neophytes in Belgium" (1800-2005) (Verloove 2006) or the various reports produced by scientists of the Research Institute for Nature and Forest. Most of these publications are referred to in the *Harmonia* information system of the Belgian Forum on Invasive Species and in the data centre of the Flanders Marine Institute.

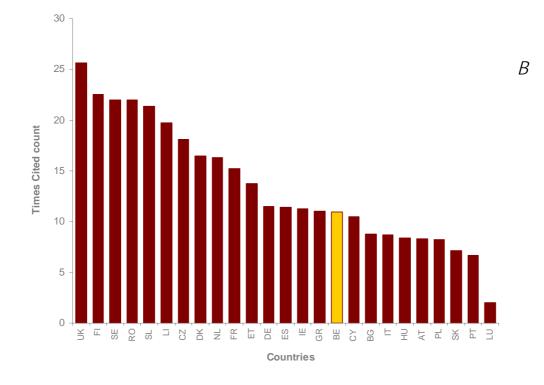
Twenty percent of Belgian papers published in WoS-indexed journals result from BelSPO funded projects. This concerns mainly research teams that are member of the INPLANBEL-PERINBEL-ALIEN IMPACT suite of projects. The following laboratories also contributed significantly to the production of Belgian publications related to invasion ecology: Animal Ecology (UA), Behavioural and Evolutionary Ecology (ULB), Biological Control and Spatial Ecology (ULB), Forestry (UGent) and Forest, Nature and Landscape (KUL).

Despite the high level of the scientific research dedicated to invasion ecology in Belgium, only few research teams have been able to exploit European funding sources. The only institutions involved in European Research and Technology Development (RTD)









*Figure 5: Country ranking based on the two performance indicators: number of WoS publications corrected for population size (A) and Times Cited count of WoS publications (B).* 





Program	Acronym	Title of the project	Starting year	Belgian partners
FP5	GIANT ALIEN	Giant Hogweed ( <i>Heracleum mantegazzianum</i> ) a pernicious invasive weed: Developing a sustainable strategy for alien invasive plant management in Europe	2002	(NBGB)
FP6	ALARM	Assessing LArge-scale environmental Risks with tested Methods	2004	(KUL, UCL)
FP6	MarBEF	Marine Biodiversity and Ecosystem Functioning	2005	VLIZ
FP6	DAISIE	Delivering Alien Invasive Species Inventories for Europe	2005	-
FP6	IMPASSE	Environmental impacts of alien species in aquaculture	2006	-
FP6	REBECA	Registration of Biological Control Agents	2006	-
FP6	ALTER-Net	A Long-Term Biodiversity, Ecosystem and Awareness Research Network	2006	INBO
FP6	FORTHREATS	European Network on emerging diseases and threats through invasive alien species in forest ecosystems	2007	ULB
FP7	PRATIQUE	Enhancements of Pest Risk Analysis Techniques	2008	-

*Table 1 – Presentation of the different European RTD projects related to biological invasions and partnership with Belgian research teams.* 

projects are VLIZ (MarBEF), INBO (ALTER-Net) and ULB (FORTHREATS). No Belgian partner was involved in the large EU projects that focused on biological invasions (ALARM, DAISIE, IMPASSE, etc.). The poor integration of Belgian scientists in EU research network is probably due to the publication time lag. On the other hand, many Belgian scientists are today involved in EU policy-oriented networks, linked to, amongst others, the Bern Convention, the European Environmental Agency (EEA), the European Food Safety Agency (EFSA) and the European Plant Protection Organisation (EPPO). The *Harmonia* information system and the ISEIA assessment protocol elaborated by the Belgian Forum on Invasive Species were welcomed by those initiatives (see e.g. EPPO 2008, Copp et al. 2009, Essl et al. submitted). But the contribution of Belgian scientists to the European alien species inventory has been on an exclusively voluntary basis, which is unsustainable in the long term.

#### **Perspectives for research**

Today, Belgian research dedicated to biological invasions enters a phase of maturity and is conducted according to high quality standards. Some research topics are well developed by Belgian teams and can be considered as very competitive within the international arena, e.g., studies dedicated to the evolutionary and ecological mechanisms of plant invasions or to those focusing on the spatial dynamics of invasions. Research effort should capitalise on that basis and try to integrate as much as possible within international networks. Scientists should also try to address the remaining gaps and consider invasion issues in less-studied ecosystems, such as freshwater and marine environments.





The Science Facing Aliens conference generated lively discussions on the future priorities for research on invasive alien species. If society wishes to limit the impact of biological invasions, then setting up an Early Detection and Rapid Response (EDRR) system in Belgium will be needed. This implies developing adequate monitoring activities, correctly identifying new invasive species, performing rapid risk analyses and implementing adequate management responses. Scientific research activities in support of EDRR should be promoted within emergent disciplines like bio-informatics, DNA barcoding and risk analysis. Best practices for the management of invasive species should also be identified.

Furthermore, the invasion history of new invasive alien species will need to be documented, especially in the case of non-native species that have so far not or hardly been recognised as posing a threat in other countries. We need to quantify the impact of such species on biodiversity and ecosystem functioning, using both observational and experimental studies. To be effective, the results of these studies should be actively communicated to field managers and experts from Belgium and other countries. We therefore invite scientists involved in research on invasive species to participate to the risk assessment activities co-ordinated by the Belgian Forum on Invasive Species, and to attend meetings organised by EEA, EFSA, EPPO and other international initiatives.

Finally, we advocate that invasion ecologists liaise with colleagues from other disciplines to reinforce interdisciplinary and integrative studies. Key areas where improved links with invasion ecology are needed are global change biology, restoration ecology, weed science as well as plant, animal and human health science, as exemplified by BelSPO MODIRISK and EPI-STIS projects.

#### Acknowledgements

The authors would like to acknowledge all the Belgian scientists who were so kind as to provide information about their research projects in the BioBel database, without which this analysis would have been impossible.

#### References

- Copp G.H. et al., 2009. Calibration of FISK, an Invasiveness Screening Tool for Non-native Freshwater Fishes. Risk analysis 29: 457-467.
- EPPO, 2008. Impact assessment of invasive alien plants in Belgium. EPPO Reporting service 2008/087.
- Essl F., Klingenstein F., Milasowszky N., Nehring S., Otto C. & Rabitsch W., submitted. The German-Austrian black list information system (GABLIS): a tool for assessing biodiversity risks of invasive alien species in Europe. Journal of Nature Conservation.
- Richardson D.M. & Pysek P., 2008. Fifty years of invasion ecology : the legacy of Charles Elton. Diversity and Distribution 14 : 161-168.
- Verloove F., 2006. Catalogue of the Neophytes in Belgium (1800-2005). Scripta Botanica Belgica 39, 89 pp.













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Edited by H. Segers & E. Branquart

**BIOLOGICAL DIVERSITY** 

22 MAY 2009



