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Risk analysis of the Louisiana Crayfish, *Procambarus clarkii* (Girard, 1852)

Risk analysis report of non-native organisms in Belgium

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Adopted in date of : 11 March 2013

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Etienne Branquart (Cellule Espèces Invasives, Service Public de Wallonie) developed the risk analysis template that was used for this exercise.

The general process of drafting, reviewing and approval of the risk analysis for selected invasive alien species in Belgium was attended by a steering committee, chaired by the Federal Public Service Health, Food chain safety and Environment. RBINS/KBIN was contracted by the Federal Public Service Health, Food chain safety and Environment to perform PRA's for a batch of species. ULg was contracted by Service Public de Wallonie to perform PRA's for a selection of species. INBO and DEMNA performed risk analysis for a number of species as in-kind contribution.

Steering committee members were:

Tim Adriaens	Research Institute for Nature and Forest (INBO)
Olivier Beck	Brussels Environment (BIM)
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Rationale and scope of the Belgian risk analysis scheme

The Convention on Biological Diversity (CBD) emphasises the need for a precautionary approach towards non-native species. It strongly promotes the use of robust and good quality risk assessment to help underpin this approach (COP 6 Decision VI/23). More specifically, when considering trade restrictions for reducing the risk of introduction and spread of a non-native organisms, full and comprehensive risk assessment is required to demonstrate that the proposed measures are adequate and efficient to reduce the risk and that they do not create any disguised barriers to trade. This should be seen in the context of WTO and free trade as a principle in the EU (Baker et al. 2008, Shine et al. 2010, Shrader et al. 2010).

This risk analysis has the specific aim of evaluating whether or not to install trade restrictions for a selection of absent or emerging invasive alien species that may threaten biodiversity in Belgium as a preventive risk management option. It is conducted at the scale of Belgium but results and conclusions could also be relevant for neighbouring areas with similar eco-climatic conditions (e.g. areas included within the Atlantic and the continental biogeographic regions in Europe).

The risk analysis tool that was used here follows a simplified scheme elaborated on the basis of the recommendations provided by the international standard for pest risk analysis for organisms of quarantine concern[1] *produced by the secretariat of the International Plant Protection Convention (FAO 2004). This logical scheme adopted in the plant health domain separates the assessment of entry, establishment, spread and impacts. As proposed in the GB non-native species risk assessment scheme, this IPPC standard can be adapted to assess the risk of intentional introductions of non-native species regardless the taxon that may or not be considered as detrimental (Andersen 2004, Baker et al. 2005, Baker et al. 2008, Schrader et al. 2010).*

The risk analysis follows a process defined by three stages : (1) the initiation process which involves identifying the organism and its introduction pathways that should be considered for risk analysis in relation to Belgium, (2) the risk assessment stage which includes the categorization of emerging non-native species to determine whether the criteria for a quarantine organism are satisfied and an evaluation of the probability of organism entry, establishment, spread, and of their potential environmental, economic and social consequences and (3) the risk management stage which involves identifying management options for reducing the risks identified at stage 2 to an acceptable level. These are evaluated for efficacy, feasibility and impact in order to select the most appropriate. The risk management section in the current risk analysis should however not been regarded as a full-option management plan, which would require an extra feasibility study including legal, technical and financial considerations. Such thorough study is out of the scope of the produced documents, in which the management is largely limited to identifying needed actions separate from trade restrictions and, where possible, to comment on cost-benefit information if easily available in the literature.

This risk analysis is an advisory document and should be used to help support Belgian decision making. It does not in itself determine government policy, nor does it have any legal status. Neither should it reflect stakeholder consensus. Although the document at hand is of public nature, it is important to realise that this risk assessments exercise is carried out by (an) independent expert(s)

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A weed or a pest organism not yet present in the area under assessment, or present but not widely distributed, that is likely to cause economic damages and is proposed for official regulation and control (FAO 2010).

who produces knowledge-based risk assignments sensu Aven (2011). It was completed using a uniform template to ensure that the full range of issues recognised in international standards was addressed.

To address a number of common misconceptions about non-native species risk assessments, the following points should be noted (after Baker et al. 2008):

- *Risk assessments are advisory and therefore part of the suite of information on which policy decisions are based;*
- *The risk assessment deals with potential negative (ecological, economic, social) impacts. It is not meant to consider positive impacts associated with the introduction or presence of a species, nor is the purpose of this assessment to perform a cost-benefit analysis in that respect. The latter elements though would be elements of consideration for any policy decision;*
- *Completed risk assessments are not final and absolute. New scientific evidence may prompt a re-evaluation of the risks and/or a change of policy.*

Figure 1 : Habitus of *Procambarus clarkii*. (Source: Duloup – http://commons.wikimedia.org/).

Executive summary

PROBABILITY OF ESTABLISHMENT AND SPREAD (EXPOSURE)

- Entry in Belgium

If the sea food industry may have played a minor role during the 1980 - 1990s, current introductions in Belgium mainly result from the release of live animals used as pets by aquarium owners. Based on the current distribution of *P. clarkii* in France and the Netherlands, and on the dispersal capacity of the species, it is reasonable to assume that natural colonisation from neighbouring countries will soon become an additional pathway of introductions in Belgium.

- Establishment capacity

Most freshwater bodies and slow flowing streams of the territory, including endangered and sensitive areas, are potentially suitable to *P. clarkii* establishment. This observation is true under current climatic conditions, and also under predicted increasing temperatures due to climate change.

- Dispersion capacity

The dispersal capacity of *P. clarkii* is high. It is capable of natural dispersion over relatively long distances (up to several tens of kilometres in a few days). The species frequently disperses overland and it is hence not limited to connected waters for spreading. Dispersion by birds is also suspected to occur. In addition, it is particularly subjected to repeated release by humans because it is one of the most popular crayfish species in the sea food and aquarium trade. The latter is nowadays particularly popular in Europe, including Belgium, where living specimens are easily found on the Internet or in aquarist centres.

EFFECT OF ESTABLISHMENT

- Environmental impacts

Procambarus clarkii may severely impact native biodiversity. It may cause adverse effect on native species by competition for food or shelters, by predation, by herbivory and by pathogen transmission. It can also alter ecosystem functioning by modification of physical and chemical characteristics of water and sediments, and by cascade effects through the food web. Native crayfish are particularly threatened but *P. clarkii* may also have strong impacts on fishes, amphibians, invertebrates, macrophytes and algae because of its omnivory and trophic plasticity. After its introduction, water turbidity generally increases, nutrients and energy flows are often greatly shortened and simplified, and local extinction of some organims may occur. *Procambarus clarkii* can thus be considered as a keystone species, which profoundly affects the ecosystem functions. Finally, *P. clarkii* may also accumulate heavy metals, radioactive elements and other pollutants, and may therefore potentially transmit them to higher trophic levels.

RISK MANAGEMENT

The main current pathway of introductions of *P. clarkii* in Belgium is the release of animals by people. <u>Information, education, prevention and control</u> seem the most promising management measures to reduce the negative impact of invasive crayfish species. Fortunately, several education actions were (and are) already carried out at the European level, and in Belgium.

A unified, strengthened legislation should be established in Europe to ensure a total ban on import, trade and holding of live *P. clarkii* and other (potentially) invasive crayfish. Indeed, the potential to become invasive exists for most crayfish species nowadays present in the aquarist catalogues, especially for those originating from temperate regions.

Unfortunately, for *P. clarkii*, prohibition of importation, trade and holding in Belgium could not be enough to prevent its entry and establishment because (1) the species is already spreading in the country (especially in the Flemish Region), and (2) natural spreading from neighbouring countries are expected since dense populations are now close to the Belgian border. Nevertheless, such measures can certainly limit secondary releases in the environment and slow down its current spread and prevent colonization of some natural districts of the country.

As for other non-native aquatic species, any introduction and translocation of *P. clarkii* for use in aquaculture is subjected to the issue of a permit according to the Council Regulation (EC) No 708/2007 of 11 June 2007. Such introduction should theoretically not be allowed unless the applicant may demonstrate that the probability of crayfish escape is very low due to drastic security measures around the aquaculture facilities (closed system).

National surveys should be regularly performed to detect early-stage invasions (and thus to allow authorities and managers to take rapid eradication or control actions). In Belgium, surveys specifically directed towards alien crayfish are lacking.

Being a freshwater species, *P. clarkii* can be difficult to detect at early stages of invasion. Nonetheless two traits may facilitate its detection: (1) individuals regularly wanders over land during dispersion periods, and (2) burrows are often obvious enough to be easily and rapidly detected.

Examples of effective eradication of *P. clarkii* are very few and limited to small populations at an early stage of invasion. A combination of intense trapping, release of native fish species, and building of barriers around the invaded waterbody seems the most efficient method to control and limit further spread of *P. clarkii* populations. Dewatering small ponds can also be effective locally. Use of chemicals and pathogens to control *P. clarkii* should not be considered because these methods have strong negative impacts on native crayfish and other components of the ecosystem. New control techniques based on the use of sexual pheromones and sterile male release are currently under development and may provide interesting results in the future. Use of chemicals and pathogens to control *P. clarkii* should not be considered because these methods have strong negative impacts on native crayfish and other components of the ecosystem.

Résumé

PROBABILITE D'ETABLISSEMENT ET DE DISSEMINATION (EXPOSITION)

- Introduction en Belgique

Si l'industrie des fruits de mer peut avoir joué un rôle mineur dans les années 1980-1990, les introductions actuelles en Belgique sont principalement le résultat de la libération d'animaux vivants utilisés comme animaux de compagnie par les amateurs d'aquariums. Sur base de la distribution actuelle de *P. clarkii* en France, aux Pays-Bas et de la capacité de dispersion de cette espèce, il est raisonnable de penser que la colonisation naturelle à partir des pays voisins puisse bientôt devenir une voie d'introduction supplémentaire de l'espèce en Belgique.

- Capacité d'établissement

La plupart des plans d'eau douce et des cours d'eau à débit lent du territoire, y compris les zones menacées et sensibles, constituent des zones d'établissement appropriées potentielles de *P. clarkii.* Cette observation est valable dans les conditions climatiques actuelles et dans les conditions annoncées d'une hausse des températures liée aux changements climatiques.

- Capacité de dispersion

La capacité de dispersion de *P. clarkii* est élevée. Cette espèce est capable de se déplacer sur de grandes distances (jusqu'à plusieurs dizaines de kilomètres en quelques jours). La dispersion de cette espèce est fréquemment observée sur la terre ferme et n'est donc pas limitée aux environnements aquatiques. On suspecte aussi une dispersion par les oiseaux. Cette espèce est aussi sujette aux rejets répétés par l'homme dans la nature en raison du fait qu'il s'agit d'une des espèces d'écrevisses les plus populaires en aquariophilie. Aujourd'hui, elle est particulièrement populaire en Europe, y compris en Belgique, où il est facile de la commander sur Internet ou dans les commerces qui vendent des aquariums.

EFFET DE L'ETABLISSEMENT

- Impacts environnementaux

Procambarus clarkii peut avoir un impact sévère sur la biodiversité indigène. Cette espèce peut provoquer des effets indésirables sur les espèces indigènes par compétition pour la nourriture ou l'habitat, par prédation, par broutage et par transmission d'agents pathogènes. Elle peut aussi perturber le fonctionnement de l'écosystème en modifiant les caractéristiques physiques et chimiques de l'eau, la sédimentation et peut également affecter la chaîne alimentaire aquatique. Les écrevisses indigènes sont particulièrement menacées par la présence de *P. clarkii* qui peut aussi avoir des impacts majeurs sur les poissons, les batraciens, les invertébrés, les macrophytes et les algues en raison de son caractère omnivore et de sa plasticité trophique étendue. Après son introduction dans les plans d'eau et cours d'eau, la turbidité de l'eau augmente généralement, les cycles des nutriments et de l'énergie sont souvent largement raccourcis et simplifiés et on peut observer l'extinction locale de certains organismes. *Procambarus clarkii* peut donc être considérée comme une espèce clé qui affecte profondément les fonctions de l'écosystème. Enfin, *P. clarkii* peut aussi accumuler les métaux lourds, les éléments radioactifs et d'autres polluants et a ainsi la capacité de les transmettre à des niveaux trophiques supérieurs.

GESTION DES RISQUES

La principale voie d'introduction actuelle de *P. clarkii* en Belgique est le rejet d'animaux par l'homme dans l'environnement. L'information, l'éducation, la prévention et le contrôle du commerce semblent les mesures de gestion les plus prometteuses pour réduire l'impact négatif des espèces d'écrevisse envahissantes sur les écosystèmes. Heureusement, plusieurs actions d'éducation ont été (et sont) menées au niveau européen et en Belgique.

Un renforcement conjoint de la législation devrait être mis en place en Europe pour assurer une interdiction totale d'importation, de commerce ou de détention d'individus vivants de *P. clarkii* et d'autres espèces d'écrevisses potentiellement envahissantes. En effet, aujourd'hui, la majorité des espèces d'écrevisse qu'on trouve dans les catalogues des aquariophiles et plus particulièrement les espèces en provenance des régions tempérées, démontrent un certain potentiel d'envahissement.

Malheureusement, pour *P. clarkii,* l'interdiction d'importation, de commerce et de détention en Belgique ne suffira pas pour empêcher son introduction et son établissement du fait que (1) l'espèce est déjà présente et établie et que (2) on s'attend à sa dispersion naturelle à partir des pays voisins en raison de la présence de populations denses à proximité des frontières belges. Malgré cela, de telles mesures pourront néanmoins très certainement limiter les rejets secondaires dans l'environnement et ralentir sa dispersion actuelle ainsi que prévenir la colonisation de certaines zones naturelles du pays.

Comme pour les autres espèces aquatiques exotiques, toute introduction et tout transfert de *P. clarkii* dans le cadre de l'aquaculture sont soumis à l'obtention d'un permis conformément au Règlement (CE) n° 708/2007 du Conseil du 11 juin 2007. Cette introduction doit théoriquement être interdite sauf si le demandeur peut apporter la preuve que la probabilité que l'écrevisse ne s'échappe est très faible en raison de mesures de sécurité draconiennes prises au niveau des installations aquacoles (à l'aide d'un système fermé).

Des études nationales doivent être régulièrement effectuées pour détecter les envahissements dès leurs stades précoces (et donc permettre aux autorités et aux responsables de prendre rapidement des mesures d'éradication ou de contrôle). En Belgique, des études spécifiquement axées sur les écrevisses exotiques font défaut.

Etant donné que *P. clarkii* est une espèce d'eau douce, il peut être difficile de la détecter à ses stades précoces d'invasion. Deux éléments peuvent toutefois faciliter sa détection : (1) pendant les périodes de dispersion, des individus sont régulièrement rencontrés sur la terre ferme et (2) les terriers sont souvent faciles à repérer.

Les exemples d'éradication efficace de *P. clarkii* sont très peu nombreux et limités à de petites populations à un stade précoce d'invasion. La méthode la plus efficace pour contrôler et limiter la dispersion des populations de *P. clarkii* semble être la combinaison d'une capture intensive, l'apport d'espèces de poissons indigènes et la construction de barrières autour des plans d'eau envahis. La vidange des petits étangs peut aussi s'avérer efficace localement. L'utilisation de substances chimiques et d'agents pathogènes pour lutter contre *P. clarkii* n'est pas envisageable en raison des impacts négatifs importants de ces méthodes sur les écrevisses indigènes et autres éléments de l'écosystème. De nouvelles techniques de contrôle basées sur l'utilisation de phéromones sexuelles ou la stérilisation des mâles sont actuellement en développement et pourraient donner des résultats intéressants à l'avenir.

Samenvatting

WAARSCHIJNLIJKHEID VAN VESTIGING EN VERSPREIDING (BLOOTSTELLING)

- Introductie in België

Terwijl de schaaldierenindustrie tijdens de jaren 1980-1990 een onbeduidende rol speelde, zijn de huidige introducties in België overwegend een gevolg van levende dieren die door aquariumhouders in de natuur vrijgelaten werden. Uitgaande van de huidige verspreiding van *P. clarkii* in Frankrijk en Nederland, en van het verbreidingsvermogen van de soort, kan redelijkerwijze aangenomen worden dat ook natuurlijke kolonisatie vanuit de buurlanden weldra een bijkomende introductieweg voor de soort in België wordt.

- Vestigingsvermogen

De meeste zoetwaterlichamen en traag stromende waterlopen op het grondgebied, met inbegrip van bedreigde en kwetsbare gebieden, vormen een potentiële vestigingsplaats voor *P. clarkii*. Dit geldt zowel onder de huidige klimaatomstandigheden als bij de voorspelde stijgende temperaturen door de klimaatverandering.

- Verspreidingsvermogen

Procambarus clarkii heeft een hoog verspreidingsvermogen. Ze kan zich over relatief grote afstanden natuurlijk verspreiden (tientallen kilometer op een paar dagen tijd). De soort verbreidt zich bovendien frequent over land, wat betekent dat ze niet afhankelijk is van aan het land aansluitende waterpartijen. Vermoed wordt dat de soort ook door vogels verspreid wordt. Daarnaast wordt deze soort veelvuldig vrijgelaten door de mens, aangezien het een van de meest populaire rivierkreeftsoorten in de schaaldieren-aquariumhandel betreft. De soort is bijzonder populair in Europa, waaronder België, waar levende specimens gemakkelijk op het internet of in aquariacentra aangeboden worden.

EFFECTEN VAN DE VESTIGING

- Milieu-impact

Procambarus clarkii kan de inheemse biodiversiteit ingrijpend beïnvloeden. Ze kan nadelige gevolgen hebben op de inheemse soorten door wedijver voor voedsel of schuilplaatsen, door predatie, herbivorie en door overdracht van pathogenen. Ze kan ook de werking van de ecosystemen aantasten door een wijziging van de fysische en chemische eigenschappen van water en sedimenten, en door watervaleffecten via het voedselweb. Vooral de inheemse rivierkreeft wordt bedreigd maar *P. clarkii* kan ook een grote impact hebben op vis, amfibieën, ongewervelden, macrofyten en algen omwille van zijn trofische plasticiteit en omdat hij een alleseter is. Na introductie neemt de troebelheid van het water doorgaans toe, worden de nutriënten- en energiestromen verregaand beperkt en vereenvoudigd, en kunnen bepaalde organismen lokaal zelfs uitsterven. *Procambarus clarkii* kan bijgevolg worden beschouwd als een ecosysteemingenieur die de ecosysteemfuncties drastisch kan aantasten. Tot slot kan de soort ook zware metalen, radioactieve elementen en andere polluenten opstapelen, die potentieel naar de hogere trofische niveaus worden overgebracht.

RISICOBEHEER

De voornaamste introductieweg van *P. clarkii* in België is momenteel de vrijlating van dieren door de mens. Informatie, educatie, preventie en controle blijken de meest beloftevolle beheersmaatregelen te zijn om de negatieve impact van invasieve exotische rivierkreeften te beperken. Gelukkig werden (en worden) al verschillende educatieacties op Europees niveau en binnen België op het getouw gezet.

Binnen Europa dient een eenvormige en strengere wetgeving te komen om een totaal verbod op de invoer, handel en het bezit van levende *P. clarkii* en andere (potentieel) invasieve rivierkreeftsoorten te verzekeren. Voor de meeste soorten rivierkreeften die momenteel voorkomen in de catalogi van aquariumliefhebbers, en met name voor die soorten afkomstig uit gematigde regio's, bestaat de kans dat ze invasief worden.

Helaas volstaan voor *P. clarkii* een verbod op de invoer, handel en het bezit in België niet om de intrede en de vestiging te voorkomen omdat (1) de soort reeds verspreid is in het land (met name in Vlaanderen), en (2) de natuurlijke verspreiding vanuit buurlanden wordt verwacht aangezien er zich aan de Belgische grenzen populaties ophouden. Toch kunnen dergelijke maatregelen secundaire vrijlatingen in het milieu beperken, de huidige verspreiding vertragen en de kolonisatie van sommige natuurlijke districten in het land voorkomen.

Zoals dit ook voor andere niet-inheemse aquatische soorten geldt, dient in overeenstemming met de Verordening van de Raad (EG) Nr. 708/2007 van 11 juni 2007 voor de introductie en translocatie van *P. clarkii* voor gebruik in aquacultuur een vergunning te worden afgeleverd. In theorie is dergelijke introductie niet toegestaan, tenzij de aanvrager kan aantonen dat de ontsnappingskans van de rivierkreeft door drastische veiligheidsmaatregelen rond de aquacultuurvestiging (afgesloten systeem) bijzonder laag is.

Er dient regelmatig nationaal onderzoek te worden verricht om invasies in een vroeg stadium op te sporen (zodat overheid en beheerders snel tot uitroeiing of bestrijding kunnen overgaan). In België ontbreekt het momenteel aan een monitoringprogramma dat zich specifiek richt op uitheemse rivierkreeftsoorten.

Omdat het een zoetwatersoort betreft, kan *P. clarkii* maar moeilijk in een vroeg invasiestadium opgespoord worden. Toch kunnen twee karakteristieken van de soort de detectie ervan vereenvoudigen: (1) individuen zwerven tijdens de verbreidingsperiodes regelmatig over land, en (2) tunnels zijn vaak voldoende zichtbaar om vlot en snel te worden gedetecteerd.

De zeldzame voorbeelden van een effectieve uitroeiing van *P. clarkii* zijn beperkt tot kleine populaties in een vroeg stadium van invasie. Een combinatie van het intens uitzetten van vallen, het vrijlaten van inheemse vissoorten en het isoleren van waterlichamen waar er zich een invasie voordoet door het bouwen van barrières , blijken de meest doeltreffende methodes om de verdere verspreiding van *P. clarkii* populaties te controleren en te beperken. Ook het dreggen van kleine vijvers kan lokaal doeltreffend zijn. Het gebruik van chemische middelen en pathogenen om de *P. clarkii* onder controle houden, komt niet in aanmerking omdat deze methoden bijzonder negatieve gevolgen kunnen hebben voor de inheemse rivierkreeft en andere biota in het ecosysteem. Momenteel worden nieuwe bestrijdingstechnieken ontwikkeld gebaseerd op geslachtsferomonen en het uitzetten van onvruchtbare mannelijke dieren. Hiervan worden voor de toekomst beloftevolle resultaten verwacht.

STAGE 1: INITIATION

Precise the identity of the invasive organism (scientific name, synonyms and common names in Dutch, English, French and German), its taxonomic position and a short morphological description. Present its distribution and pathways of quarantine concern that should be considered for risk analysis in Belgium. A short morphological description can be added if relevant. Specify also the reason(s) why a risk analysis is needed (the emergency of a new invasive organism in Belgium and neighboring areas, the reporting of higher damages caused by a non native organism in Belgium than in its area of origin, or request made to import a new non-native organism in the Belgium).

1.1 ORGANISM IDENTITY

Scientific name : *Procambarus clarkii* (Girard, 1852)

Synonyms: *Cambarus Clarkii* Girard, 1852;
Cambarus clarkii Faxon 1898;
Procambarus clarkii Hobbs 1942.

Common names : American, Red swamp or Louisiana crayfish/crawfish (English); Ecrevisse de Louisiane, Ecrevisse rouge de Lousiane, Ecrevisse rouge des marais, Ecrevisse rouge d'Amérique (French); Rode rivierkreeft (Dutch); Roter Amerikanischer Sumpfkrebs, Louisiana-Flusskrebs (German); Gambero rosso della Louisian (Italian); Lagostim vermelho (Portuguese); Cangrejo de las marismas, Cangrejo Americano (Spanish).

Taxonomic position: Domain Eukaryota » Kingdom Animalia » Phylum Arthropoda » Subphylum Mandibulata » Superclass Crustacea » Class Malacostraca » Subclass Eumalacostraca » Superorder Eucarida » Order Decapoda » Suborder Pleocyemata » Superfamily Astacoidea » Family Cambaridae » Genus *Procambarus* » Subgenus *Scapulicambarus* » Species *clarkii*
Remark: No subspecies are currently recognized.

1.2 SHORT DESCRIPTION

Diagnosis: Crayfish (Figures 1 and 2) with chelipeds (1) red on both dorsal and ventral faces, (2) armed with a strong spur at the inner side of the carpus and (3) with strong spines and tubercles on the propodus (Numbers refer to Figure 2).

Body description: Crayfish with a rough carapace, particularly behind cervical groove. One pair of post-orbital ridges is present. Cervical spines are present. There is no areola between branchiocardic grooves. The latter converge dorsally. Chelae are elongate, typically S-shaped, red on both surfaces, and covered in spines and tubercles, which are more prominent on the upper side. Two tubercles are present on the inner side of the fixed finger. Rostrum is prominent, triangular with borders tapering to a small, triangular acumen (Souty-Grosset *et al.*, 2006; Gherardi and Panov, 2006; Huner, 2011; ISSG, 2011).

P. clarkii exhibits distinct secondary sexual characteristics once the species has reached maturity. Males have inflated chelae and distinct ischial hooks at the bases of the 2^{nd} and 3^{rd} pairs of walking legs (the 3^{rd} and 4^{th} pairs of pereopods) that are used to hold females during copulation. Male gonopods, the first pair of abdominal appendages (pleopods) modified for sperm transfer, become hardened (Figure 3A). Chelae of females inflate somewhat as well and the sperm receptacle, located between the walking legs, cornifies and develops a species-specific morphology that is receptive only to the species-specific terminal ends of the male gonopods (Figure 3B).

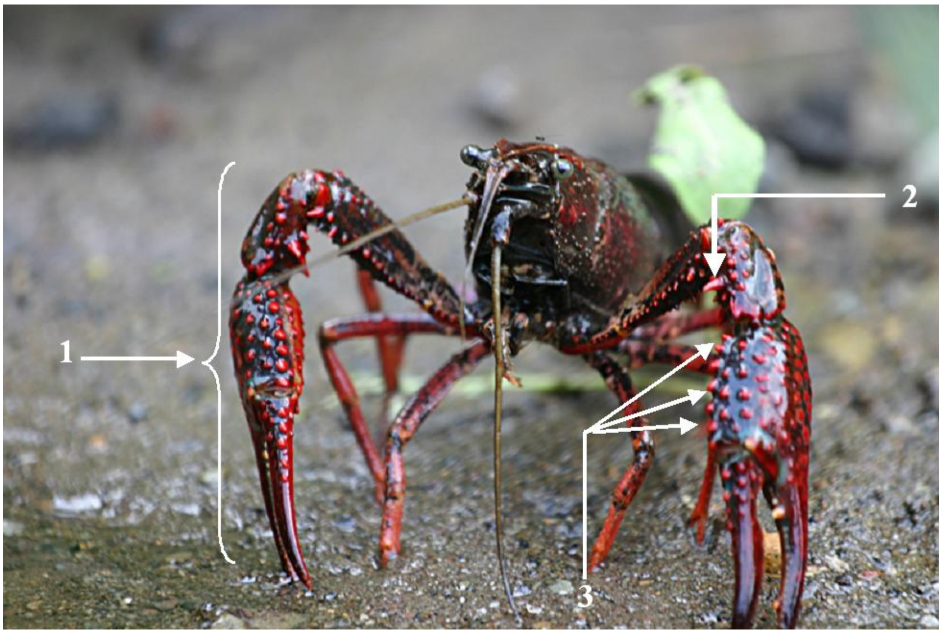

Figure 2: Main morphological criteria used for identifying *Procambarus clarkii* (see paragraph 1.2 -"diagnosis"). Modified from Mike Murphy - Wikipedia.fr.

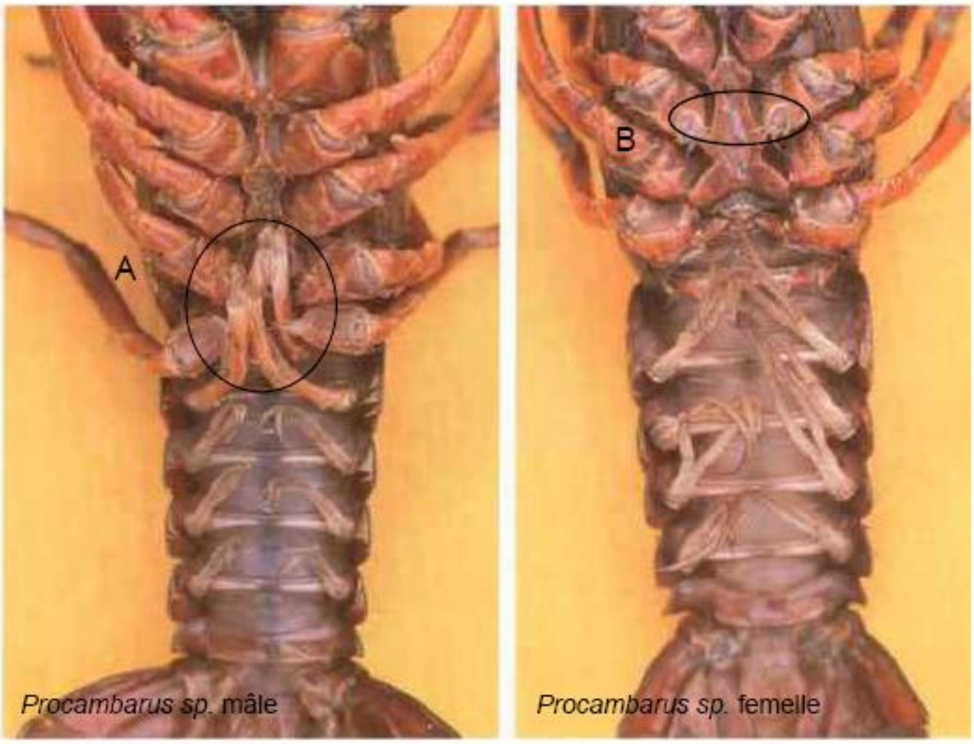

Figure 3: Ventral view of male and female *Procambarus* crayfish, illustrating sexual dimorphism: (A) gonopods; (B) sperm receptacle (annulus ventralis). (Source: Al Kaddissi, 2012).

Body size: Total body length at maturation is about 5.5 to 12 cm, up to 15 cm. Weight at maturation ranges from 5 to 60 g. (*Remark:* size is not a satisfactory criterion for ascertaining the maturation status or age of any crayfish species).

Colour: Adults are usually dark red, orange, or reddish brown but blue, yellow, white and black varieties are known. Coloration intensity is dependent on the habitat, with darker individuals found in clear, acid-stained waters and lighter ones in opaque, muddy waters. Juveniles are mainly greenish-brown (with intensity dictated by water clarity), with a narrow dark band on either side of the abdomen, and a broader, lighter band along the dorsal surface. They appear very similar to other *Procambarus* species (Boets *et al.* 2009). However, red pigment can generally be detected on appendages, especially where walking legs join the body.

Supplementary information: An illustrated key for identification of native and introduced crayfish species in Europe is available in Souty-Grosset *et al.* (2006). Photographs of both juveniles and adults of *Procambarus clarkii* are available in Koese (2008). The latter also offers an illustrated key (in Dutch) for identification of crayfish species present in the Netherlands, which includes all the species currently found in Belgium. The « Maison Wallonne de la Pêche » recently published a booklet with a simplified key for crayfish identification in Belgium. It is available at:
http://www.maisondelapeche.be/telechargements/DepliantEcrevisse_light.pdf.
Another illustrated booklet with information about the five crayfish species currently found in Belgium (4 introduced and 1 native species) and other European crayfish was produced by the "Association Theutoise pour l'Environnement" and is available at: http://www.aspei.be/documentation/EcrevissesBrochure.pdf. Finally, a book about crayfish species in Wallonia is in preparation (Roger Cammaerts, pers. comm.).

1.3 ORGANISM DISTRIBUTION

Native range:
Procambarus clarkii is native to north-eastern Mexico and south-central USA, extending westward to Texas, eastward to Alabama, and northward to Tennessee and Illinois (Figure 4).

Figure 4: Native range of *Procambarus clarkii* (black area) ©Keith A. Crandall *et al.*, 2001; Tree of Life web project.

Introduced range

Belgium:

Procambarus clarkii is not yet widely established in Belgium but is now regularly observed around Brugge (West Vlanderen), and less regularly in Limburg, Oost Vlanderen, Vlaams Brabant and in the Namur region (Figure 5).

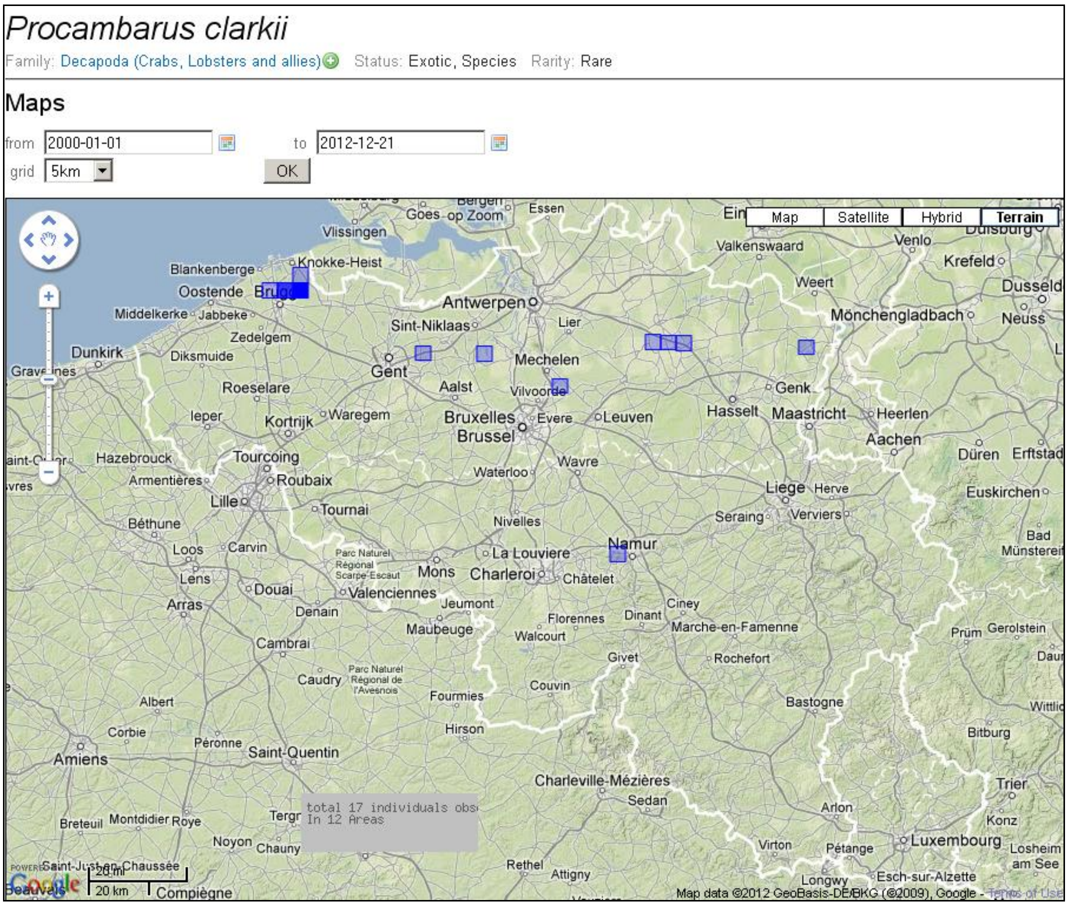

Figure 5: Localization of documented observations of *Procambarus clarkii* in Belgium from Jan. 2001 to Dec. 2012. During this period, 17 records in 12 areas (5 x 5-km squares) were entered in the database of "observations.be" (Aves-Natagora and Stichting Natuurinformatie), 12 of them (71%) were carried out in 2012. Source: observations.be [accessed on 21 December 2012].

Rest of Europe:

From the middle of the 19^{th} century, a disease now commonly known as crayfish plague entered the waters of the Po Valley in Italy and gradually spread throughout Europe, killing off many populations of indigenous crayfish (Holdich 1999, 2003). The causative agent of the crayfish plague is an oomycete species, a fungus-like organism, *Aphanomyces astaci*, indigenous to North America. As crayfish were a valuable commodity in Europe in the 19^{th} century, *Procambarus clarkii* (immune to the disease) was legally introduced into southern Spain in 1973 from Louisiana (USA) to boost European stocks (Souty-Grosset *et al.* 2006; Holdich and Pöckl 2007). Within ten years, *P. clarkii* had become an important commercial species in Spain where it is now the most abundant crayfish. In the 1970s and 1980s, it was illegally introduced throughout Spain, France and Italy. It later colonized other European countries either naturally or through introductions linked to human activities (for live food trade, as aquarium pet, as supplies for science classes, or as live bait used by anglers). As a result, *Procambarus clarkii* is now widely present in Central and Western Europe (Figure 6). It is currently found in Austria, Belgium, England, France, Germany, Italy, the Netherlands, Spain, and Switzerland. It is also present on various islands (Azores, Balearic Islands, Canary Islands, Cyprus, Sardinia and Sicily). Stloukal and Vitázková (2009 in Holdich *et al.*, 2009) mention the fact that in Slovakia *P. clarkii* occurs in garden ponds and presents a high invasion risk. Some of the introductions of *P. clarkii* in European countries originate from Spain, but recent genetic studies suggest that others have come from extra-European sources such as East Africa (Kenya) and the Far East. The species is increasing in many areas (Souty-Grosset *et al.* 2006; Crandall 2010; Holdich *et al.*, 2009; Gherardi 2011).

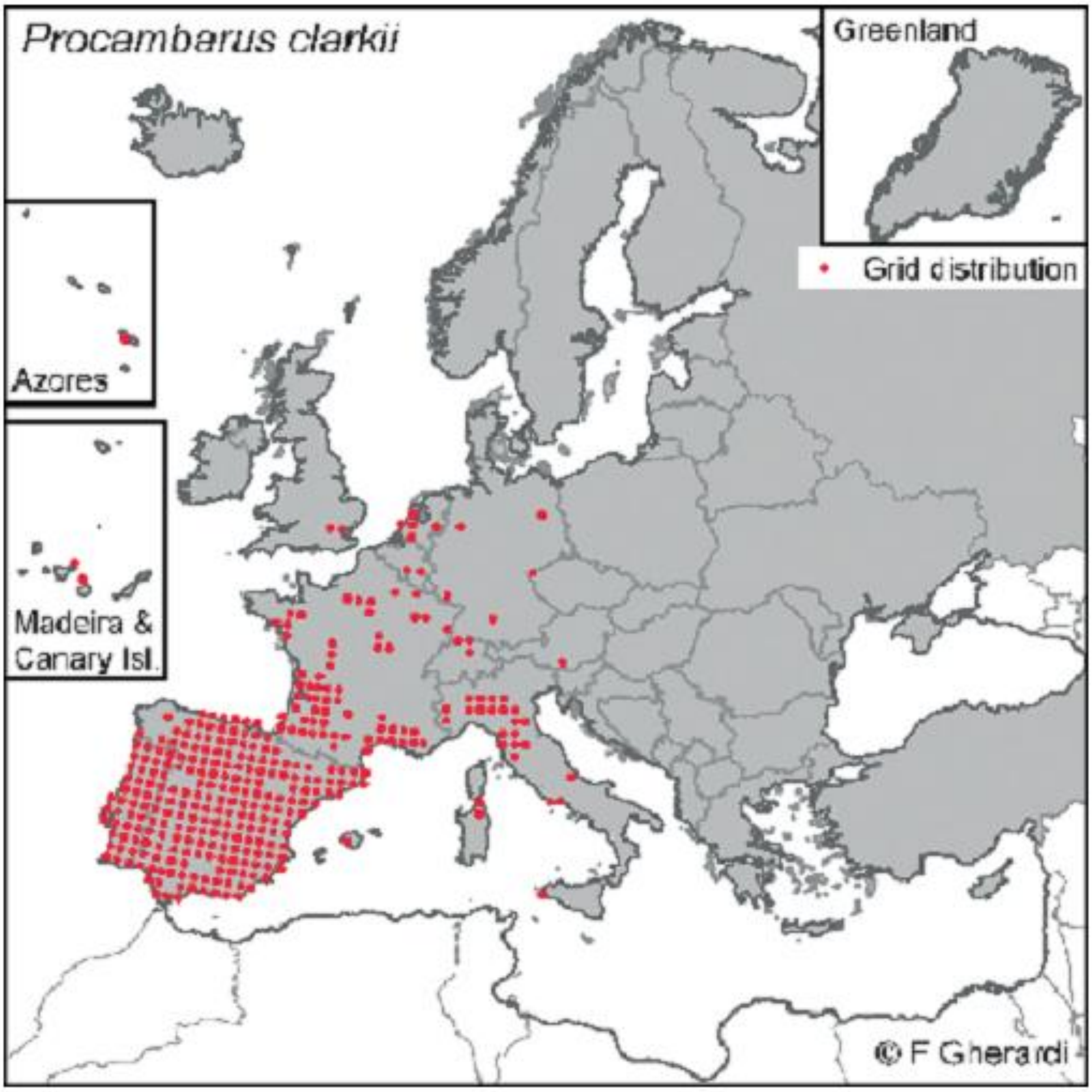

Figure 6: European distribution of *Procambarus clarkii*. Each dot corresponds to a CGRS (Common European Chorological Grid Reference System) square in which the species was recorded. (Source: Gherardi and Panov, 2006).

Other continents:

Procambarus clarkii was massively translocated, mainly for aquaculture purposes, but sometimes for additional reasons. For instance, it was imported to Japan in 1927 as a food for the bullfrog, *Rana catesbeiana*. From the 1960s onward, many releases of the species in Africa were aimed at controlling freshwater snails that host *Schistosoma* spp., the agents of human schistosomiasis. In fact, *Procambarus clarkii* accounts for over 40 percent of all crayfish introduction events recorded (n = 56). As a result, it is currently the most cosmopolitan crayfish, being found in the wild in all continents except Australia and Antarctica (Huner, 1977; Huner and Avault, 1979) (Figure 7). It was widely introduced in the USA (Alabama, Arizona, Arkansas, California, Georgia, Hawaii, Idaho, Indiana, Maryland, Nevada, North Carolina, Ohio, Oregon, South Carolina, Utah, Virginia, West Virginia), in South and Central America (Belize, Brazil, Colombia, Costa Rica, Dominican Republic, Ecuador, Mexico, Venezuela), Asia and Eurasia (China, Georgia, Israel, Japan, Philippines, Taiwan), and Africa (Egypt, Kenya, Uganda, South Africa, Sudan, Zambia) (Huner, 2002; Magalhães *et al.,* 2005; Souty-Grosset *et al.,* 2006; Wizen *et al.*, 2008; Crandall, 2010; Gherardi 2011). Both field and molecular evidence show that the expansion of *P. clarkii* was accelerated by direct or indirect human-induced dispersions across natural barriers (Barbaresi and Gherardi, 2000; Yue *et al.*, 2010).

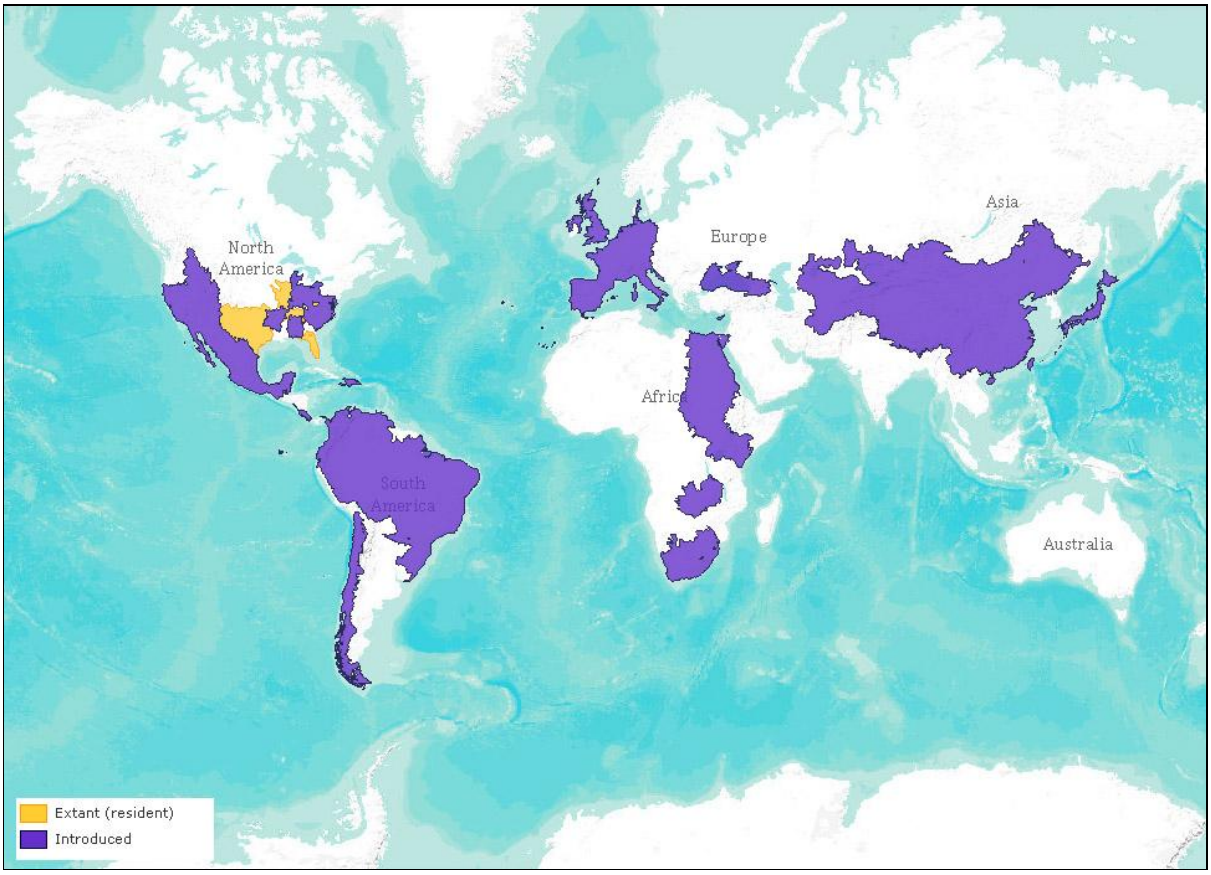

Figure 7: Countries in which *Procambarus clarkii* is currently found in the wild (Crandall, K.A. 2010. *Procambarus clarkii*. In: IUCN 2012. IUCN Red List of Threatened Species. Version 2012.2. <www.iucnredlist.org>. Downloaded on 21 December 2012.)

1.4 REASONS FOR PERFORMING RISK ANALYSIS

Procambarus clarkii has been found to exhibit properties of an r-selected species[2], including early maturity at small body size (Paglianti and Gherardi, 2004; Scalici and Gherardi, 2007), rapid growth rates (Paglianti and Gherardi, 2004), large number of offspring at a given

[2] A species is considered r-selected if it invests more heavily in reproduction than in survival to adulthood. The offspring produced are numerous, mature rapidly and require little or no postnatal care. As a result, individuals grow fast, reproduce quickly and die quickly. The opposite life strategy (i.e. production of less offspring but extensive investment in postnatal care) characterizes K-selected species.

parental size, and relatively short life spans (Lindqvist and Huner, 1999). It is also characterized by a wide plasticity of life cycle (e.g. Gutiérrez-Yurrita *et al.*, 1999) that allows the species to invade diversified environments. *Procambarus clarkii* can indeed persist in habitats and climates far different from those associated with its natural range. For instance, it thrives now in tropical regions such as Ecuador and Uganda and cold temperate regions such as Germany. Its invasive potential is therefore high, being enhanced by its outstanding dispersal capability (e.g. Gherardi and Barbaresi, 2000). The species is also adapted to extreme environments, such as temporary streams (Gherardi *et al.*, 2002a) and polluted habitats (Gherardi *et al.*, 2000). (Hentonnen and Huner, 1999; Holdich *et al.*, 1999; Huner, 2002). *P. clarkii* is listed among the "100 of the worst" invasive species by the Delivering Alien Invasive Species in Europe (DAISIE) project (http://www.europe-aliens.org/speciesTheWorst.do).

The following table summarizes the biological and ecological characteristics of successful invaders shared by *P. clarkii* (-: absent; + low; ++ medium; +++ high; Source: Geiger *et al.*, 2005). (*Remark:* in 2008, Yue *et al.*, discovered four natural clones in *Procambarus clarkii* in China, which indicates that the species is sometimes able to reproduce asexually. This ability increases even more the invasive potential of the species).

Biological characteristics of invaders	*Procambarus clarkii*
High dispersal capability through seeds, eggs or highly mobile larval stages	+
Ability to reproduce both sexually and asexually	–
High fecundity	++
Short generation and juvenile development times	++
Fast adaptation to environmental stress	+++
High tolerance to environmental heterogeneity	+++
Desirability to and association with humans (edibility, game species)	+++
Additional features	
Omnivory	+++
Brood care	+++

The impacts of *P. clarkii* introductions or invasions are characterized in detail in the following references Hobbs *et al.* (1989), Ackefors (1999), Holdich (1999), Holdich *et al.* (1999), Geiger *et al.* (2005), Souty-Grosset *et al.* (2006), and Gherardi, (2007). To sum up:

- **Ecosystem Impact**
  - *P. clarkii* is omnivorous, causing significant destruction of macrophytes and preying heavily on insects and molluscs (Acquistapace *et al.*, 2006), thus reducing resources available for native species, including crayfish, fishes, and waterbirds (Geiger *et al.,* 2005). Because of its aggressiveness and predatory ability, it also directly threats the native fauna (e.g. amphibians, Gherardi *et al.*, 2001; Renai and Gherardi, 2004). Its introduction therefore causes important changes in the structure of food webs, and in some cases has led

to the elimination of some species (Geiger *et al.,* 2005; Rodríguez *et al.,* 2005; Souty-Grosset *et al.*, 2006).

- It is a vector for a number of helminth parasites of vertebrates (Souty-Grosset *et al.*, 2006).

- *P. clarkii* has contributed to the decline of the native European crayfish (Family Astacidae) because it outcompetes them and acts as a vector for the transmission of the crayfish fungus-like plague, *Aphanomyces astaci* (*P. clarkii* is highly resistant to this disease, nevertheless it may die from it under stressful conditions and high spore concentrations (Cerenius and Söderhäll, 1992; Diéguez-Uribeondo and Söderhäll, 1993).

- *P. clarkii* also reduces the value of invaded freshwater habitats by degrading riverbanks because of its burrowing activity, and by increasing water turbidity, with the consequent inhibition of primary production (Rodríguez *et al.*, 2003, 2005) and structural damages to cultivated rice fields (Correia and Ferreira, 1995; Huner, 2002).

- **Health and Social Impact**

  - *P. clarkii* accumulates heavy metals (Gherardi *et al.*, 2002b) and toxins (e.g. microcystin) produced by Cyanobacteria, such as *Microcystis aeruginosa*, and can transfer them to superior trophic levels, including humans (Souty-Grosset *et al.*, 2006).

  - *P. clarkii* is an intermediate host of trematodes of the genus *Paragonimus*, which are potential pathogens of humans and their pets if raw or undercooked crayfish are consumed (Souty-Grosset *et al.*, 2006).

- **Economic Impact**

  - If present in irrigation structures, such as reservoirs or channels, *Procambarus clarkii* may cause significant economic losses. This is due to both its burrowing activity, which alters soil hydrology and causes water leakage, and its feeding habit, which causes damage to plants.

  - Introductions of *P. clarkii* have also had positive economic impacts, mainly linked to the development of major fisheries, for instance in China and Spain, and to a smaller extent in Kenya and California (USA).

STAGE 2 : RISK ASSESSMENT

2.1 PROBABILITY OF ESTABLISHMENT AND SPREAD (EXPOSURE)

Evidence should be available to support the conclusion that the non-native organism could enter, become established in the wild and spread in Belgium and neighbouring areas. An analysis of each associated pathways from its origin to its establishment in Belgium is required. Organisms intentionally imported maybe maintained in a number of intended sites for an indeterminate period. In this specific case, the risk may arise because of the probability to spread and establish in unintended habitats nearby intended introduction sites.

2.1.1 Present status in Belgium

Specify if the species already occurs in Belgium and if it makes self-sustaining populations in the wild (establishment). Give detail about species abundance and distribution within Belgium when establishment is confirmed together with the size of area suitable for further spread within Belgium.

Boets *et al.* (2009) precisely described the first records of *Procambarus clarkii* in Belgium (Figure 8) : "the first specimen of *P. clarkii* was found dead in the reservoir of Vielsalm (Ardennes, alt. 375m) during the first Belgian crayfish distribution survey, made by the Station de Recherches Forestières et Hydrobiologiques of Groenendaal during the years 1983 - 1985. This specimen might have originated from a nearby restaurant. More interesting was the discovery of a living individual in a pond nearby Cerfontaine (Fagne-Famenne, alt. 245m) on 3 September 1996 during a large scale distribution survey of crayfish in Wallonia, funded by a grant from the Ministry of the Walloon Region during the years 1994-1996. More recently, on 2 July 2008, *P. clarkii* was found in the nature reserve Zammelsbroek in Zammel (Kempen, alt. 15m). On 28 January 2009, populations of *P. clarkii* were found there in three ponds situated northeast of the nearby River Grote Nete although the ponds were not directly connected to this river. The ponds were sampled using a handnet with a mesh size of 0.5mm. Without much effort, several individuals including juveniles were caught, indicating that the species is well established and that it reproduces."

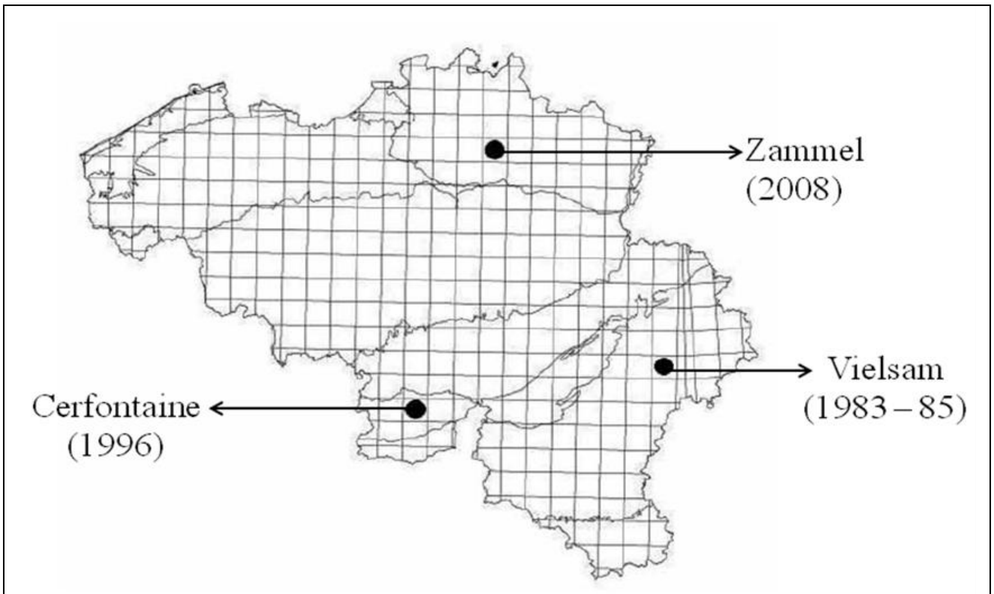

Figure 8 : Historical records of *Procambarus clarkii* in Belgium, on a 10 x 10 km UTM grid (modified from Boets *et al.,* 2009).

Vielsam and Cerfontaine populations went naturally extinct (Roger Cammaerts, pers. comm.) but the species is in expansion in Flanders (Boets *et al.,* 2012) and *Procambarus clarkii* is now regularly observed around Brugge (West Vlanderen), and less regularly in Limburg, Oost

Vlanderen, Vlaams Brabant and in the Namur region (Figure 5), where some individuals were introduced in an oxbow lake ("bras mort") of the Sambre river by anglers ten years ago. The population has now, however, almost entirely disappeared (Roger Cammaerts, comm. pers.). The largest population currently documented in Belgium is present in the Damse Vaart canal (Boets *et al.,* 2012) but appears now, however, to be strongly reduced (Roger Cammaerts, pers. comm.).

Climatic and other environmental conditions (e.g. water quality and elevation) in Belgium fit well with the range tolerated by the species. Due to its high dispersal capability and adaptability to a wide range of environmental conditions, it is highly probable that *P. clarkii* will establish in Belgium and spread throughout the country in the near future if current prevention, control and management actions are not reinforced.

2.1.2 Present status in neighbouring countries

Mention here the status of the non-native organism in the neighbouring countries.

<u>France:</u> Established and spreading.

Procambarus clarkii is reported for the first time in France in 1990, during a national survey aiming at documenting crayfish distribution in the country. However its introduction seems to date back from 1975 (Laurent et al., 1991). In 2006, *P. clarkii* was present in 61 departments (Figure 9) (Collas *et al*., 2007). High densities of the species were found along the Atlantic coast, from the Loire-Atlantique to the Landes department, and in the Mediterranean region (Hérault, Gard, Bouches-du-Rhône). In departments with high densities, it was reported that populations tended to stabilize, except in newly invaded sites, where they were exploding (Collas *et al*., 2007, 2008).

The species continues to extend its range in France. It was found in the Vosges department in 2008, along the upper part of the Meurthe river (Collas *et al*., 2008). In the Ardennes department, it is now present in several rivers, including the Chiers river (CETE de l'EST, 2011). (This river also flows in the Grand Duchy of Luxembourg and in Belgium, and is a tributary to the Meuse river. This therefore represents a potential entrance gate for *P. clarkii* into the Belgian river system). Presence of the species in the Nord department is also suspected (CETE de l'EST, 2011).

The French law now forbids, since 1983, the introduction, transport and sale of live *Procambarus clarkii* specimens ("Arrêté du 21 juillet 1983 [modifié] relatif à la protection des écrevisses autochtones").

<u>The Netherlands:</u> Established and spreading.

In the Netherlands, a total of nine invasive crayfish species, including *P. clarkii*, have been observed in the wild so far (Koese and Evers, 2011). Six of them, including *P. clarkii,* are established (Koese and Evers, 2011). Results of a recent national survey indicate that *Procambarus clarkii* is now the second most abundant and widely distributed species in the Netherlands, after *Orconectes limosus* (commonly called the Spiny-check Crayfish), another introduced species from North America (Koese and Evers, 2011).

The first specimens of *Procambarus clarkii* known in the wild were released by a restaurant owner in 1985, in Den Haag (Soes and van Eekelen, 2006; Soes and Koese, 2010). Other introductions probably followed and helped the species to spread rapidly (Figure 10). It is now regularly reported in a number of ponds and streams, especially in the west of the country, around Amsterdam, Utrecht and Den Haag, but also near the Belgian border (Breda, Tilburg), and from some localities in the east (Koese 2008; Koese and Evers, 2011) (Figure 11). The distribution of *P. clarkii* is closely associated with urban concentrations, reflecting the fact that the species mainly entered in the Netherlands through the consumption and aquarium trades (Soes and Koese, 2010).

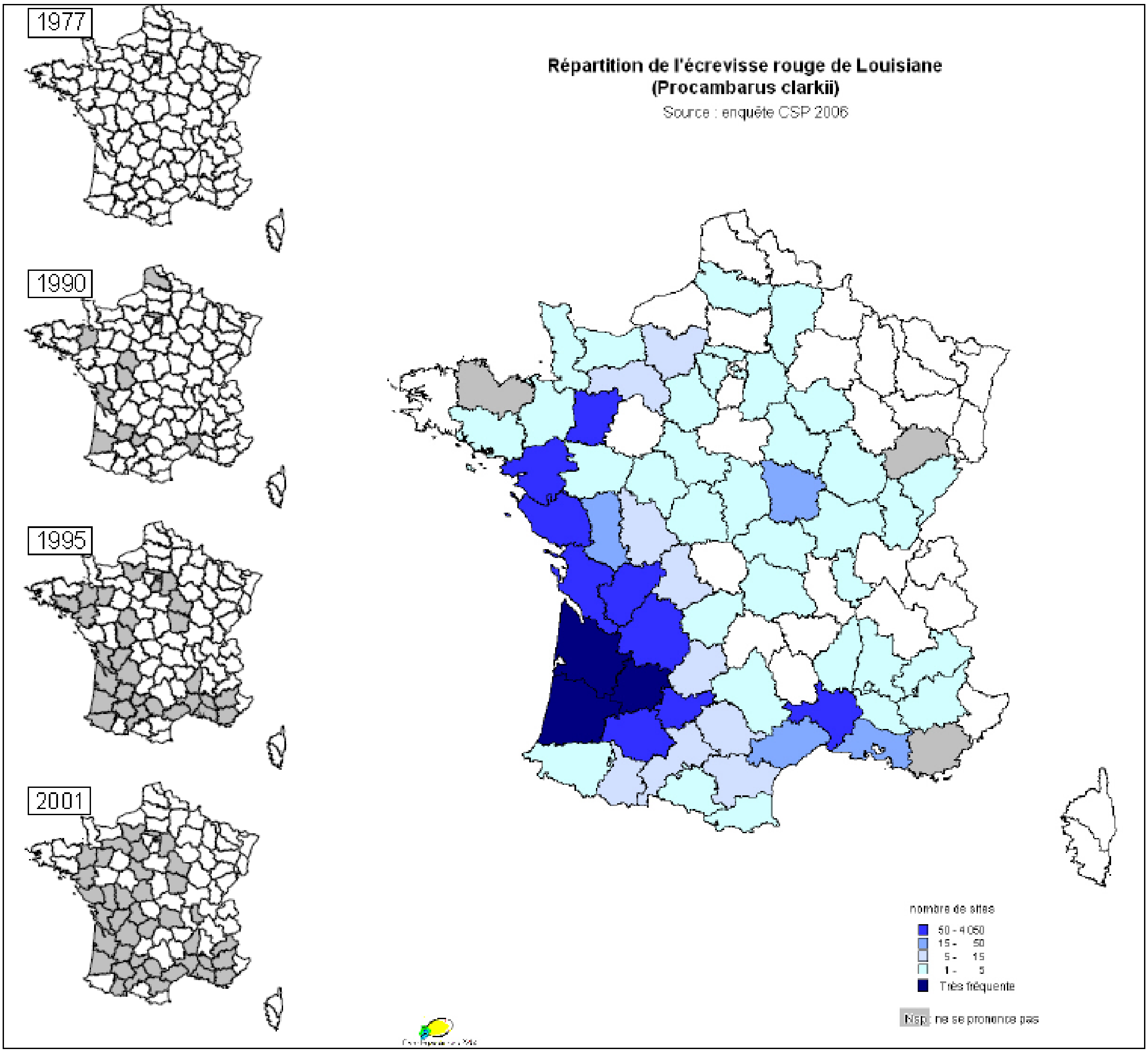

Figure 9: Evolution of the geographical distribution of *Procambarus clarkii* in France. On the 1977 to 2001 maps, the departments where the species was observed is shaded in grey. On the 2006 map, the intensity of the blues shows the abundance of the species inside each department. (Source: Collas *et al.,* 2008).

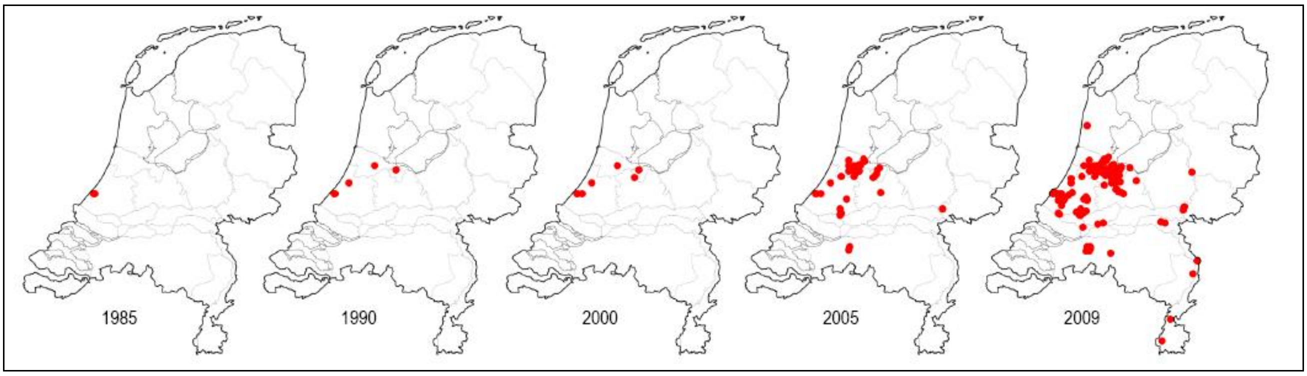

Figure 10: Evolution of the geographical distribution of *Procambarus clarkii* in the Netherlands, from 1985 to 2009. (Source: Soes and Koese, 2010).

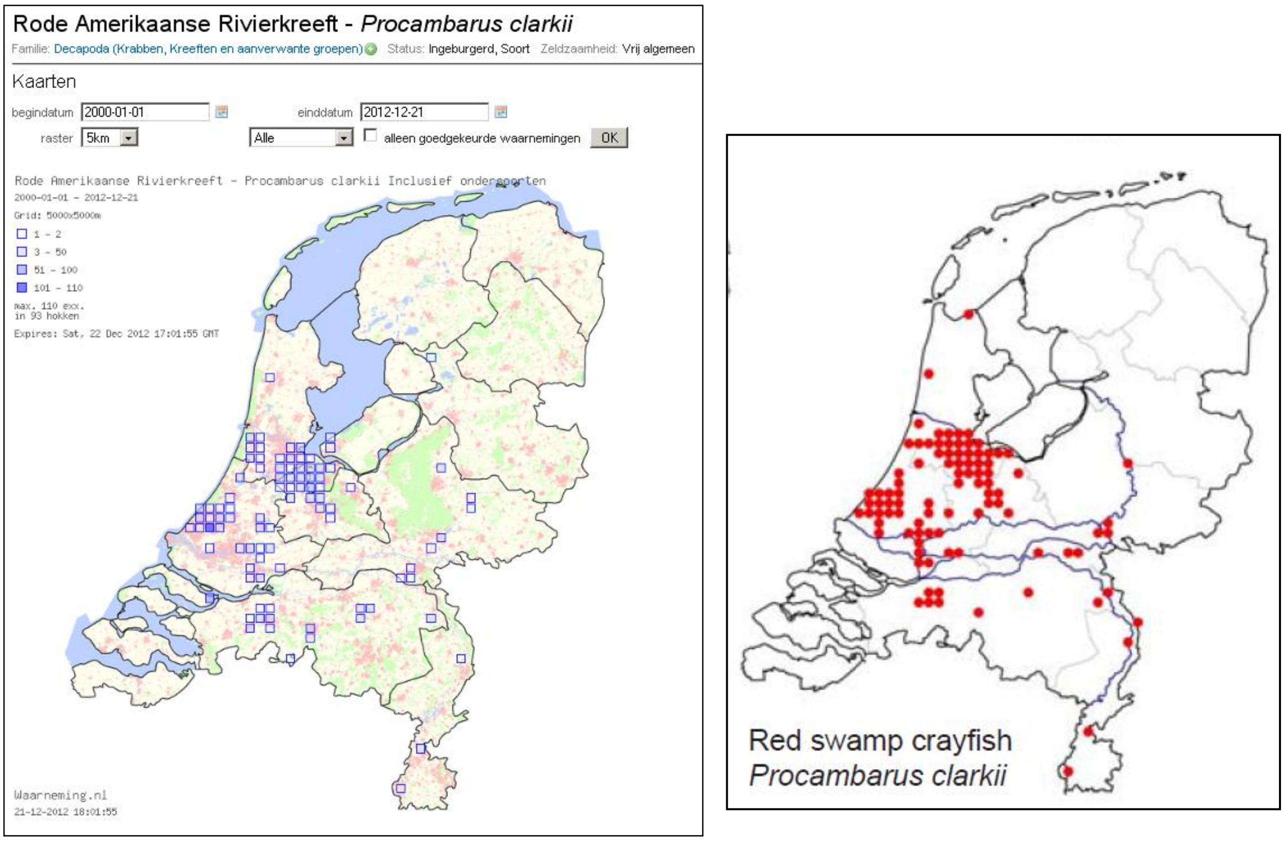

Figure 11: Localization of documented observations of *Procambarus clarkii* in the Netherlands from 2000 till present. Two different sources are presented as each one contain a few localities which are not documented by the second (Source for the map on the left: waarneming.nl; source for the map on the right: Koese & Evers, 2011).

<u>Germany:</u> Established, scattered distribution

Some records are scattered across the country (Figure 6). In some localities, the species is well established and actively spread, such as along the River Danube in Southwestern Germany (Chucholl, 2011a).

<u>Grand Duchy of Luxembourg:</u> Probably absent

To our knoweldge, *P. clarkii* is absent from the Grand Duchy of Luxembourg. For instance, the species is not listed in Holdich *et al*. (2009) for the country and local naturalists consider it absent (Mireille Molitor, [natur&ëmwelt / Fondation Hëllef fir d'Natur] pers. comm.). However, due to the fact that some populations have established themselves in neighbouring countries [for instance in the German Rhineland-Palatinate land, where the species is in expansion (Mireille Molitor, pers. comm.)], it is possible that the species is already present but not yet detected in some ponds or rivers.

<u>United Kingdom:</u> Established, restricted distribution.

Procambarus clarkii was first recorded in Britain in 1991 in the Men's Bathing Pond at Hampstead Heath in North London. In 2000, it was found in Regents Canal, less than 3 km apart from the first site. During surveys carried out between 2008 and 2010 the species was found in four other ponds within Hampstead Heath park (Ellis *et al.*, 2012). The population is growing and the species is expected to spread in nearby catchments. However, its future range could remain small, except if further deliberate or accidental introductions by humans occur (Ellis *et al.,* 2012). In 2010, *P. clarkii* was listed on schedule 9 of the Wildlife and Countryside Act of 1981, which prohibits its release into the wild.

2.1.3 Introduction in Belgium

Specify what are the potential international introduction pathways mediated by human, the frequency of introduction and the number of individuals that are likely to be released in Europe and in Belgium. Consider potential for natural colonisation from neighbouring areas where the species is established and compare with the risk of introduction by the human-mediated pathways. In case of plant or animal species kept in captivity, assess risk for organism escape to the wild (unintended habitats).

Initial introductions of *Procambarus clarkii* in Belgium were probably the result of accidental or deliberate releases in relation with sea food or aquarium trades. Nowadays, the international trade in living specimens for consumption is small but nearly confined to the Red Swamp Crayfish (along with the Narrow-Clawed Crayfish, *Astacus lepdodactylus*) (Soes and Koese, 2010), and thus may still represent an important issue. The trade of live ornamental crayfish has grown rapidly in the last decade and has become the major pathway for non-indigenous crayfish introductions in Europe (Chucholl, 2013), and probably in Belgium as well. *Procambarus clarkii* is one of the most popular species in the aquarium trade due to its attractive colour and its tolerance to a wide range of conditions (Soes and Koese, 2010; Chucholl, 2013). However, it also grows rapidly, and is hence frequently released into the wild when individuals become too large for the owner (Chucholl, 2013).

In the near future, it is highly probable that natural colonisation from neighbouring countries (mainly France and the Netherlands) will occur more and more frequently. The species is rapidly extending its range in these countries and is already present in ponds and streams close to the Belgian border, some of them being directly connected to the Belgian water system through natural catchments or canals. In addition, the Red Swamp Crayfish is hardly limited to connected waters, as it is an active species capable to walk many kilometres overland per night under favourable conditions (Gherardi and Barbaresi, 2000).

ENTRY IN BELGIUM
If the sea food industry may have played a minor role during the 1980s - 1990s, current introductions in Belgium mainly result from the release of live animals used as pets by aquarium owners. However, based on the current distribution of *P. clarkii* in France and the Netherlands, and on the dispersal capacity of the species, it is reasonable to assume that natural colonisation from neighbouring countries will soon become an important pathway of introductions in Belgium.

2.1.4 Establishment capacity and endangered area

Provide a short description of life-history and reproduction traits of the organism that should be compared with those of their closest native relatives (A). Specify which are the optimal and limiting climatic (B), habitat (C) and food (D) requirements for organism survival, growth and reproduction both in its native and introduced ranges. When present in Belgium, specify agents (predators, parasites, diseases, etc.) that are likely to control population development (E). For species absent from Belgium, identify the probability for future establishment (F) and the area most suitable for species establishment (endangered area) (G) depending if climatic, habitat and food conditions found in Belgium are considered as optimal, suboptimal or inadequate for the establishment of a reproductively viable population. The endangered area may be the whole country or part of it where ecological factors favour the establishment of the organism (consider the spatial distribution of preferred habitats). For non-native species already established, mention if they are well adapted to the eco-climatic conditions found in Belgium (F), where they easily form self-sustaining populations, and which areas in Belgium are still available for future colonisation (G).

A/ Life-cycle and reproduction

Souty-Grosset *et al.* (2006) explained: "Under laboratory conditions, the maximum life span of *P. clarkii* is about four years, but it rarely exceeds 12-18 months in nature. *Procambarus clarkii* is a typical r-selected species with a short life cycle and high fecundity. It undergoes cyclic dimorphism with sexually active "FI" [Form I] individuals being present in autumn, winter and spring, and non-breeding "FII" [Form II] individuals present in the summer. At least two generations per year are possible at lower latitudes, particularly in localities with an extended flooding period. *Procambarus clarkii* can reach weights in excess of 50 g in 3-5 months. Size at maturity ranges from less than 45 mm to more than 125 mm (Total Length). Up to 600 eggs may be produced by females of 10 cm (Total Length). Embryonic development is temperature-dependent, taking 2-3 weeks at 22°C and being effectively arrested below 10°C. *Procambarus clarkii* populations may contain individuals that are incubating or carrying juveniles throughout the year, which allows them to reproduce at the first opportunity, thus contributing to their success as colonizers." Besides, high prevalence of multiple paternity was recently demonstrated by genetic analyses in introduced populations in China (Yue *et al.*, 2010).

P. clarkii is a burrowing crayfish, and although mating occurs in open water, the burrow provides protection while the eggs and offspring are attached to the female abdomen. The eggs are fertilized externally with sperm that has been stored in the female seminal receptacle. Although crayfish can survive in a high humid environment within the burrow, standing water is necessary for spawning. Hatchlings remain attached to the female's abdomen through two moults, after which they become free and can forage on their own (FAO, 2012).

In comparison, the Belgian native species, the Noble Crayfish (*Astacus astacus*), is a K-selected species, which may live for more than 20 years. Males normally reproduce once a year but females often remain reproductively inactive in year(s) between reproductive periods (Souty-Grosset *et al.,* 2006).

In the following table, reproductive traits of *Procambarus clarkii,* observed under favourable environmental conditions, are compared with those of European crayfish (i.e. genera *Astacus* and *Autropotamobius*) (Collas *et al.*, 2008). The reproductive superiority of *P. clarkii* is apparent.

	European crayfish species	*Procambarus clarkii*
Body size (and age) at first reproduction	5 cm (2 -4 years)	4 - 5 cm (4 - 6 months)
Number of reproductions per year	1	1 or 2
Number of eggs per female	30 - 50	700 - 800
Incubation time	5 -6 months	2 months

Recently, Yue *et al.* (2008) reported on the occurrence of four natural clones in *Procambarus clarkii* from China, suggesting that parthenogenesis may occur in the species.

B/ Climatic requirements[3]

Procambarus clarkii is usually considered as a "warm water" species (Henttonen and Huner, 1999). Optimal temperatures are 21-27°C and growth inhibition occurs at temperatures below 12 °C (Espina *et al.*, 1993; Ackefors, 1999; Mazlum, 2007 in Ellis *et al.*, 2012). Moreover, the activity of *P. clarkii* is greatly reduced at temperatures below 10°C and the species could hardly move at temperatures below 4°C (Vletter 2008 in Soes and Koese 2010). In laboratory conditions, *P. clarkii* was found to grow faster in warmer water but its survival was higher at lower temperatures, with ten times higher mortality at 18-26°C than at 10°C (Mazlum, 2007 in Ellis *et al.*, 2012).

Procambarus clarkii is in fact an eurythermal species (FAO, 2012). Indeed, the ecological plasticity of *P. clarkii* is high and the species has been able to adapt to a wide range of climatic conditions, from tropical to temperate climates. In particular, the species is able to cope well with new cold habitats by modulating its life history (Chucholl, 2011a). In Germany, for instance, reproduction is univoltine and occurs from late summer to autumn, while a small proportion of females carries eggs throughout the winter. This contrasts with the species' multi-voltine life cycle at lower latitudes, with year-round breeding and several reproduction peaks per year (see paragraph 2.1.4 - A). Growth was estimated to be slower in Germany than at lower latitudes, whereas longevity, mean lifetime and size increased (Chucholl, 2011a).

Moreover, its habit of burrowing (Barbaresi *et al.,* 2004a) aids the crayfish to withstand environmental extremes like freezing temperature (Souty-Grosset *et al.*, 2006).

The potential distribution of *P. clarkii* under different climate change scenarios has been predicted using ecological niche models (ENMs). Results indicate that global increasing temperature would allow *P. clarkii* to shift to higher latitudes in continents of both the northern and southern hemispheres, and to expand in Europe (Liu *et al.,* 2011).

In addition, it has been shown that niche based models (NBM) used to predict *P. clarkii* invasions into new areas strongly underestimate invasive range when they are based on data from native range only (Capinha *et al.*, 2011; *in press*) because a "niche shift" of considerable magnitude from its native range to its invasive one is observed (Larson and Olden 2012).This result means that the realized niche of *P. clarkii* in its native range (i.e. the actual space, with corresponding climatic and other abiotic conditions that *P. clarkii* inhabits

[3] Organism's capacity to establish a self-sustaining population under Atlantic temperate conditions (Cfb Köppen-Geiger climate type) should be considered, with a focus on its potential to survive cold periods during the wintertime (e.g. plant hardiness) and to reproduce taking into account the limited amount of heat available during the summertime.

and the resources it can access as a result of limiting pressures from other species such as predators, parasites and competitors) is greatly inferior to its fundamental niche (i.e. the space and full range of abiotic and biotic conditions and resources in which *P. clarkii* could survive and reproduce if it was free of interference from other species or not limited by physical barriers). As a result, it is not possible to correctly estimate the range of climatic tolerance of *P. clarkii* based on our current level of knowledge, and thus to correctly predict its potential invasive range in Europe. Because populations of the species already occur at higher latitudes and elevations, and in sites presenting harsher climatic conditions than those encountered in Belgium, it is probable that Belgian climatic conditions will not prevent the colonisation and spread of the species throughout the country. This is especially true if introduced specimens would originate from cold-adapted populations.

C/ Habitat preferences[4]

Procambarus clarkii mainly inhabits lentic waters but may be found in a wide variety of freshwater habitats including rivers, lakes, ponds, streams, canals, seasonally flooded swamps and marshes, slow flowing waters, reservoirs, irrigation systems and rice fields (Oliveira and Fabião, 1998; Henttonen and Huner, 1999; Souty-Grosset *et al.*, 2006). It is very tolerant and adaptable to a wide range of aquatic conditions including moderate salinity, low oxygen levels, extreme temperatures, and pollution (Collas *et al.*, 2007; Cruz and Rebelo, 2007; Gherardi and Panov, 2006). It is also well adapted to life in seasonally flooded wetlands, where standing water may be absent for part of the year (Souty-Grosset *et al.*, 2006; Bernardo *et al.*, 2011).

In the cooler regions of Europe, it prefers small, permanent ponds because it is unable to survive predation by fishes in large water bodies (Henttonen and Huner, 1999). Another non-exclusive explanation is that lentic[5] habitats offer more favorable temperature conditions in comparison with lotic habitats (Chucholl, 2011b).

In the Netherlands, *P. clarkii* was mainly found in waters with oxygen concentrations < 5 mg/l, appearing to be more tolerant of oxygen limitations than other crayfish species (Koese and Evers, 2011). However, when dissolved oxygen levels drop to less than 3 mg/l, stress reactions, including climbing to the surface to obtain atmospheric oxygen, have been observed (Souty-Grosset *et al.*, 2006).

Populations have been negatively correlated with high elevation and water flow velocity (Gil-Sánchez and Alba-Tercedor, 2002; Cruz and Rebelo, 2007). In fact, altitude seems to be the main physical barrier to the establishment of new populations in Europe, although the species has been found at 1200 m above sea level in Spain (Souty-Grosset *et al.*, 2006).

In the Netherlands, the minimal pH value where the Red Swamp Crayfish was found was 5.9, but the species is still expanding its range and it is not possible to determine whether the absence of the species in waters with lower pH value is caused by ecological constraints of an unfinished expansion (Koese and Evers, 2011). Nevertheless, Soes and Koese (2010) consider that *P. clarkii* cannot established viable populations in fast-flowing streams and in water bodies with a pH value lower than 5.5. In this direction, Yue *et al.* (2009) found an optimum pH value of 7.8 for survival and growth of the species in aquaculture in China.

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[4] Including host plant, soil conditions and other abiotic factors where appropriate.

[5] Lentic refers to standing or relatively still water habitats as lakes or ponds, whereas lotic refers to flowing water ones (e.g. rivers, streams).

D/ Food habits[6]

Table: Diet of *Procambarus clarkii* in the River Guadiana (Spain), in % of total gut contents (from Pérez-Bote, 2005).

	%F	%N	%V	RI
a. General composition				
Plants	73.24	56.80	52.58	50.34
Animals	58.12	24.92	30.33	31.25
Amorphous material	18.21	13.12	12.97	12.21
Sand	13.21	5.16	4.12	6.20
b. Animal components				
Fish	35.07	5.30	11.82	11.40
Crayfish	29.20	17.23	27.32	14.14
Mollusca	16.55	5.95	1.91	5.33
Diptera	73.24	47.14	38.01	34.59
Ephemeroptera	42.82	13.70	12.80	16.10
Coleoptera	25.12	5.30	4.64	7.66
Hemiptera	21.20	2.28	1.30	5.41
Odonata	14.76	3.10	2.20	4.38

%F, frequency of occurrence; %N, percentage number; %V, percentage volume; RI, relative importance index.

Procambarus clarkii is polytrophic (Gutiérrez-Yurrita *et al.*, 1998). For instance, the analysis of the gut contents of 1225 *P. clarkii* individuals from the Guadiana River (Spain) showed a diet dominated by plants and animals, followed by amorphous material and sand (Pérez-Bote, 2005; Table on the right). The animal portion of the diet was dominated by insects (mainly dipterans and ephemeropterans), crayfish, and fish (Pérez-Bote, 2005). Similar analyses from other populations of *P. clarkii* in Europe and elsewhere have also demonstrated the generalist diet of *P. clarkii*, although the identity of dominant food items may vary (Correia, 2002, 2003; Smart *et al.*, 2002).

P. clarkii displays a wide plasticity in its feeding behaviour and modifies is diet according to the seasons (Correia 2002; Pérez-Bote, 2005; Gherardi and Barbaresi, 2007), and life stages. Adult individuals mainly feed on plants and detritus, while juveniles consume a higher proportion of animal food (Correia, 2003; Geiger *et al.*, 2005; Pérez-Bote, 2005). Diet differences among sexes are not significant (Correia, 2003; Pérez-Bote, 2005). The species is also sometimes cannibalistic (Correia, 2003; Souty-Grosset *et al.*, 2006).

For plants, *P. clarkii* seems to base its feeding decisions on plant availability and on multiple plant traits, such as morphology, structure, chemical defences, and nutritive value. Often, it feeds upon plants whose finely branches or filamentous morphologies make them easier to handle and to consume, and not because of their nutritional value (Gherardi and Barbaresi, 2007; Gherardi, 2007).

A singular observation in Africa (Lake Naivasha, Kenya) highlights the opportunistic trophic behaviour of *P. clarkii* (Grey and Jackson, 2012). After the crash of submerged macrophytes and associated macroinvertebrates in the lake, and during a natural drawdown of the water level, crayfish take advantage of the footprints of hippopotamus to extend their range during the night beyond the lake up to 40 m into the riparian zone, and to consume living terrestrial plants in the vicinity of the footprints. This study was the first one to demonstrate direct use of terrestrial resources by an aquatic crayfish species.

E/ Control agents

Large birds (e.g. cormorants, crows, herons, storks, some hawks and even owls), predaceous fishes (e.g. eels, perches), carnivorous mammals (e.g. otters, minks, raccoons), turtles, snakes, and alligators may predate on *P. clarkii*. Large invertebrate predators (e.g. dytiscid beetles, aquatic hemipteran bugs, dragonfly naiads, and fishing spiders) may

[6] For animal species only.

consume juveniles (Hobbs, 1993; Correia, 2001; Gherardi and Panov, 2006; Souty-Grosset *et al.*, 2006).

Procambarus clarkii is usually resitant to the crayfish plague caused by the oomycetes *Aphanomyces astaci* (Saprolegniaceae family) but it can die from it under stressful conditions and high spore concentrations (Souty-Grosset *et al.*, 2006). Another Saprolegniaceae, *Saprolegnia parasitica*, has been experimentally found to be infective and lethal for *P. clarkii* (Diéguez-Uribeondo *et al.* 1994).

Psorospermium spp. is a crayfish parasite (belonging to a clade close to the animal-fungus dichotomy) frequently encountered in the abdominal musculature of *P. clarkii ;* its pathogenicity is, however, unclear (Henttonen *et al.*, 1992, 1994, 1997; Souty-Grosset *et al.*, 2006). *Procambarus clarkii* is also susceptible to "burn spot" disease, a fungal infection (Souty-Grosset *et al.*, 2006) and to "white spot" syndrome, a viral infection (Du *et al.*, 2007, 2008; Baumgartner *et al.,* 2009).

Many epibiontic protozoans are found on the gills of *P. clarkii* but are shed at each moult. However, during periods of hard environmental conditions and during the reproductive period, the state of moulting slows down and ectocommensal protozoans may reach harmful levels and lead to mortalities as they reduce the amount of oxygen that can be obtained by the crayfish (Souty-Grosset *et al.*, 2006). Entocytherid ostracods and copepods may also be found living ectocommensally in the gill chamber, but their impact on the ecology of *P. clarkii* remain to be studied (Souty-Grosset *et al.*, 2006; Aguilar-Alberola *et al.*, 2012).

Branchiobdellidans are leech-like obligate ectosymbionts, which may occur on the exoskeletal surface of crayfish species and clean it from diatoms and detritus. In Europe *Branchiobdella parasita, B. italica* and *Cambarincola mesochoreus* have been found on *P. clarkii*. Whether Branchiobdellidans may sometimes negatively impact their host is unclear (Souty-Grosset *et al.*, 2006).

Exhaustive reviews of crayfish diseases and commensal organisms, including those of *P. clarkii,* were carried out by Edgerton *et al.* (2002) and Longshaw (2011).

Based on the literature, it appears that predators or pathogens are rarely able to control establishment and expansion of *P. clarkii* populations in its invasive range (e.g. Neveu, 2001; Gherardi *et al.*, 2011b).

F/ Establishment capacity in Belgium

The life history traits of *P. clarkii*, its opportunistic and generalist trophic behaviour, its ecological plasticity, its tolerance to a wide range of environmental conditions (including temperature, pH, salinity, oxygen level and pollution), and its dispersal capacity contribute to explain its worldwide invasive success, and make the species highly susceptible to establish dense populations in potentially all freshwater habitats in Belgium.

G/ Endangered areas in Belgium

Most freshwater bodies and, to a lesser extent, streams of the territory, including endangered and sensitive areas, are potentially suitable to *P. clarkii* establishment. This observation is true under current climatic conditions, and also under predicted increasing temperatures due to climate change.

Establishment capacity in the Belgian geographic districts:

Districts in Belgium	***Environmental conditions for species establishment[7] under current climatic conditions***	***Environmental conditions for species establishment[8] under increasing temperatures due to climate change***
Maritime	Optimal	Optimal
Flandrian	Optimal	Optimal
Brabant	Optimal	Optimal
Kempen	Optimal	Optimal
Meuse	Optimal	Optimal
Ardenne	Optimal	Optimal
Lorraine	Optimal	Optimal

Note: This table does not mean that, at local scale, every water body and stream would present optimal conditions for the species. For instance, it is probable that *P. clarkii* cannot established viable populations in acid or fast-flowing water habitats (Soes and Koese, 2010).

ESTABLISHMENT CAPACITY AND ENDANGERED AREAS IN BELGIUM
Most freshwater bodies and streams of the territory, including endangered and sensitive areas, are potentially suitable to *P. clarkii* establishment. This observation is true under current climatic conditions, and also under predicted increasing temperatures due to climate change.

[7] For each district, choose one of the following options : optimal, suboptimal or inadequate.

[8] For each district, choose one of the following options : optimal, suboptimal or inadequate.

2.1.5 Dispersion capacity

Specify what is the rate of dispersal once the species is released or disperses into a new area. When available, data on mean expansion rate in introduced territories can be specified. For natural dispersion, provide information about frequency and range of long-distance movements (i.e. species capacity to colonise remote areas) and potential barriers for spread, both in native and in introduced areas, and specify if the species is considered as rather sedentary or mobile. For human-assisted dispersion, specify the likelihood and the frequency of intentional and accidental movements, considering especially the transport to areas from which the species may easily colonise unintended habitats with a high conservation value.

A/ Natural spread

P. clarkii displays stationary phases (during which the species maintains an "ephemeral home range" (*sensu* Robinson *et al.,* 2000)) interposed with nomadic bursts of movement (Gherardi *et al.,* 2000; Barbaresi *et al*., 2004b). It is probable that dispersion phases are density-dependent (Barbaresi *et al*., 2004b, Ellis *et al*., 2012) and/or are under control of various abiotic factors such as water level (for instance, linked to the amount of drainage), temperature, relative humidity and the period of the day (Ramalho and Anastácio, 2012). It was shown that both sexes disperse (Barbaresi *et al*., 2004b).

In the Maçãs river, in Portugal, Bernardo *et al.* (2011) documented an upstream progression *of P. clarkii* ranging from 0 to 3,1 km/yr. In Regents Canal, a mean rate of spread of at least 0,5 km/yr was estimated (Ellis *et al*., 2012). The species is however capable of natural dispersion over relatively longer distances (Gherardi and Barbaresi, 2000; Siesa *et al*., 2011). For instance, distance of 3 km can be covered per night, occasionally overland, during invasive periods (Souty-Grosset *et al*., 2006). A radio-tracked specimen even moved almost 17 km within 4 days in a rice field (Gherardi and Barbaresi, 2000). Because the species can walk overland, it is hardly limited to connected waters for expanding its range. The natural spread of invasive crayfish is however mainly directed downstream (Kerby *et al*., 2005; Bernardo *et al*., 2011). In addition, results of a recent set of experiments suggest that juveniles of *P. clarkii* may be passively transported by birds over potentially long distances, e.g. 150 km (Anástacio *et al.,* 2012).

B/ Human assistance

Genetic studies, using amplification polymorphic DNA (RAPD) markers (Barbaresi *et al.,* 2003), or CO1 mtDNA sequences and microsatellite loci (Barbaresi *et al*., 2007), have revealed high levels of genetic variability in European populations of *P. clarkii*, suggesting multiple introductions of individuals coming from different source locations. ***Procambarus clarkii* appears therefore particularly subject to repeated releases by humans.** Similar results were obtained from Chinese populations (Li *et al*., 2012).

- The **aquarium trade** is the most likely source of crayfish species introductions into Europe (Textbox 1) (Peay, 2009; Soes and Koese, 2010; Chucholl 2013). It is especially true for *P. clarkii* since it is one of the most popular crayfish in the aquarium trade worldwide (Soes and Koese, 2010). In the Netherlands, *P. clarkii* is by far the most available crayfish at Internet sales points (Figure 12). In Belgium, the species is also easily found in aquarist catalogues on the Internet (Figure 13). The problem arises when owners of *P. clarkii* specimens discard them into the freshwater environment when they grow too big, or when individuals escape. New introductions can therefore be expected everywhere, especially around urban areas.

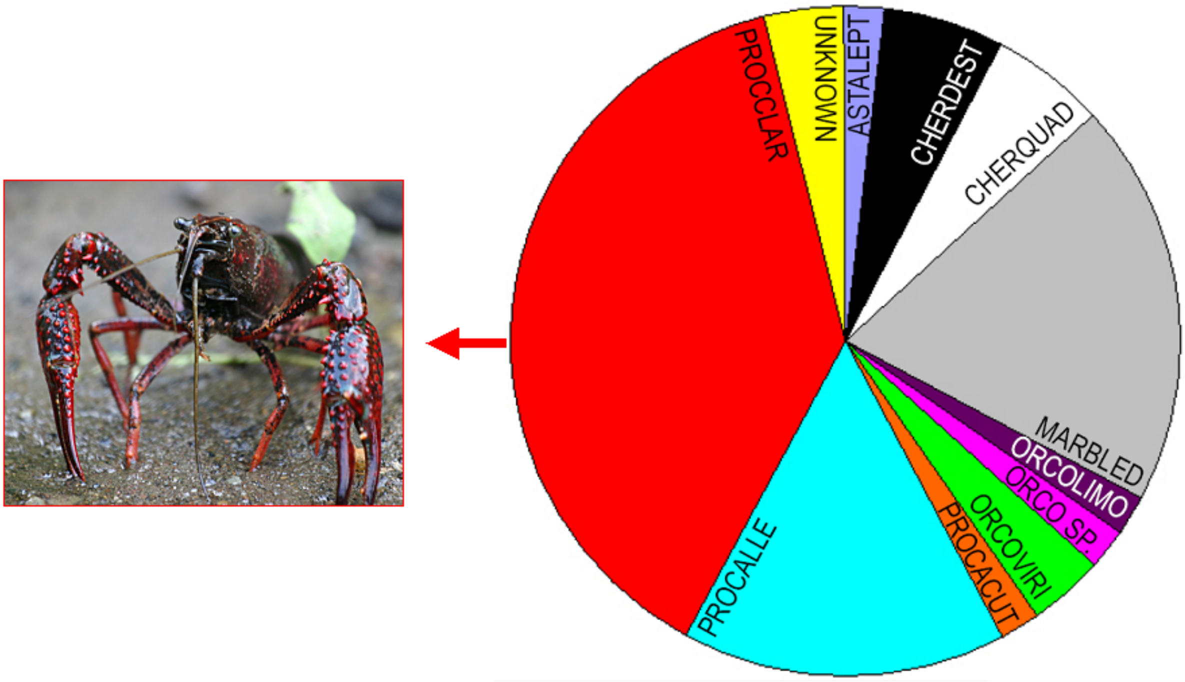

Figure 12: Relative availability of *Procambarus clarkii* and other exotic crayfish species at Internet sales points in 2010 in the Netherlands (*n* = 54). *P. clarkii* (PROCCLAR code) is by far the most traded species. Other species documented are *Astacus leptodactylus* (ASTALEPT), *Cherax destructor* (CHERDEST), *Cherax quadricarinatus* (CHERQUAD), the parthenogenetic form of *Procambarus fallax* (MARBLED), *Orconectes limosus* (ORCOLIMO), *Orconectes* sp. (ORCO SP.), *Orconectes virilis* (ORCOVIRI), *Procambarus acutus* (PROCACUT), *Procambarus alleni* (PROCALLE) (modified from Soes and Koese, 2010. Crayfish photograph from Mike Murphy - Wikipedia.fr).

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Code	Genus	Species	Common Name	Size
9028	Procambarus	clarkii red	Red swamp Crayfish	4 - 5 cm
9029	Procambarus	clarkii red	Red swamp Crayfish	> 6 cm
9130	Procambarus	falax		4 - 5 cm
9026	Procambarus	troglodites blue	Blue Crayfish	4 - 5 cm
9027	Procambarus	troglodites blue	Blue Crayfish	> 6 cm

Figure 13: Extract of an on-line catalogue from a Belgian aquarist with available living *Procambarus clarkii* individuals, illustrating the ease to find the species in Belgium.

Textbox 1 : Pet crayfish and introduction of invasive species in Europe.

The market for ornamental crayfish as pet species has grown rapidly in the last decade. New species have been imported at a very high rate, and keeping crayfish is now widespread in Europe. The 'exotic invertebrates boom' began in the late 1990s, when only a small number of species were available, chiefly *Procambarus clarkii* and the Australian species *Cherax destructor* and *Cherax quadricarinatus* imported for commercial food sales (Chucholl, 2013). By 2005, a total of 74 non-indigenous crayfish species had been imported to Central Europe and further imports of new species were likely to occur (Pekny and Lukhaup, 2005 in Chucholl, 2013). Along with tropical species, many species of crayfish from the Americas and Australia are advertised for sale on the Internet and in some aquarist catalogues in Europe, e.g. *Procambarus alleni, P. clarkii, P. sp., P. spiculifer, P. toltecae, Orconectes durelli, O. luteus, Cambarus coosae, C. manningi, C. rusticiformis, C. speciosus, Cambarellus chapalanus, C. montezumae, C. patzcuarensis, C. puer, C. shufeldtii, Cherax destructor, C.

holthuisi, C. lorentzi, C. quadricarinatus, Cherax spp. of various colour varieties, and *Samastacus spinifrons* (Holdich *et al.*, 2009). **The potential exists for any of these species to be released into the inland waters of Europe** (Holdich *et al.*, 2009). **Although the present Risk Assessment focuses on *Procambarus clarkii*, it is therefore very important to keep in mind that the issue of additional crayfish species introduction in Europe (including Belgium) through the aquarium trade is not at all restricted to this species alone!**

- *P. clarkii* is generally considered to be a delicacy. It is hence one of the most popular crayfish in the **sea food trade** worldwide (Soes and Koese, 2010). As for the aquarium trade, accidental or voluntarily releases may occur. For instance, restaurant owners or crayfish anglers may release crayfish in freshwater habitats to expand or facilitate its exploitation. In France, Collas *et al.* (2007) noted:

"L'activité halieutique autour de cette espèce peut être qualifiée d'active, elle génère dans de nombreux départements où l'espèce est abondante, un véritable engouement. Cette ressource fait en effet l'objet d'un prélèvement à la fois par les pêcheurs amateurs, mais aussi par les pêcheurs professionnels qui la commercialisent en « mort-frais » sur le Lac de Grand Lieu (BRAMARD C., com. pers.). Cette activité peut être considérée comme un vecteur de dispersion de l'espèce (Charente-Maritime). BRAMARD M. (com.pers.) signale également que les vacanciers (…) n'hésitent pas à rapporter chez eux cette espèce et à l'introduire dans des mares, bassins… d'où elles s'échappent rapidement pour gagner les eaux libres."

- **Other minor pathways** of introductions have been reported. For instance, *P. clarkii* may be introduced unintentionally as unused bait by anglers (Hobbs *et al.*, 1989) or may have escaped from science classes or research laboratories, as the species is widely used as biological model, mainly in physiological and ecotoxological studies (Al Kaddissi, 2012). Besides, Soes and Koese (2010), reported from the Netherlands the occasional dispersion of *Procambarus clarkii* with sediment after dredging. They wrote :

"Several specimens were crawling in a sediment deposit after a discharge of mud. The individuals were collected by the employees and released alive in a nearby lake (Klinkenbergerplas, Oegstgeest) in spring 2008".

DISPERSAL CAPACITY

Dispersal capacity of *Procambarus clarkii* is high. It is capable of natural dispersion over relatively long distances (up to several tens of kilometres in a few days). The species frequently disperses overland and it is hence not limited to connected waters for spreading. Dispersion by birds is also suspected to occur. In addition, it is particularly subjected to repeated release by humans because it is one of the most popular crayfish species in the sea food and aquarium trades. The latter is nowadays particularly popular in Europe, including Belgium, where living specimens are easily found on the Internet or in aquarist centres.

2.2 EFFECTS OF ESTABLISHMENT

Consider the potential of the non-native organism to cause direct and indirect environmental, economic and social damages as a result of establishment. Information should be obtained from areas where the pest occurs naturally or has been introduced, preferably within Belgium and neighbouring areas or in other areas with similar eco-climatic conditions. Compare this information with the situation in the risk analysis area. Invasion histories concerning comparable organisms can usefully be considered. The magnitude of those effects should be also compared with those caused by their closest native relatives.

2.2.1 Environmental impacts

Specify if competition, predation (or herbivory), pathogen pollution and genetic effects is likely to cause a strong, widespread and persistent decline of the populations of native species and if those mechanisms are likely to affect common or threatened species. Document also the effects (intensity, frequency and persistency) the non-native species may have on habitat peculiarities and ecosystem functions, including physical modification of the habitat, change to nutrient cycling and availability, alteration of natural successions and disruption of trophic and mutualistic interactions. Specify what kind of ecosystems are especially at risk.

A/ Competition **[HIGH]**

Procambarus clarkii contributes to the decline of indigenous European crayfish due to its strong competitive pressure, both for food and for shelters (Barbaresi *et al.*, 2007; Gherardi, 2007). When exposed in the laboratory to *P. clarkii*, indigenous crayfish changed their posture and behaviour, quickly assuming the role of subordinates. When, however, stable dominance hierarchies between *P. clarkii* and indigenous crayfish species cannot be formed, as observed between *P. clarkii* and *Austropotamobius pallipes*, the repeated fights may lead to injuries usually suffered by the weaker indigenous crayfish, followed by its likely death (Gherardi and Cioni, 2004; Gherardi, 2007).

Only one native crayfish species, *Astacus astacus,* is present in Belgian freshwaters (Textbox 2). To our knowledge, interactions between *A. astacus* and *P. clarkii* was never studied but, based on the biology of the two species, interspecific competition is very likely to occur. For the moment, the range of the two species is disconnected : *P. clarkii* is mainly found in Flanders and remaining populations of *A. astacus* only occur in Wallonia (Boets *et al.*, 2012). The two species thus do not compete for resources so far. As *A. astacus* is a threatened species, in need of special conservation actions, it is essential to avoid further spread of *P. clarkii*.

Few studies have analyzed the resource competition effects induced by *P. clarkii* on non-crayfish species. However, its ability to outcompete some fish species, for instance by expelling individuals from their shelters and therefore by making them more vulnerable to piscivorous fish species, may have detrimental effects (Gherardi, 2007). Moreover, because *P. clarkii* is polytrophic (feeding on detritus, as well as plants, insects and other animals [see paragraph 2.1.4.D]), it is potentially in competition for food with animals belonging to a wide range of feeding guilds and trophic levels (Geiger *et al.*, 2005).

<u>Textbox 2</u>: *Astacus astacus:* the single native but threatened species of crayfish in Belgium.

Along with *Procambarus clarkii*, four crayfish species occur in Belgium. Only one is native, the Noble Crayfish, *Astacus astacus* (also called "Ecrevisse à pieds rouges", "Rivierkreeft", and "Edelkrebs", in French, Dutch and German, respectively). Over the last decade, the Noble Crayfish has disappeared from approximately 20% of the sites from which it was previously known (Edsman *et al.*, 2010). Arrignon *et al.* (1999) already reported a 67% reduction in this species between 1989 and 1999. *Astacus astacus* is now extirpated from Flanders, but can still be found in *ca.* 40-50 water bodies in Wallonia (Edsman *et al.*, 2010;

Boets *et al.*, 2012; ASPEI website: http://www.aspei.be/). Its decrease in Europe, including Belgium, is due to a generalized degradation of water and habitat quality, the impact of invasive crayfish and its susceptibility to the crayfish plague (Souty-Grosset *et al.*, 2006). This species is assessed as Vulnerable by the IUCN Red List Index of Threatened Species (Edsman *et al.*, 2010), is listed in Annex V of the EU Habitats Directive (i.e. its exploitation and harvesting can be restricted by European law). Despite its endangered status and its need of conservation, it remains possible to harvest the species in several European countries, including Belgium (see: http://www.maisondelapeche.be/telechargements/DepliantEcrevisse_light.pdf). It is indeed argued that "*its exploitation is a prerequisite for its conservation. (...) If local people are allowed to catch and benefit from this noble crayfish, this is the best protection against illegal stocking of lower-value non-indigenous* [and thus also against spread of crayfish plague]" (Souty-Grosset *et al.*, 2006). However, catching regulations on season and minimum size exist in Belgium, and anglers are advised to favour the release of caught animals. The "Association pour la Sauvegarde et la Promotion des Ecrevisses Indigènes" (ASPEI; http://www.aspei.be/) exists in Belgium since November 2008 and aims at promoting the conservation of *Astacus astacus*, through reintroductions and public education.

B/ Predation/herbivory **[HIGH]**

- Predation on fishes: Although incidental predation might be expected (especially in ephemeral shallow pools where prey faces vulnerable confined conditions [Ilhéu *et al.*, 2007]) *P. clarkii* is not believed to be important predator of adult fishes. They are too slow in their responses and healthy fishes will in general be able to escape. *P. clarkii* does however predate on fish eggs and larvae (Nyström, 2002; Ilhéu *et al.*, 2007; Soes and Koese, 2010). Moreover, the destruction of macrophyte beds by *P. clarkii* indirectly affects fish populations.

- Predation on amphibians: *P. clarkii* predates on eggs, tadpoles, juveniles and even sometimes adults of most amphibian species (Gherardi *et al.*, 2001; Ficetola *et al.*, 2011, 2012; Nunes *et al.*, 2013). It may also injure the tail of tadpoles at very high rates, which may affect tadpole morphology and survival, and may have delayed fitness costs (Nunes, 2011). As a result, severe declines in populations of native amphibians, ultimately leading to local extinctions, were observed in invaded habitats across Europe (Thirion, 2007; Cruz and Rebelo 2005; Rodríguez *et al.*, 2005; Cruz *et al.*, 2006a, 2006b). It was experimentally proven that some amphibian species altered their behaviour in the presence of *P. clarkii* in order to avoid predation (Nunes *et al.*, 2013 but see Gomez-Mestre and Díaz-Paniagua, 2011). This was largely mediated by chemical cues from consumed conspecifics (Nunes *et al.*, 2013). Similarly, newts may avoid invaded wetlands for breeding (Ficetola *et al.*, 2011). Nonetheless, because most amphibian species or populations are already vulnerable or threatened by extinction in Europe, including Belgium, the predatory impact of *P. clarkii* is likely to accelerate their global decrease.

- Predation on insects and other invertebrates: *P. clarkii* feeds on many invertebrate species (insects, worms, molluscs, crustacean), and may strongly reduce their diversity and abundances (Correia *et al.*, 2005; Rodríguez *et al.*, 2005; Crehuet *et al.*, 2007; Soes and Koese, 2010; Banha and Anastácio, 2011; Klose and Cooper, 2012, *in press*).

- Herbivory: *P. clarkii* heavily consumes plants and algae, causing often profound changes in composition, diversity, biomass and coverage of these communities (Gherardi and Lazzara, 2006; Gherardi, 2007; Matsuzaki *et al.*, 2009; Geiger *et al.*, 2005 and references therein, Klose and Cooper, *in press*)[9]. For instance, after the introduction of *P. clarkii* in a shallow lake in North-West Spain, the vegetation coverage decreases from 95% to < 3%, and diversity from 16 to 11 plant species, in only four years (Rodríguez *et al.*, 2005). Exclusion experiments showed recovering of the vegetation up to 95%, confirming the role of *P. clarkii* on macrophyte destruction. Similarly, more than 80% of macrophyte biomass was lost about 20 years after the introduction of *P. clarkii* in the Doñana National Park (Spain) (Gutiérrez-Yurrita *et al.*, 1998). Comparable impact has been reported in several instances in- and outside Europe (Matsuzaki *et al.*, 2009; Geiger *et al.*, 2005, Nyström, 1999, 2002). However, it was not possible to detect a negative impact of *P. clarkii* on the vegetation cover in Dutch waters (Soes and Koese, 2010), perhaps because *P. clarkii* densities and range are still increasing in the Netherlands.

- *P. clarkii* as prey for higher trophic levels: The species is heavily preyed upon by various species of animals, even in its introduced range (see paragraph 2.1.4.E). Its appearance has sometimes been considered positive for its predators, which are sometimes otherwise rare or endangered animals (Tablado *et al.*, 2010). For instance, in Doñana National Park, *P. clarkii* has become the most common prey of the Otter, *Lutra lutra* (Delibes and Adrian, 1987). Populations of a number of avian species, mainly piscivorous species like some Ardeidae (Figure 14), Ciconiidae, and Phalacrocoracidae, may increase after the introduction of *P. clarkii* (Barbaresi and Gherardi, 2000; Rodríguez *et al.*, 2005; Poulin *et al.*, 2007; Tablado *et al.*, 2010). For instance, it is suggested that the recent increase in Great Bittern (*Botaurus stellaris*) numbers in the Camargue region (France), while other French populations are decreasing, could in part be related to *P. clarkii* abundance (Poulin *et al.*, 2007). However, care should be taken before concluding that all piscivorous bird species might experience no negative impact (Soes and Koese, 2010). Montesinos *et al.* (2008) found in the Doñana National Park that, although *P. clarkii* has become very abundant and is now a predominant part of their diet, both the Purple Heron, *Ardea purpurea*, and the Night Heron, *Nycticorax nycticorax*, did not feed them to their chicks. It is suggested that the crayfish might be too hard for the chicks to handle (Montesinos *et al.*, 2008). Because *P. clarkii* may negatively impact populations of preys preferentially used by these birds for their chicks, its introduction might affect the recruitment of the bird population. Such a negative impact would need long-term monitoring

Figure 14: Grey Heron, *Ardea cinerea,* feeding on *Procambarus clarkii* in the Netherlands (Photograph from Ton Döpp, in Soes and Koese, 2010).

[9] Along with direct herbivory, *P. clarkii* may also affect macrophyte vegetation with non-consumptive cuttings of roots and seedlings, and by modifying water turbidity and quality (Gherardi, 2007; Soes and Koese, 2010).

before being revealed. Furthermore, piscivorous species which rely on eyesight for catching their prey (such as the Great Cormorant, *Phalacrocorax carbo*) can be negatively affected due to significant increase of turbidity caused by the plant consumption and burrowing activity of *P. clarkii* (Soes and Koese, 2010). Finally, its introduction may promote the establishment and spread of other invasive species (= facilitation process), leading to an invasional meltdown (*sensu* Simberloff and von Holle, 1999). For instance, *P. clarkii* forms a large part of the diet of the introduced Sacred Ibis, *Threskiornis aethiopicus*, in French sites where the two species flourish (Marion, 2007). Experimental studies also indicated that *P. clarkii* and *Gambusia holbrooki*, the invasive Eastern Mosquitofish, may strongly interact (i.e. adult crayfish consume mosquitofish and adult mosquitofish consume recently hatched crayfish). As a result, invasions of one species is facilitated by previous invasions of the other (Anastácio *et al.*, 2011). It is therefore important that future management plans include the complexity of interactions between invasive species and the entire native community (Tablado *et al.*, 2010), as well as interactions among invasive species to avoid, for instance, a "mesopredator release" effect (e.g. Miyake and Miyashita, 2011).

C/ Genetic effects and hybridization **[LOW]**

As every predatory or herbivorous (invasive) species, *P. clarkii* may exert indirect genetic impacts on indigenous species on which it feeds, resulting from altered patterns of natural selection or gene flow within indigenous populations (Gherardi, 2007). For instance, local extinction of prey species may reduce connectivity (and hence gene flow) among populations of prey metapopulations.

Hybridization between *P. clarkii* (Cambaridae) and one of the European crayfish species (Astacidae) was never observed.

D/ Pathogen pollution **[HIGH]**

Procambarus clarkii is a vector of several crayfish diseases, and may have hence a severe impact on the preservation and reintroduction of native crayfish in Europe (Geiger *et al.*, 2005). In particular, it is an asymptomatic carrier of *Aphanomyces astaci*, the infectious agent of the so-called crayfish plague (Aquiloni *et al.*, 2011). This Oomycetes is the main cause of the sharp decline in European crayfish species (such as *Astacus astacus*, the single native crayfish in Belgium) since its introduction in Europe from North America around 1860, i.e. well before the first documented introduction of American crayfish species (Pârvulescu *et al.*, 2012, and references therein). Indeed, this disease does not require a host in order to spread, as the spores of this oomycetes can become attached to damp surfaces (e.g. mud or angler equipment) and be transported in this manner (Gherardi, 2007). It is likely that *Aphanomyces astaci* has contributed to the invasion success of *P. clarkii* in Europe (Strauss *et al.*, 2012) [although the introduction of other nonindigenous epizootic diseases may also have severely impacted European crayfish (Edgerton *et al.*, 2004)]. The two other American crayfish species, *Orconectes limosus* and *Pacifastacus leniusculus*, already widely distributed in Belgian freshwaters, are also natural reservoirs for crayfish plague. Nevertheless, the strain hosted by *P. clarkii* appeared to be more tolerant to higher temperatures (Diéguez-Uribeondo *et al.*, 1995). Indeed, growth rate and sporulation capacity of the strain found in *P. clarkii* were higher at temperatures above 20°C (compared with the strains found in other crayfish species, namely *Astacus astacus, Astacus leptodactylus* and *Pacifastacus leniusculus*). This observation emphasizes the necessity of controlling the introduction of *P. clarkii* since it may increase the risk of introducing new *A. astaci* strains with different genotypes, higher levels of virulence in warmer water, and likely also other different characteristics (Diéguez-Uribeondo *et al.*, 1995).

P. clarkii is a vector for a number of helminth parasites of vertebrates (Souty-Grosset *et al.*, 2006). *P. clarkii* is also an intermediate host of trematodes of the genus *Paragonimus*, which are potential pathogens of humans and their pets if raw or undercooked crayfish are consumed (Souty-Grosset *et al.*, 2006).

P. clarkii is highly tolerant to heavy metals, radioactive elements, other pollutants, and toxins produced by Cyanobacteria (e.g. microcystin), which accumulate in its organs and body tissues and are potentially transmitted to higher trophic levels, humans included (Bollinger *et al.*, 1997; Gherardi *et al.*, 2002b; Geiger *et al.*, 2005; Al Kaddissi, 2012).

E/ Effects on ecosystem functions **[HIGH]**

Procambarus clarkii affects the ecosystem processes by recycling sediment bound nutrients (resulting in an increased turnover of nutrients), by increasing aeration of underwater sediments, and by re-suspending sediments associated with its foraging, burrowing, and locomotion activity (e.g. walking, tail flipping) (Angeler *et al.,* 2001; Soes and Koese 2010). As a result, physical and chemical characteristics of both water and sediments are modified (Angeler *et al.,* 2001), with cascade impacts on the different compartments of the ecosystem (Rodríguez *et al.*, 2005; Gherardi, 2007).

For instance, in a floodplain wetland in Spain, enclosures with crayfish showed a significant increase in both dissolved inorganic nutrients (soluble reactive phosphorus and ammonia) and total suspended solids as a result of crayfish bioturbation, compared to the control site. At the same time, crayfish reduced the content of organic matter in the sediment and slightly increased total phosphorus and nitrogen content in sediments as an effect of its benthic activity (Angeler *et al.,* 2001).

Cascade impacts were well documented in Chozas, a small shallow lake in North-West Spain (Figure 15) (Rodríguez *et al.*, 2005). Turbidity, caused by *P. clarkii* activity and destruction of macrophytes, reduces light penetration and plant productivity. Direct and indirect plant destruction (99% plant coverage reduction) was directly related to the decrease of invertebrates (71% losses in macroinvertebrate genera), amphibia (83% reductions in species), and waterbirds (52% reduction). Plant-eating birds were negatively affected (75% losses in ducks species), while presence of birds feeding on fish and crayfish increased since the introduction of *P. clarkii*.

Introduction of *P. clarkii* hence produces both top-down and bottom-up effects in the food web of native ecosystems, restructuring interspecific interactions, and modifying nutrients and energy flows. Because of its omnivorous nature and wide plasticity in its feeding behaviour, *P. clarkii* can profoundly alter the trophic structure of freshwater communities at several levels, often acting as keystone species (Gherardi, 2007). Most sensitive ecosystems are those where detritivores are rare and which are dominated by autotrophs as in temporary freshwater marshes (Geiger *et al.*, 2005). In such systems, crayfish put the detritus energy pool directly at the disposal of higher trophic levels. This greatly shortens the energy pathways and simplifies their structure (Figure 16). Experiments in Italy have shown that *P. clarkii* strongly affects the native community even at relatively low density (4 crayfish/m²), although a high crayfish density (8 crayfish/m²) had a more pronounced impact in a shorter time (Gherardi and Acquistapace, 2007).

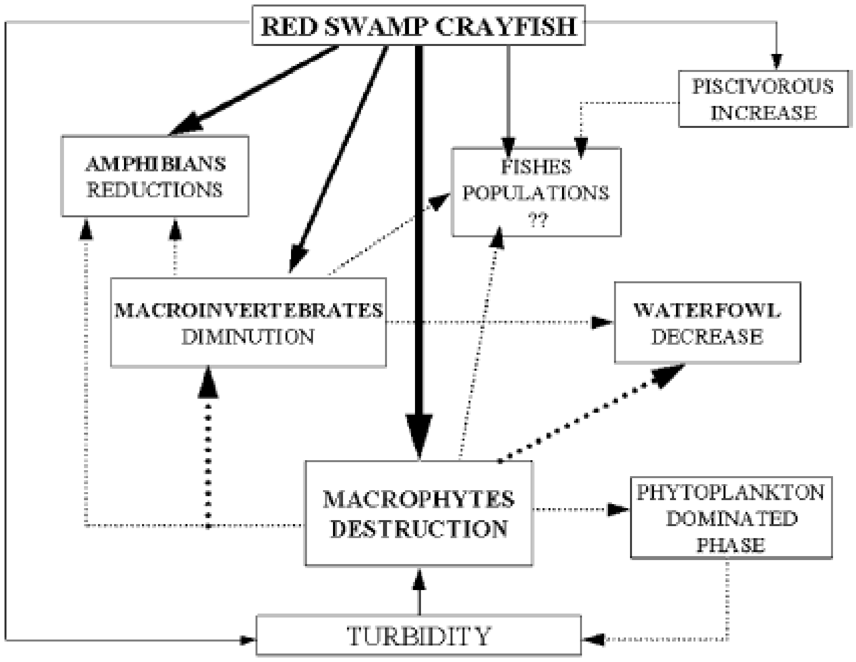

Figure 15: Direct (continuous arrows) and indirect (dotted arrows) effects of *Procambarus clarkii* on ecosystem of Clozas lake, Spain. (Source: Rodríguez *et al.*, 2005).

ENVIRONMENTAL IMPACTS

Procambarus clarkii **may severely impact the native flora and fauna, either directly (by competition for food or shelters, by predation, by herbivory and by pathogen transmission) or indirectly (by modification of physical and chemical characteristics of water and sediments, and by cascade effects through the food web). Native crayfish are particularly threatened but, because of its omnivory and trophic plasticity,** ***P. clarkii*** **may also have strong impacts on fishes, amphibians, invertebrates, macrophytes and algae. After its introduction, water turbidity generally increases, nutrients and energy flows are often greatly shortened and simplified, and local extinction of some organims may occur.** ***Procambarus clarkii*** **can thus be considered as a keystone species, which profoundly affects the ecosystem functions. Finally,** ***P. clarkii*** **may also accumulate heavy metals, radioactive elements and other pollutants, and may therefore potentially transmit them to higher trophic levels.**

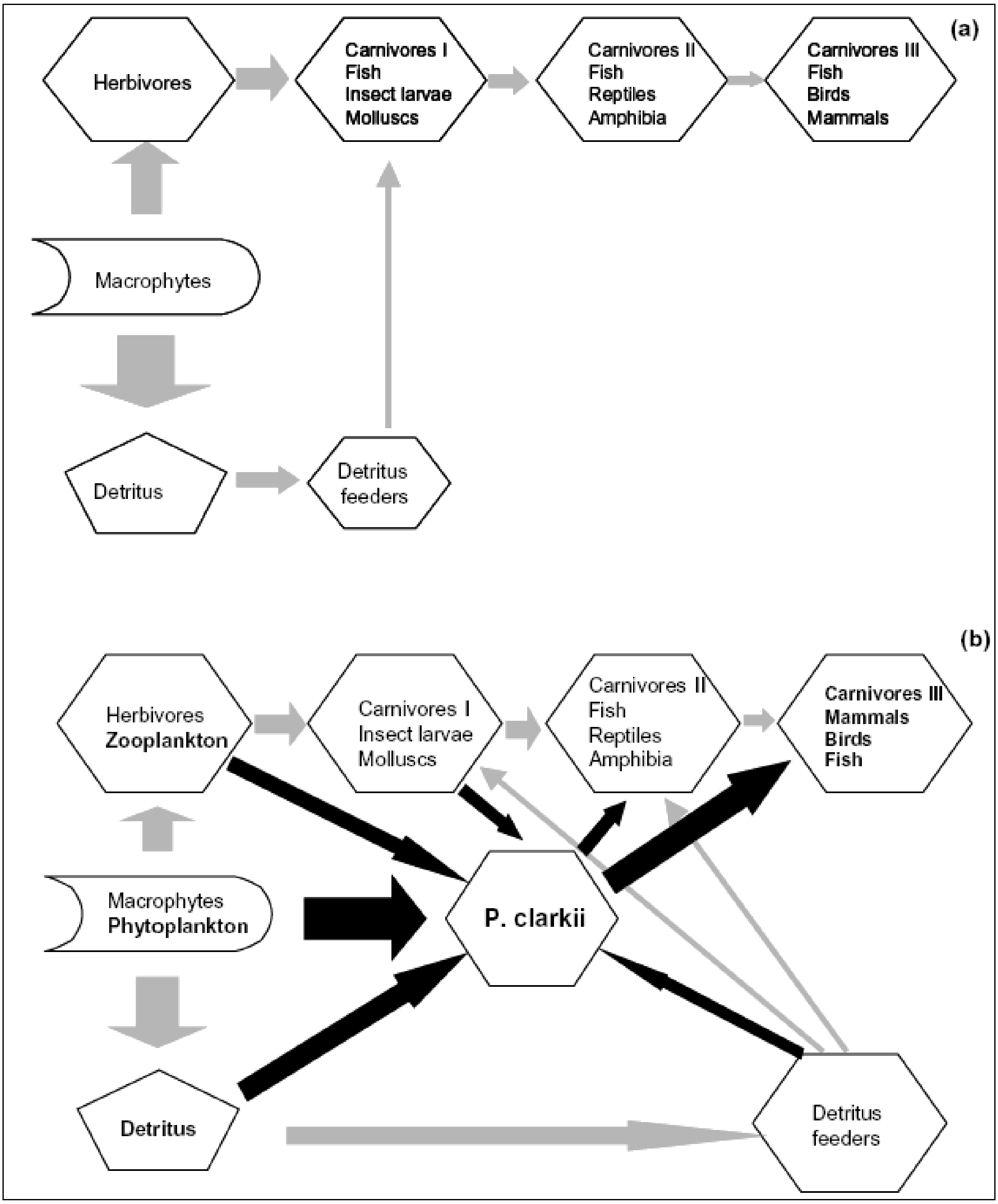

Figure 16: Simplified representation of the energy flow in waterbodies of a freshwater marshland (a) before and (b) after the introduction of *Procambarus clarkii* (Geiger *et al.*, 2005). Before the introduction of *P. clarkii* (a), macrophytes and the associated periphyton were the dominant primary producers. Only a small portion of the energy was transmitted from them to herbivores, whereas most of it was lost to the detritus pool, which accumulated large amounts of organic matter. Detritivores, mainly macroinvertebrates (oligochaetes, chironomids) and meiofauna (nematodes, ostracods), used only a small fraction of the deposited material. This system was characterized by a high diversity of herbivores and consisted of a minimum of four levels of consumers. Due to the large number of trophic levels and losses of energy to the detritus pool, the energy transferred to top predators such as birds and mammals was comparatively low. After the introduction of *P. clarkii* (b), much of the detritus was consumed by the crayfish and the energy gained was directly transferred to the top predator level (fishes, birds, and mammals). This resulted in a decreased importance of macrophytes, herbivores, and primary carnivores but offered a larger availability of energy for vertebrate predators.

2.2.2 Other impacts

A/ Economic impacts

Describe the expected or observed direct costs of the introduced species on sectorial activities (e.g. damages to crops, forests, livestock, aquaculture, tourism or infrastructures).

- **Burrowing activity.** Most crayfish species are known to create burrows in the absence of enough cover (Barbaresi *et al.*, 2004a). There are several reports of damage of dikes, irrigation channels and other water works due to the intense burrowing behaviour of *Procambarus clarkii* (up to 2 meters long), which can result in bank collapse and consequently in severe damage to both agricultural fields and natural ecosystems (Fonseca *et al.,* 1996; Correia and Ferreira, 1995; Huner, 2002; Barbaresi *et al.*, 2004a).

- **Damage in rice fields.** *Procambarus clarkii* was often introduced in rice fields in order to increase rice-farmers profits by double cropping rice and crayfish. However the crayfish feeds on young parts of the rice plant and damages seeds and seedlings (Anastácio *et al.*, 2005). As a result, biodegradable surfactants, which inhibit oxygen consumption thus leading to decreased *P. clarkii* activity, were sometimes used (e.g. in Spain) to limit the damage being done by *P. clarkii* to rice crops, whilst still enabling production of crayfish (Fonseca *et al.*, 1996) but were rarely effective (Anastácio *et al.,* 2000).

- **Impact on aquaculture and fishery.** *Procambarus clarkii* may have a positive impact on aquaculture and fishery since juveniles and adults of the species could constitute important prey items for freshwater predatory fishes. However it is probably only a transient effect since *P. clarkii* may predate on eggs and fish juveniles, and may strongly alter habitat conditions.

- **Sea food industry.** China is currently the largest producer of *Procambarus clarkii*, followed by the USA (70,000 and 50,000 tonnes in 1999, respectively) (Ackefors, 2000; Wickins and Lee, 2002). Minor fisheries are established in Kenya, Portugal and Spain (FAO, 2012). In the southern USA, mainly in Louisiana, the red swamp crayfish is the main species cultured together with the White River Crayfish, *Procambarus zonangulus*. Total production of these two species in aquaculture in 1999 was approximately 35,000 tonnes, of which 85% consisted of *P. clarkii*. The total production of crayfish in Europe currently varies between 3,000 and 5,000 tonnes per annum (with 80% coming from Spain). Two native species (*Astacus astacus* and *A. leptodactylus*) and two introduced species (*Pacifastacus leniusculus* and *Procambarus clarkii*) are the most important crayfish species produced (Ackefors 1998, 2000; Wickins and Lee, 2002; Skurdal and Taugbøl, 2002). However, nowadays, Europe produces less and imports more crayfish than before (the species mainly arrives from the Chinese market, often already cooked and dressed) (Soes and Koese, 2010). For instance, European culture fisheries yielded about 160 tonnes in 1994, 40% of this was *P. clarkii* (32% *Pa. leniusculus*, 17% *A. astacus*, 8% *A. leptodactylus* and 2% other introduced American species) (Ackefors, 1998).

In 2005, China production had risen to over 88,000 tonnes, with a value exceeding USD 303 million, bringing the global aquaculture production of *P. clarkii* in that year to nearly 105,000 tonnes (Figure 17) (FAO, 2012). Afterwards, the global production rapidly rose and was over 600,000 tonnes in 2010 (Figure 17) (FAO, 2012).

In Africa, very few of the several projects that led to crayfish importations since the 1960s can be regarded as successful (de Moor, 2002). For instance, in Lake Naivasha, Kenya, only about 40 tonnes of *P. clarkii* are now caught annually exclusively for local consumption (mainly tourism), after their first harvests in 1975 of several hundred tonnes per year (Smart *et al.*, 2002). Crayfish were reported to spoil valuable fish caught in gillnets (up to 30% of the catch) and to damage fish nets (de Moor, 2002). They are also responsible for the decline of the rooted vegetation and therefore of the increase of phytoplankton, decrease of water transparency, and reduction of fish, including commercial species, in the littoral zone (de Moor, 2002).

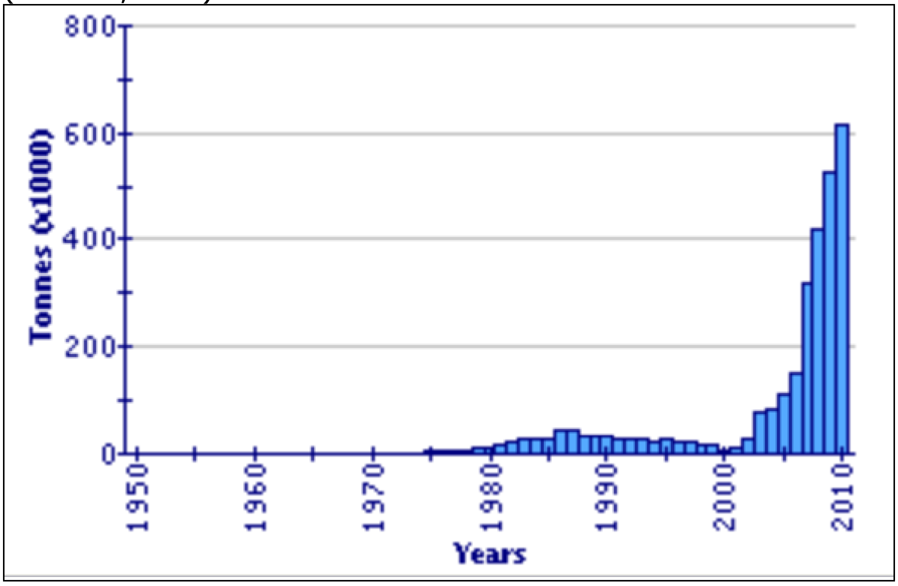

Figure 17: Global aquaculture production of *Procambarus clarkii* from 1950 to 2010 (Source: FAO Fishery Statistic, 2012).

B/ Social impacts

Describe the expected or observed effects of the introduced species on human health and well-being, recreation activities and aesthetic values.

- **Impact on human health.** Little attention has been paid to the potential harm that non-indigenous crayfish pose to human health. These crayfish, such as *Procambarus clarkii*, often live in areas contaminated by sewage and toxic industrial residues and may have high heavy metal concentrations in their tissues (Geiger *et al.,* 2005). They were found to highly tolerate metals such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn), and to bioaccumulate these metals in their tissues and organs at a significantly higher rate than the indigenous species (Bini and Chelazzi, 2006; Bollinger *et al.*, 1997; Gherardi *et al.*, 2002b; Suárez-Serrano *et al.*, 2010; Al Kaddissi, 2012). Their potential to transfer metals and other contaminants to their consumers, including man, is obviously high (Geiger *et al.*, 2005; Gherardi, 2007).

The finding that *P. clarkii* may consume Cyanobacteria is of increasing concern for human health (Gherardi and Lazzara, 2006). Several Cyanobacteria release a wide range of toxins that may produce lethal animal and human intoxications (e.g. Cox *et al.*, 2005). *P. clarkii* was found to accumulate such toxins in its tissues (Vasconcelos *et al.*, 2001; Gherardi, 2007), being therefore able to transfer them to more sensitive organisms, man included.

Moreover, *P. clarkii* is an intermediate host of trematodes of the genus *Paragonimus*, which are potential pathogens of humans and their pets if raw or undercooked crayfish are consumed (Souty-Grosset *et al.*, 2006).

However, *P. clarkii* may sometimes have positive impact on human health. For instance, it is able to control, through predation and competition, populations of the pulmonate snails *Biomphalaria* and *Bulinus* known to host *Schistosoma mansoni* and *S. haematobium*, the agents of human schistosomiasis, one of the most widespread diseases in Africa. In Kenya alone, it is known to affect 3.5 million individuals with 12 million more at risk of infection. As suggested by Mkoji *et al.* (1999), due to the quick spread of this crayfish in African waterbodies, the epidemiology of schistosomiasis is expected to be significantly altered with time although the possibilities remain that African snails will soon evolve measures to avoid crayfish predation before their extinction or that the parasite will change its host (Gherardi, 2007).

- **Cultural and traditional values.** Some crayfish species, such as *Procambarus clarkii*, are highly valued as food, and in some countries or regions, like Scandinavia and Louisiana, feasting on them has become a cultural icon (Gherardi, 2007; Souty-Grosset *et al.*, 2006). In Sweden, *P. clarkii* has mostly replaced the native *Astacus astacus*, after the sharp decrease of the latter caused by outbreaks of crayfish plague during the last century.

- **Recreation activities and aesthetic values.** Crayfish are widely appreciated by anglers and aquarists. The aquarium trade is even increasing in Europe (Holdich *et al.*, 2009; Chucholl, 2013). *Procambarus clarkii* is one of the most priced species because of its beautiful colours and its ease to maintain in captivity. In Belgium, the species is easily found for sale on the Internet or in aquarist centres.

STAGE 3 : RISK MANAGEMENT

The decision to be made in the risk management process will be based on the information collected during the two preceding stages, e.g. reason for initiating the process, estimation of probability of introduction and evaluation of potential consequences of introduction in Belgium. If the risk is found to be unacceptable, then possible preventive and control actions should be identified to mitigate the impact of the non-native organism and reduce the risk below an acceptable level. Specify the efficiency of potential measures for risk reduction.

3.1 RELATIVE IMPORTANCE OF PATHWAYS FOR INVASIVE SPECIES ENTRY IN BELGIUM

The relative importance of intentional and unintentional introduction pathways mediated by human activities should be compared with the natural spread of the organism. Make use e.g. of information used to answer to question 2.1.3.

The trade of live *Procambarus clarkii* by the sea food industry is now anedoctal in Europe (except perhaps in Spain and Nordic countries). Sold specimens are now imported, mainly from China and the USA, often cooked and dressed.

The main current pathway of introductions of *Procambarus clarkii* in Europe, including Belgium, is thought to be the release of animals used as pets by aquarists. Subsequent dispersion by anglers may also exist. Indeed, although natural dispersal capacity of *Procambarus clarkii* is relatively high (for a crayfish), it can hardly alone explain the rapid spreading of the species (as observed in France, for instance) or its appearance in sites highly distant from established populations (as documented in the Netherlands and in Belgium, for instance). Nevertheless, natural colonisation from neighbouring countries (mainly France and the Netherlands) can soon become regular since dense populations of the species are now close to the Belgian border and spread rapidly in these territories. The fact that the species is already present in waterbodies or streams connected to Belgian freshwater habitats through natural catchments or canals is particularly worrying. For instance, introduction of specimens from the Dutch North Brabant to the Belgian Campine may have already occurred or is very likely in near future (Roger Cammaerts, pers. comm.).

3.2 PREVENTIVE ACTIONS

Which preventive measures have been identified to reduce the risk of introduction of the organism? Do they reduce the risk to an acceptable level and are they considered as cost-effective? Specify if the proposed measures have undesirable social or environmental consequences. Consider especially (i) the restrictions on importation and trade and (ii) the use of specific holding conditions and effect of prohibition of organism introduction into the wild.

(i) Prohibition of organism importation, trade and holding

Information, education, prevention and control seem the most promising management measures to reduce the negative impact of invasive crayfish species, such as *Procambarus clarkii,* and other potentially invasive crayfish as well (Geiger *et al.*, 2005; Harlioğlu and Harlioğlu, 2006; Peay, 2009).

- **Information and education.** Government authorities, fisherman and public should be educated on the endangered status of native crayfish, and on the potential serious dangers of non-native crayfish introductions and crayfish diseases.

To achieve this objective, the European thematic network CRAYNET "European crayfish as keystone species-linking science, management and economics with sustainable environmental quality" started in 2002 under the auspices of the European Commission (Souty-Grosset *et al.*, 2006). The project was completed in November 2005. At that time, the network was constituted by over 500 researchers and freshwater managers from 11 European countries.

CRAYNET aimed to establish for the first time a network of aquatic crayfish researchers and managers ('stakeholders') to (1) identify trends in land use and consequent water in European waters, and their probable impact on biodiversity, as assessed by bio-indicators (crayfish are powerful bio-indicators for water quality and are also keystone species controlling ecosystems); (2) discuss ways to harmonise national and regional legislation and to improve it at European level; (3) identify research needed to solve management problems in crayfish survival and habitat and water quality protection; (4) produce handbooks on crayfish management solutions; bio-monitoring protocols; wise recreational use of native and alien species; (5) produce publicity (website, documents, videos) aimed at stakeholders and the general public (http://www.edinburgh.ceh.ac.uk/projectpages/craynet_page.htm).

The CRAYNET programme diversified the means of dissemination by editing a series of leaflets, aimed at widest diffusion to the general public, a poster "Crayfish of Europe" giving in a single view the best pictures of indigenous (ICS) and non-indigenous crayfish species (NICS) for exhibitions across Europe (Figure 18), and a booklet "Identifying native and alien crayfish species in Europe" directed particularly towards managers, decision makers and third-level students.

Figure 18: Poster produced by the CRAYNET network and aiming at educating the European general public, freshwater managers and decision makers in order to prevent introduction and dispersion of non-indigeneous crayfish species (including *Procambarus clarkii*) and to improve conservation of native species (such as *Astacus astacus*).

In Belgium, similar efforts of education and information were (and still are) carried out by associations, naturalists, researchers, and even crayfish anglers. In November 2008 was created the "Association pour la Sauvegarde et la Promotion des Ecrevisses Indigènes (ASPEI; http://www.aspei.be/), supported by the "Région wallonne" and by the "Ministères de l'Environnement et de l'Emploi et du Travail". This association aims at promoting the conservation, reintroduction, and wise use of the Belgian single native species, *Astacus astacus*, and at avoiding further spread of invasive species (e.g. *Procambarus clarkii*), thanks to the education of public, anglers, managers, and decision makers (Figure 19). Such efforts should be maintained.

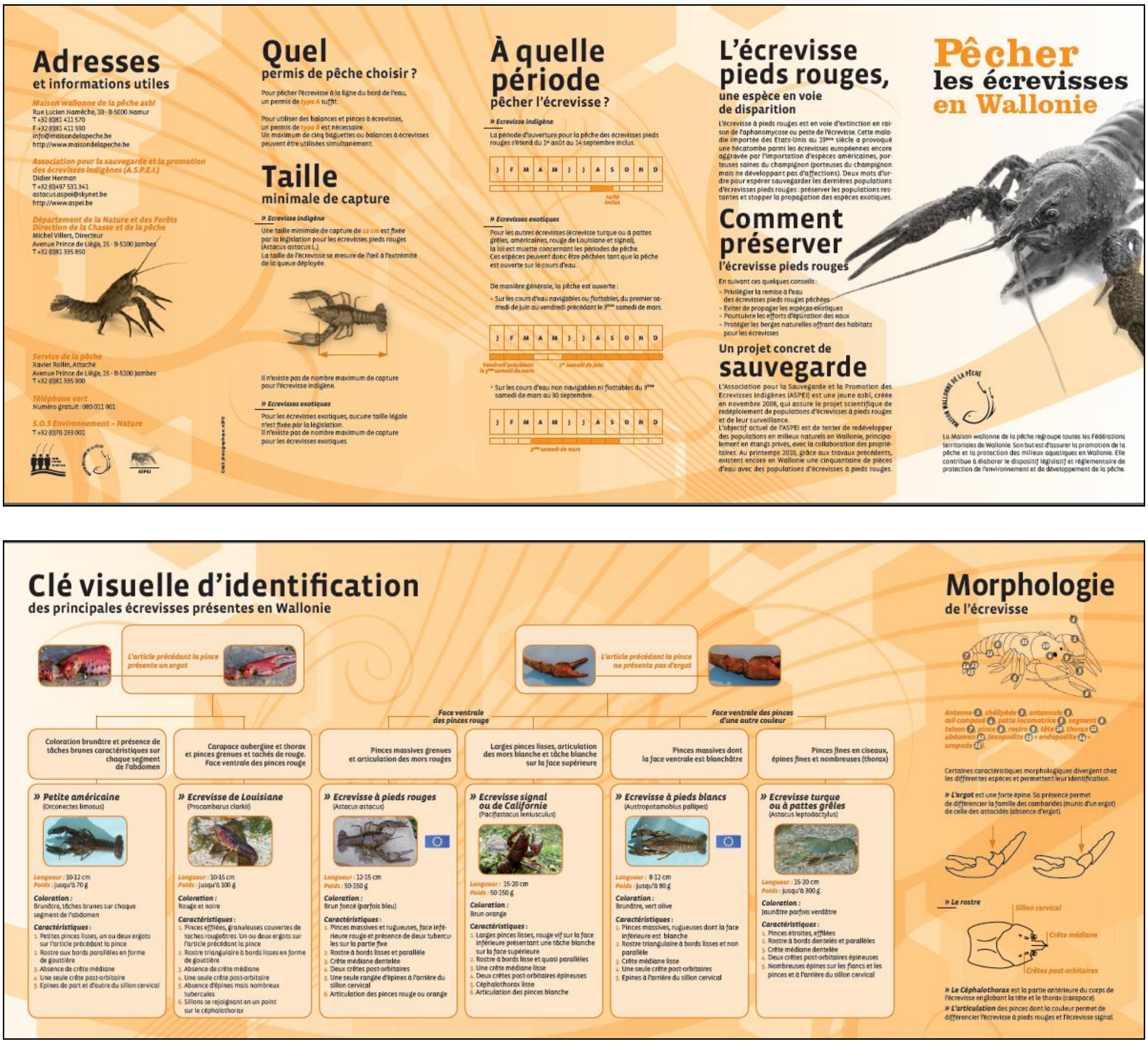

Figure 19: Example of a booklet produced in Belgium in order to educate the general public and anglers. It was published by the « Maison Wallonne de la Pêche » (with the support of the ASPEI association among others) and present a simplified key for crayfish identification. The aims were to explain crayfish harvesting rules in Belgium, to promote conservation of native species, and to avoid spreading of invasive crayfish (e.g. *Procambarus clarkii*). It is available at: http://www.maisondelapeche.be/telechargements/DepliantEcrevisse_light.pdf.

- **Prohibition of organism importation, trade and holding.** Sale and importation of live non-native crayfish, *Procambarus clarkii* in particular, should be banned by the Belgian government for any purposes.

In addition, a unified, strengthened legislation should be established in Europe in order to ensure a total ban on import, trade and holding of live *Procambarus clarkii* and other (potentially) invasive crayfish. Indeed, legislation concerning live non-native crayfish sale and importation currently differs among different European countries. For instance, in England, *Procambarus clarkii* is listed since 2010 on schedule 9 of the Wildlife and Countryside Act of

1981, which prohibits its release into the wild. Similarly, the French law forbids the introduction, transport and sale of live *P. clarkii* specimens ("Arrêté du 21 juillet 1983 [modifié] relatif à la protection des écrevisses autochtones") but other exotic, potentially invasive, crayfish are not concerned (Collas, 2012). At the opposite, importation and trade of exotic crayfish are possible in Belgium and the Netherlands. This absence of unity facilitates the introduction and spread of invasive crayfish, even in countries with constraining laws.

(ii) Use of specific holding conditions and effect of prohibition of organism introduction into the wild

Although introduction of non-native species into the wild is strictly forbidden by regional nature conservation laws (Etienne Branquart, pers. comm.), it is nearly impossible to ensure that crayfish aquarists will never release their pets into the wild or to prevent that some individuals will not escape from captivity, even under specific holding condition rules (as advocated by the Council Regulation (EC) No 708/2007 of 11 June 2007 concerning use of alien and locally absent species in aquaculture). Since involuntary dissemination is almost impossible to avoid, preventive measures including restriction of its sale and holding as well as increasing public awareness about its environmental and economic impacts is determinant in the control or *Procambarus clarkii* in Belgium and other non-native countries.

Prohibiting the introduction of *Procambarus clarkii* into the wild would limit the spread of this highly invasive keystone species, as well as the spread of the crayfish plague.

3.3 CONTROL AND ERADICATION ACTIONS

Which management measures have been identified to reduce the risk of introduction of the organism? Do they reduce the risk to an acceptable level and are they considered as cost-effective? Specify if the proposed measures have undesirable social or environmental consequences. Consider especially the following questions.

(i) Can the species be easily detected at early stages of invasion (early detection)?

Being a freshwater species, *Procambarus clarkii* can be difficult to detect at early stages of invasion. Nonetheless two traits may facilitate its detection:

- *P. clarkii*, contrary to some other invasive crayfish such as *Orconectes limosus*, regularly wanders over land during dispersion periods (Figure 20). As noted by Koese and Evers (2011), crayfish species that regularly break through the water surface have a much larger probability of being recorded compared to species that almost never leave the water. However, wandering activity is mainly nocturnal.

- The high burrowing activity of the species greatly modifies the aspect of river and pond banks. Burrows are often obvious enough to be easily and rapidly detected by naturalists, anglers or freshwater managers (Figure 21).

Finally, crayfish rearing is the object of a growing fondness in Belgium (e.g. the ASPEI association). Crayfish habitats and populations are thus relatively well known and monitored (at least in some areas).

In the Netherlands, a national inventory of crayfish was conducted in 2010 (Koese and Evers, 2011), facilitating the detection of new small populations of *Procambarus clarkii*. Similarly, past national surveys have allowed biologists to detect the presence of the species in Belgium (Boets *et al.*, 2009). Such initiatives have to be regularly performed to be effective at detecting early-stage invasions. In Wallonia, a survey is carried out every six years to monitor populations of *Astacus astacus* (Roger Cammaerts, pers. comm.). This kind of survey also facilitates the discovery of invasive species.

Figure 20 : A *Procambarus clarkii* crayfish wandering over land in the Netherlands. (Photo by Bart Noort, source: Koese and Evers, 2011).

(A)

(B)

Figure 21: Examples of *Procambarus clarkii* burrows. (A) Burrows in the bank of a small clay dike in a Spanish rice field (Source: Soes and Koese, 2010). (B) A male in its burrow in the bank of a groundwater-filled pits in an abandonned quarry in Israel (Wizen *et al.*, 2008).

(ii) Are they some best practices available for organism local eradication?

The side effect of chemicals and even biological control means can often be as detrimental

or even worse for the environment at large, native species and human health.

The precautionary principle should be applied as a general rule.

- **Trapping** is usually an insufficient tool for eradication of crayfish populations (Hyatt, 2004). For instance, in Switzerland, attempts have been made to control an illegal introduction of *P. clarkii* from a pond using trapping. This was unsuccessful and a combination of methods have been considered, such as trapping, biocides, and introducing predatory fish species (Frutiger and Müller, 2002). Predatory fishes were thought to have had the greatest impact on the Red Swamp Crayfish population (Frutiger and Müller, 2002). However, physical measures, including the use of traps, nets and electro-fishing, can sometimes control populations if used intensively enough. Eradication is unlikely as the crayfish may hunker down in their burrows. In fact, culling may stimulate earlier maturation age and result in greater egg production (ISSG, 2011). It is advised to use semi-cylindrical traps made from 5.5 mm mesh galvanized steel wire to obtain the highest catch efficiency (Paillisson *et al.,* 2011). [Note: *P. clarkii* is a vector of the crayfish plague, a lethal illness for native crayfish caused by the invasive fungus-like *Aphanomyces astaci* (Oomycetes). Its spores can become attached to damp surfaces such as crayfish traps. It is therefore important to keep in quarantine the traps before using them in other waterbodies. Fortunately, spores are relatively short-lived (a few days or even 24 h when absolutely dry) (Souty-Grosset *et al.*, 2006)].

- **Dewatering ponds** might be a practical method (Collas, 2012), especially for ponds that are considered of major importance for threatened amphibians (Soes and Koese, 2010). Success of this method is however hardly guaranteed for every crayfish species, as some species have proven to be quite resistant to even long periods of drought in winter (e.g. *P. clarkii*). Dewatering should be accompanied by a physical isolation of the colonized pond (e.g. through the use of "crapauduc" or by liming), and by bank dredging to destruct *P. clarkii* burrows (Collas, 2012). Both artificial and natural barriers may indeed limit the spread of *P. clarkii* in invaded areas (Kerby *et al.*, 2005; Dana *et al.*, 2011).

- **Autocidal method: the Sterile Male Release Technique (SMRT).** Experimental studies recently showed that a dose of 20 Gy X-rays did not compromise either the survival or the mating ability of *Procambarus clarkii* males, but reduced the size of their testes and significantly altered spermatogenesis. The number of aborted eggs was larger in clutches sired by treated males, with a consequent reduction (by 43%) in the number of offspring. These results foster hopes in efforts to control invasive crayfish, showing the potential effectiveness of ionising irradiation to reduce male fertility in the perspective of adopting the Sterile Male Release Technique to control *P. clarkii* populations (Aquiloni *et al.*, 2009). So far, this method was not tested in nature.

- **Biological control.** Several predatory fishes feed on *Procambarus clarkii* in both its native and introduced range. As a result, it was often advocated the release of native predatory fish species in order to increase the predatory pressure on *P. clarkii* (Neveu, 2001). Among the fish species native to Europe, the eel, *Anguilla anguilla*, might be a promising biological control agent because of its benthonic feeding habit, its ability to enter in crayfish burrows and its tolerance to partially deoxygenated waters, properties that match the lifestyle of crayfish and the typical habitats they occupy (Aquiloni *et al.*, 2010). However, *Procambarus clarkii* seems to perceive a general fish odour that alerts it to possible predation risk without the need of either a direct recent experience or via sharing a common native range (Gherardi *et al.,* 2011a). Moreover, where they coexist with fish, they become able to distinguish among species, adapting the intensity of their response to the effective risk (Gherardi *et al.,* 2011a). The use of *A. anguilla* as biological control agent should also be examined with caution since the species is in rapid decline in Europe.

Biological control must generally be combined with trapping to control (very rarely to eradicate) *P. clarkii* populations (Neveu, 2001; Frutiger and Müller, 2002).

Crayfish are susceptible to various microbial pathogens and parasites (Gherardi *et al.* 2011b). The problem in using most of them as biological control agent lies in the fact that they usually lack host-specificity. There is thus the risk that microbes and other parasites will spread to non-target organisms, including native crayfish species (Gherardi *et al.*, 2011b).

- **Chemical methods** are the only methods that have proven to be successful in the eradication of exotic crayfish populations (Hyatt, 2004; Sandodden and Johnsen, 2010). Organophosphate insecticides have been proven effective in laboratory and large-scale field trials in controlling *P. clarkii* in rice ponds (Chang and Lange, 1967; Eversole and Sellers, 1997) but have resulted in the deaths of many birds! Pesticides indeed adversely impact non-target organisms due to their lack of selectivity and in certain cases also persistence in nature over longer periods. Pyrethroids seem to have the greatest potential due to their lethal effects on crayfish and their rapid breakdown (Hyatt, 2004; Sandodden and Johnsen, 2010; Cecchinelli *et al.*, 2011). A recent experiment combining dewatering and BETAMAX VEX, a synthetic pyrethroid, on an isolated pond seems to have eradicate completely a Signal Crayfish population, *Pasifastacus leniusculus,* with relatively little effort. Although these results look promising, the actual application to more complex systems might prove to be more difficult due to a less perfect dispersion of the chemical (Sandodden and Johnsen, 2010). In addition, this biocide is non-specific and affects every aquatic crustaceans (Gherardi *et al.*, 2011b).

  In Spain, the use of **biodegradable surfactants**, which inhibit oxygen consumption thus leading to decreased activity, might have some potential in limiting the damage being done by *P. clarkii* to rice crops, whilst still enabling production of crayfish in rice fields (Fonseca *et al.*, 1996).

  A field experiment in Italy was carried out to study the use of **sex pheromones** for the control of invasive populations of *Procambarus clarkii*: (Aquiloni and Gherardi, 2010). Results confirm that males are attracted by females' sex pheromones: the sex ratio of the crayfish captured by the traps containing females was significantly biased towards males. However, when overall numbers of the captured crayfish were compared among types of trap, food appeared to be the most attractive bait. The authors hypothesize that the method could be improved using purified and concentrated sex pheromone instead of live animals.

To conclude, examples of effective eradication of *Procambarus clarkii* are very few and limited to small populations at an early stage of invasion. In addition, every method possesses its advantages and limitations. They are summarized in the following table (source: Gherardi *et al.*, 2011b). A combination of intense trapping, release of native fish species, and (when possible) building of barriers around the invaded waterbody to limit further spreading, seems to be the most efficient method and certainly the most respectful one for the native ecosystems.

Table 1 Synthesis of the different methods used to control invasive NICS showing a class-level evaluation of their general efficacy according to the following criteria: population size (number of individuals in the target population), area size (dimension of the area invaded by the target population), applicability (suitable habitats for the application of the method), species-specificity (capacity to affect the target species only), selectivity (capacity to affect a specific class of individuals in the target population), impact (potential ecological damages), time (duration of the application to be effective), cost (expenses of the method), and efficacy (capacity to control the target population)

Methods	Population size	Area size	Applicability	Species-specificity	Selectivity	Impact	Time	Cost	Efficacy
Mechanical									
Trap	+++	++	+++	+	+++	+	+++	+++	+++
Electrofishing	++	+	++	++	+	+	+	+	+
By hand	+	+	+	+++	+++	+	+	+	+
Physical									
Drainage	–	+	+	+	+	+++	++	+++	+
Diversion of rivers	–	+	+	+	+	+++	++	+++	+
Barriers	–	+	++	++	+	++	+	+++	++
Biological									
Predators	+++	++	++	++	+++	+	+++	++	++
Pathogens	–	–	+++	+++	+	?	+	+	+++
Biocides									
Chemical	–	+	++	+	+	+++	+	++	+++
Natural	–	+	++	+	+	++	+	++	+++
Autocidal									
SMRT	+	+	++	+++	+	+	+++	++	?
Sex pheromones	–	++	+++	+++	+++	+	+++	+	+

+, low; ++, medium; +++, high; –, irrelevant; ?, unknown

(iii) Do eradication and control actions cause undesirable consequences on non-target species and on ecosystem services ?

Yes, especially actions based on chemicals! See paragraph (ii) above. The impact can be very severe.

(iv) Could the species be effectively eradicated at early stage of invasion?

Yes, but only under intense (both moneywise and labor intensive) efforts of trapping combined with the release of native fish species, and (when possible) the building of barriers around the invaded waterbody to limit further spreading. Dewatering small ponds can also be locally effective. See paragraph (ii) above.

Once *Procambarus clarkii* has been removed, it is important to monitor the recovery of the ecosystems and to ensure that the target crayfish species and/or other invasive species do not recolonize the site.

(v) If widely widespread, can the species be easily contained in a given area or limited under an acceptable population level?

Once widespread, *Procambarus clarkii* and other aquatic aliens are difficult if not impossible to be rid off or even controlled (Wizen *et al.,* 2008). Moreover, it is much more difficult to control *P. clarkii* in lotic habitats than in lentic ones. Fortunately, the species preferentially inhabits the latter. Control of *P. clarkii* populations can be attempted in lentic habitats with a combination of intense trapping, release of native fish species, and building of barriers around the invaded waterbody to limit further spreading.

RISK MANAGEMENT

The main current pathway of introductions of *Procambarus clarkii* in Belgium is the release of animals by people.

<u>Information, education, prevention and control</u> seem the most promising management measures to reduce the negative impact of invasive crayfish species. Fortunately, several education actions were (and are) already carried out at the European level, and in Belgium.

A unified, strengthened legislation should be established <u>in Europe</u> to ensure a total ban on import, trade and holding of live *Procambarus clarkii* <u>and other (potentially) invasive crayfish</u>. Indeed, the potential to become invasive exists for most crayfish species nowadays present in the aquarist catalogues, especially for those originating from temperate regions.

Unfortunately, for *P. clarkii*, prohibition of importation, trade and holding in Belgium could not be enough to prevent its entry and establishment because (1) the species is already spreading in the country (especially in the Flemish Region), and (2) natural spreading from neighbouring countries are expected since dense populations are now close to the Belgian border. Nevertheless, such measures would certainly slow down its current spread.

National surveys should be regularly performed to detect early-stage invasions (and thus to allow authorities and managers to take rapid eradication or control actions). In Belgium, surveys specifically directed towards alien crayfish are lacking.

Being a freshwater species, *Procambarus clarkii* can be difficult to detect at early stages of invasion. Nonetheless two traits may facilitate its detection: (1) individuals regularly wanders over land during dispersion periods, and (2) burrows are often obvious enough to be easily and rapidly detected.

Examples of effective eradication of *Procambarus clarkii* are very few and limited to small populations at an early stage of invasion.

If early detected, a combination of intense trapping, release of native fish species, and (when possible) building of barriers around the invaded waterbody to limit further spreading, seems the most efficient method to eradicate (or at least to control) invasive populations. This approach is certainly the most respectful one for the native ecosystems. Dewatering small ponds can also be locally effective.

Once widespread, *Procambarus clarkii* and other aquatic aliens are difficult if not impossible to be rid off or even controlled. Some methods (i.e. use of sex pheromones, Sterile Male Release Technique) are promising but are still under development. Use of chemicals and pathogens to control *P. clarkii* should not be considered because these methods have strong negative impacts on native crayfish and other components of the ecosystem.

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