Exotic crayfish invasions

Laurent Basilico, Jean-Patrice Damien, Jean-Marc Roussel, Nicolas Poulet, J.M. Paillisson

ONEMA Meeting

Recap

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Ecological impacts and management approaches

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The first "National meetings on invasive exotic crayfish" were organised by the INRA, the Parc naturel régional de Brière, the Forum des marais atlantiques, the Onema and the CNRS. They took place on the 19th and 20th of June, 2013 at Saint-Lyphard (Loire-Atlantique).

This issue is available for consultation on the Onema website (http://www.onema.fr/IMG/EV/cat7a-thematic-issues.html#meetingsrecap) and on the website of the PNR Brière (http://www.parc-naturel-briere.fr). It is referenced in the national portal "technical documents on water" (www.documentation.eaufrance.fr).

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Foreword

Since spiny-cheek crayfish were introduced in Europe in the late XIXth century, and above all since signal and red swamp crayfish appeared at the turn of the 1970s, the colonization of European freshwaters by exotic crayfish has imposed itself as a research topic and a growing concern for researchers and aquatic environment stakeholders. In France, the issue gained a new dimension over the last decade, as occasionally devastating effects of invasive crayfish were noted on natural environments and emblematic species.

In response to a growing need for more knowledge and management options, a joint research programme driven by the Inra, the CNRS, the Parc naturel régional de Brière (PNR) and the Onema was launched in the Brière marshes in 2010. The presentation of these studies, along with others led in France these last years, led to the first "National meetings on invasive exotic crayfish" organised on the 19th and 20th of June, 2013 in Saint-Lyphard (Loire-Atlantique). Gathering nearly 120 researchers and managers – fishing federations, natural parks, environmental associations, administrations – these two days of information and debate made it possible to establish for the first time such a broad dialogue about the subject on a national scale.

The outcome is a whole set of updated data about the biology of invasive species, the ways they colonize environments and the ecological impacts they induce. Resolutely on an operational mode, the seminar also provided a record of the means currently available to monitor and manage invasive crayfish through a number of field experiment feedbacks. The present document proposes a summary of the scientific features, field observations and management strategies presented on the occasion.

Sommaire

«Incertitude, ô mes délices,
Vous et moi nous nous en allons
Comme s'en vont les écrevisses,
À reculons, à reculons»
Guillaume Apollinaire

Introduction

In the early 2000s, the Brière marshes, in Loire-Atlantique, still harboured a remarkable aquatic life. A broad diversity of fish, amphibians, invertebrates, flourished among the vegetation beds. In 2013, these 170 km2 of wetlands now offer quite a different sight. Out of 38 plant species, 17 are not there any more, and 16 have rarefied (Parc naturel régional de Brière). Frogs have gone mute, grass snakes and water beetles are no longer found, not one water lily has been seen since 2006 in the now turbid waters. Red swamp crayfish were reported in the marshes for the first time in 1987, after a probable escape from a breeding farm. Over a ten-year time span, its populations soared and have now reached densities of around 30 individuals *per* square meter in certain ponds. Thus, within a few years the Brière marshes have become a typical case of an ecosystem transformed by an exotic crayfish species. These invasions currently affect, at various levels, a large number of aquatic habitats in France, from the Brenne ponds to the Rhone delta, and from sandpits in the Cher county to brooks in the Ardèche county.

Such a situation is rooted in the past. The **spiny-cheek crayfish** (*Orconectes limosus*), first introduced in our waters as early as 1911, has been the most widespread crayfish in France for half a century. In the early 1970s, it was joined by other species that also came from North America. Among these, signal crayfish (*Pacifastacus leniusculus*) and red swamp crayfish (*Procambarus clarkii*) are formidable invasive species: the rapid development of their populations, even faster in the last decade, has reshuffled the cards in many French streams and ponds. Meanwhile, native crayfish – white-clawed crayfish (*Austropotamobius pallipes*) to start with, once the most widespread in our streams – have undergone a worrying decline and been replaced by the newcomers in numerous habitats.

The importance of the ecological and economic impacts induced by these invasions is now broadly recognised. At a time when the recovery and the preservation of the good state of aquatic environments impose themselves as essential ecological and societal stakes, the issue of invasive crayfish constitutes a major concern for numerous water managers and stakeholders. A growing need for more knowledge and tools has been expressed in the last ten years: what is the way of life of the different invasive crayfish species? Where can they be found? How do they colonize natural environments? What are precisely the consequences of their proliferation for aquatic environments? And above all, what operational ways do we have at our disposal to tackle them, limit their propagation and restore impacted ecosystems? In order to meet these needs, and following studies already carried out on the subject, a research effort so far unprecedented in France has been led since 2010. It mobilized a number of partners (the Inra, the CNRS, the PNR Brière and the Onema) on a pilot area: the Brière marshes. The presentation of these studies, along with others carried out elsewhere in France, from the Vosges to the Camargue, were presented during the first "French meeting on invasive exotic crayfish". Orchestrated by these institutions, in collaboration with the Forum des Marais Atlantiques, the meetings gathered about 120 managers and scientists at Saint-Lyphard (Loire-Atlantique), at the heart of the Brière marshes, on the 19th and 20th of June, 2013.

The contributions to these two days, which offered a wealth of new information but also of questionings, fuelled the present summary. After on overview of the different species inventoried in France, of their dynamics and regulatory status, the summary presents a set of new data about the impacts of invasive crayfish on environments – from their modes of colonization to their effects on foodwebs. The third section gives an account of the efficiency of the different management and control methods presently available, based on the feedbacks of various field experiments.

What is an invasive species?

An invasive species, also called invading exotic species, is an exotic species that settles in natural or semi-natural habitats or ecosystems and usually gains in importance due to the development of abundant populations.

An invasive species can be detrimental to ecosystems:

- it alters the composition, the structure and /or the functioning of communities ;
- it is detrimental to crops and cattle ;
- it is harmful to human health ;
- it has important economic impacts…

1 Crayfish in France: present situation and temporal trends

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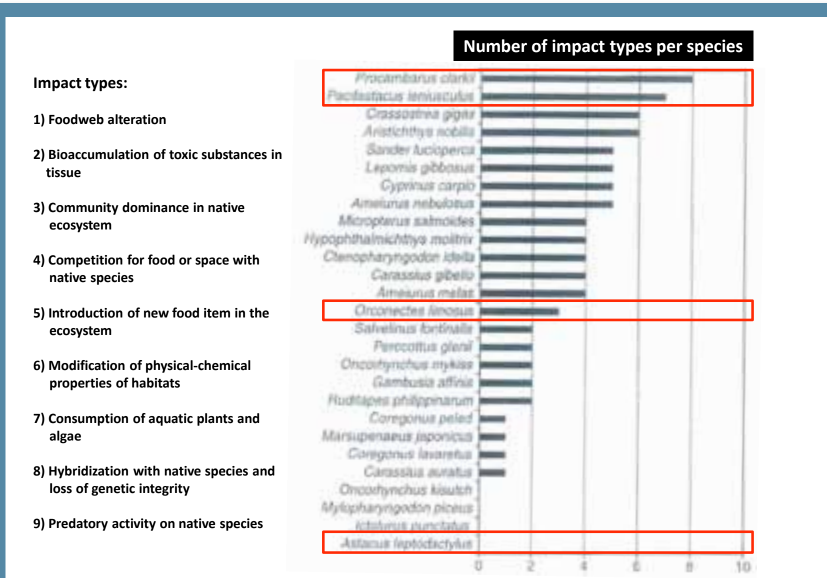
Approximately 600 crayfish species are currently inventoried around the world. These freshwater crustaceans are distributed into three families: *Astacidae*, which include all European crayfish and several species of the West coast of North America ; *Cambaridae* (two-thirds of the species), which gather species from North America and the Far East ; and finally Parastacidae, which gather species from the southern hemisphere – more particularly Chile, Madagascar, Australia and New-Zealand. As early as the late XIXth century, and especially during the XXth century, the globalisation of commercial trade, the opening of new waterways and numerous crayfish introduction and farming attempts have brought about deep changes in these original distribution areas: some species, especially in Europe, declined within their original distribution area, while others got acclimated to new environments, following repeated introduction episodes, most of them unintentional.

Part of these exotic crayfish acclimatized and some of them turned out to be very successful invasive species. A classification of animal species in terms of their invasive potential for

European aquatic environments (Savini *et al.*, 2010) ranks red-swamp crayfish and signal crayfish in the first place – far ahead of other well-known invaders such as catfish *Ameiurus melas* or pumpkinseed *Lepomis gibbosus* as regards their impacts on host environments (Figure 1).

In France, nine crayfish species are presently found in natural environments. In addition to the three native species, all three on the decline, six introduced species are also found, and among them three at least are recognised as invasive species. This first section offers a quick overview (*M. Collas, Onema*) of these species, of their distribution areas in France and their regulatory status. The evolution of their respective distributions was detailed based on a European study (Souty-Grosset *et al.*, 2006) and on five national surveys – aiming at pointing out the presence or not of species at a county scale – by the Conseil supérieur de la pêche (CSP) over 35 years (1977, 1990, 1995, 2001 and 2006), and updated by the Onema in 2013 (Collas and Burgun, 2013, unpublished data).

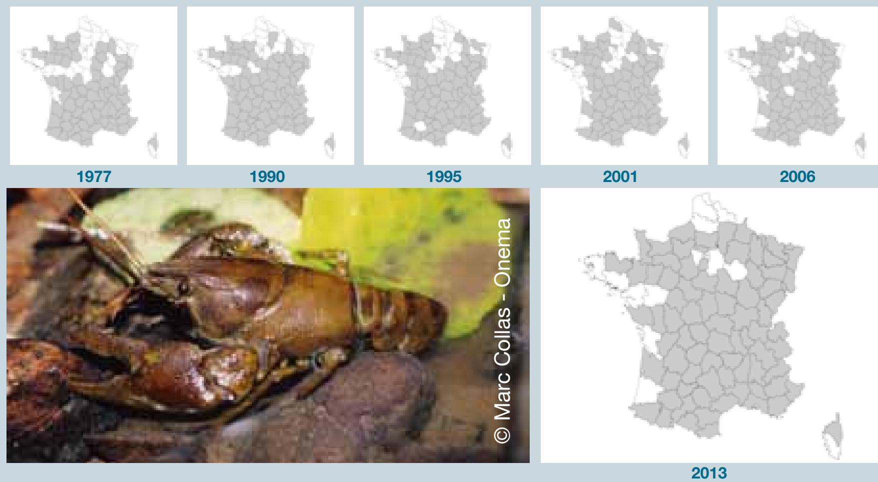
Figure 1. Ranking of the most disturbing animal invasive species for aquatic environments in Europe, according to the diversity of admitted impact types. Crayfish are in red boxes (Source: C. Souty-Grosset, Poitiers University, from Savini et al., 2010).



1.1 – Native species: a worrying situation

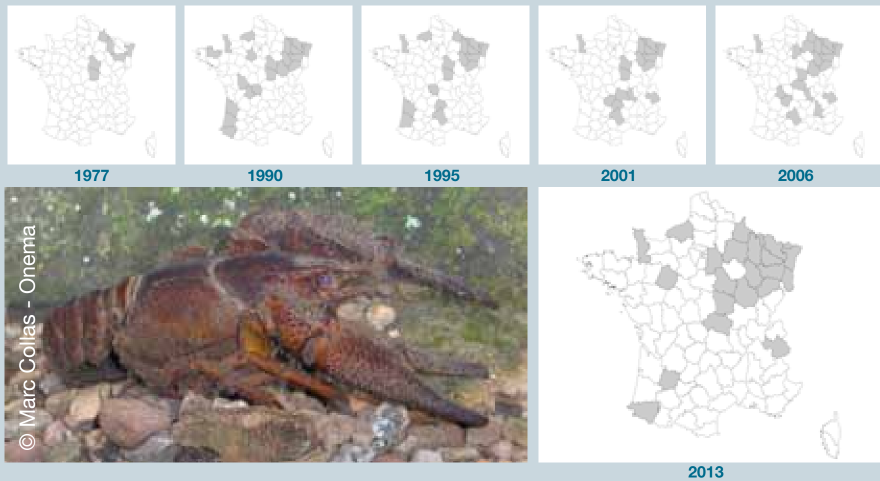
The white-clawed crayfish (*Austropotamobius pallipes*) is the best-represented native species in France. It is only found in the West of Europe – from the British Isles to ex-Yugoslavia, and from North-West Spain to Germany – it inhabits lotic, cold, clear waters. Although it is still recorded in 79 counties, this emblematic species – part of French heritage and gastronomy – has severely declined, as testified by numerous aquatic environment managers (Figure 2). Its populations are more and more fragmented and driven upstream of watersheds. It is affected by the evolution of environments toward more and more artificial systems, by chemical pollutants, and it is sensitive to water temperature. Therefore it suffers from the fierce competition with invasive species such as signal crayfish. Crayfish plague *Aphanomyces astaci* is another important factor of its decline (see section 2.2). It is considered as "vulnerable" in France by the International union for conservation of nature (IUCN). The electrofishing campaigns led by the CSP and the Onema showed a 7% decrease in its average occurrence between 1990 and 2009, and nearly 25% drop of the average density of its populations.

Figure 2. White-clawed crayfish – county distribution in France, from 1977 to 2006 (inset) and in 2013 (main map) (Source: M. Collas & V. Burgun, Onema).
NB : this map is in no way an illustration of population densities. The finding of only one population, even highly localised, is sufficient for a county to appear in grey (meaning that crayfish are present).



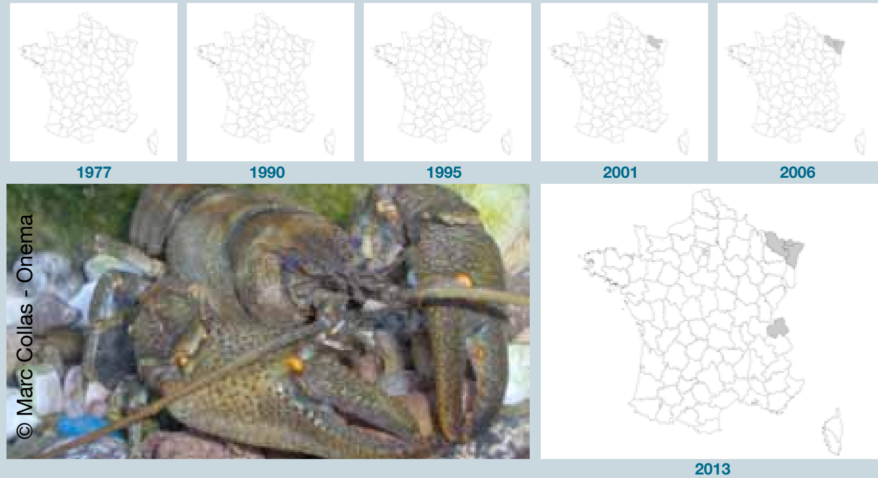
The **red-clawed crayfish**, or “noble” crayfish (*Astacus astacus*), is another native species. It was once quite widespread in Scandinavian and northern Europe streams. It is no longer found in plain rivers, which were once its choice habitats, and is now only found in private ponds and undisturbed streams, often thanks to reintroductions. It was recorded in 21 counties in France in 2013, and is considered as “endangered” in France by the IUCN (Figure 3).

Figure 3. Red-clawed crayfish – county distribution in France, from 1977 to 2006 (inset) and in 2013 (main map) (Source: M. Collas & V. Burgun, Onema).
NB : this map is in no way an illustration of population densities. The finding of only one population, even highly localised, is sufficient for a county to appear in grey (meaning that crayfish are present).



Finally, the **stone crayfish** (*Austropotamobius torrentium*) reaches in France the western limits of its distribution area - Central Europe down to the Black Sea region. Four populations are recorded in France, in three counties: the Moselle, Bas-Rhin and Haute-Savoie counties (Figure 4). This crayfish is in decline in its whole distribution area, and is consequently considered as critically endangered in France by the IUCN.

Figure 4. Stone crayfish – county distribution in France, from 1977 to 2006 (inset) and in 2013 (main map) (Source: M. Collas & V. Burgun, Onema).
NB : this map is in no way an illustration of population densities. The finding of only one population, even highly localised, is sufficient for a county to appear in grey (meaning that crayfish are present).



These three native species are globally impacted by the degradation of their natural environments and are threatened by the development of invasive crayfish, so they are the targets of specific preservation measures. Their habitats are protected by the decree of July 21st, 1983, modified by the decree of Jan. 18th, 2000, which adds stone crayfish to the list, and they are listed in annexes 2 or 5 of the European directive N° 92/43/CEE also called “Habitats, Fauna, Flora”. Yet, fishing is still allowed 10 days in the year.

1.2 – Spiny-cheek crayfish: the first invasion

Originally from the East coast of the United States, the **spiny-cheek crayfish** (*Orconectes limosus*) is present in 21 European countries (Holdich *et al.*, 2010), and has long been the most frequently found crayfish in French freshwaters. It first came to Europe in 1890: about one hundred individuals were imported into a pond by a German fish farmer, wherefrom they colonized the Oder basin. In France, an attempt to breed the species was led at the fish farming station of

Fécamp as early as 1898, but it was rapidly halted due to the burrowing activity of the species. In 1911, two thousand specimens imported from Cologne were transferred into the Cher river, near Saint-Florent du Cher (P. Buffault, 1925). The species has a broad ecological spectrum (it inhabits all types of aquatic environments, from streams to ponds and reservoirs), so it gradually spread step-by-step to the whole territory, quite helped by intentional introductions.

The surveys carried out by the CSP and the Onema in 2001, 2006 and 2013 recorded it in 95 mainland counties: only the Lozère county still appears to stand apart (Figure 5). Such a generalized and relatively long-lasting presence – it is occasionally called "common" crayfish – should not overshadow its pronounced invasive character. In particular, it greatly contributed to the decline of native crayfish during the XX[th] century by directly competing with them and transmitting the pathogen Aphanomyces astaci (see 2.2). Its average occurrence values in the CSP and Onema surveys increased by 50% between 1990 and 2009, while the mean density of its populations remained stable.

Figure 5. Spiny-cheek crayfish – county distribution in France, from 1977 to 2006 (inset) and in 2013 (main map) (Source: M. Collas & V. Burgun, Onema).
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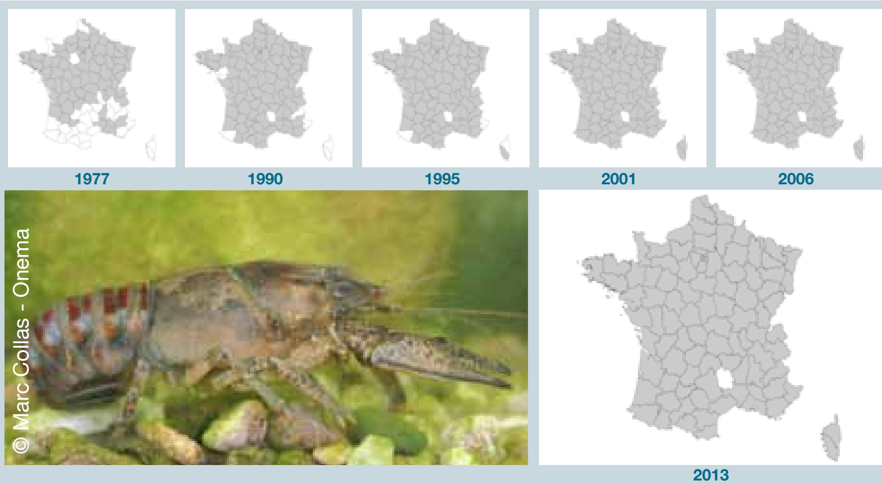


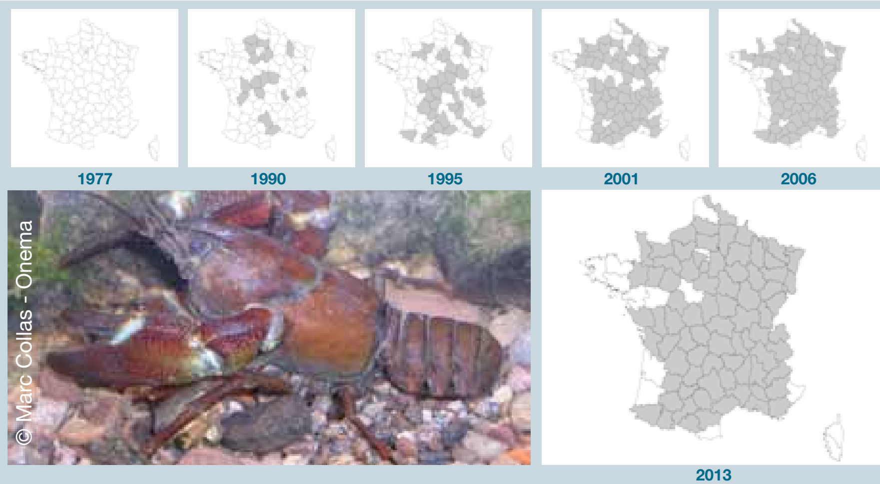
1.3 – Signal crayfish: an accelerated progress

The **signal crayfish** (*Pacifastacus leniusculus*) first arrived in Europe more recently. It originally came from the West coast of the United States. Called "signal crayfish" after the white or light-blue blotches on its claws, it was first imported into Scandinavian countries for repopulation purposes: in Sweden, where it was introduced into 260 lakes and streams between 1960 and 1982, and then into dozens of lakes in Finland between 1967 and 1974. In 1972, the first congress of the international association of astacology presented a positive record of these experiments that led to the introduction of the species into many European countries.

In France, acclimation attempts were made in the 1970s, especially in the Ain, Haute-Savoie and Yonne counties. Several fish farms started breeding it and offered live specimens for sale. Then the species rapidly colonized French waters, often starting from ponds where it had acclimated, and favoured by the growing fancy for fishing it. It was recorded in 61 counties in 2001, in 73 counties in 2006, and in 79 in 2013 (Figure 6).

Robust and aggressive, the signal crayfish is fond of quiet waters, even though deep (the lake of Geneva or the lake of Annecy harbour sizeable

Figure 6. Signal crayfish – county distribution in France, from 1977 to 2006 (inset) and in 2013 (main map) (Source: M. Collas & V. Burgun, Onema). ***NB : this map is in no way an illustration of population densities. The finding of only one population, even highly localised, is sufficient for a county to appear in grey (meaning that crayfish are present).***



Dispersing red swamp crayfish

populations), but it also develops very well in rivers by progressively spreading upstream of drainage basins. It directly competes with native crayfish populations and systematically replaces them (transmitting *Aphanomyces astaci* often helps). In the United Kingdom, it is expected to have thoroughly replaced white-clawed crayfish by 2030 (Souty-Grosset *et al.*, 2006). The development of that invasive species soared between 1990 and 2009 (CSP and Onema surveys): + 80% in average occurrence values, + 90% in average density values.

1.4 – Red swamp crayfish, a world conqueror

Originally from the South of the United States and the North of Mexico, the red swamp crayfish (*Procambarus clarkii*) is presently the most widespread crayfish in the world. It is produced in high quantities for commercial trade in the United States. It was introduced in East Africa (Kenya, Sudan, Zambia…) and in Spain as early as 1973, and was then massively imported from these countries into France for human consumption: between 1976 and 1984, Kenya and Spain respectively supplied 170 and 126 tons of live crayfish that were stored in tanks on their arrival, following the “resoaking” technique. Intentional acclimations occurred in many ponds and fish farms, before and even after the decree of July 21st, 1983 that yet forbade the introduction of the species. It can develop very abundant populations within a very short time. In France, it has been spreading faster over the last decade: Onema and CSP surveys confirmed its presence in 49 counties in 2001, in 67 counties in 2006 and in 73 counties in 2013 (Figure 7).

This invasive species has a short life cycle (2 to 3 years lifetime, versus around 10 years for signal crayfish), it is aggressive and fecund, and it stands out due to its high ecological plasticity. Its omnivorous and opportunistic feeding diet enables it to adapt to highly diverse habitats. Unlike most other crayfish, it tolerates turbid, poorly oxygenated, but also brackish waters. It is also amphibious and can thus live through prolonged dry periods and colonize new environments by overland. It is fond of stagnant waters – ponds, canals, marshes and swamps – but also colonizes quiet streams. As it has a burrowing behaviour, it digs burrows down to 2 m deep in the banks and uses them as shelters. This excavating activity increases water turbidity (it is referred to as a bioturbating species). Finally, similarly to most introduced crayfish, it is the vector of aphanomycosis but also of chytridiomycosis, a lethal infectious disease for amphibians. Despite the disastrous degradations it induces in the natural environments it colonizes, its worldwide production in fish farms has been multiplied more than 10-fold in the last decade, to reach an annual 600,000 tons in 2010 (FAO).

Figure 7. Red swamp crayfish – county distribution in France, from 1977 to 2006 (inset) and in 2013 (main map) (Source: M. Collas & V. Burgun, Onema).
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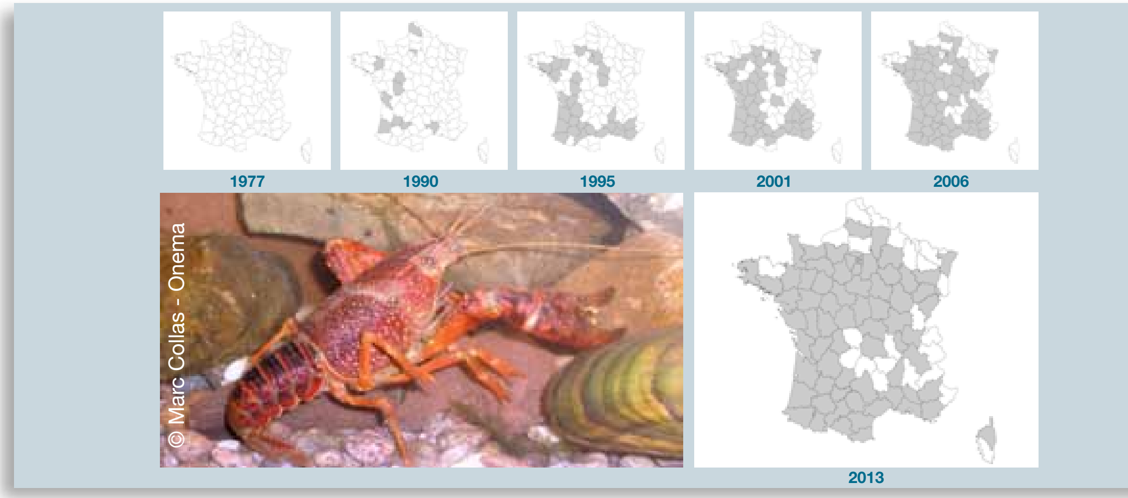

Focus

Lorraine, Morvan: a valuable contribution to the national survey

In order to refine the national survey, actions on a regional scale can provide valuable data.

Here is an example from the Lorraine region: after red swamp crayfish were first found in ponds of the Meurthe basin in 2008, the FDAAPPMA (Fédération des associations agréées pour la pêche et la protection des milieux aquatiques) launched in 2010 a study to get as thorough a view as possible of crayfish populations throughout the region. Thanks to this operation funded by the Agence de l'Eau Rhin Meuse, the DREAL (Direction régionale de l'environnement, de l'aménagement et du lodgement), the regional Council and the FNPF (Fédération nationale de la pêche en France), with technical support from several partners including the Onema and the Parc naturel régional, it was possible to lead more than 400 hunts within 18 months on the whole local hydrographic network (*P. Pommeret, FDAAPPMA Lorraine*). The survey led to the finding of numerous exotic crayfish populations (36 new stations altogether), but also of so far unheard-of local populations (11 new stations for white-clawed crayfish, 26 for red-clawed crayfish). "Exhaustive" regional mappings were drawn for each species. The operation led to the creation of a regional database and to the distribution of various first-information documents targeting fishermen and the general public, including a crayfish identification guide, edited by the Fédération de pêche de Lorraine.

Three hundred km away, another study investigated the evolution of signal crayfish populations in the Parc naturel régional du Morvan. The study (*L. Paris, PNR Morvan*) consisted in gathering all the available data, *i.e.* electrofishing data from the Onema, surveys led by the PNR Morvan since 1990, and a database from the Yonne fishing Federation, enriched and updated by more than 1,000 observations about the signal crayfish. The analysis of these data provides very concrete information about its temporal colonization dynamics. The species settles in all kinds of environments, from ponds and quiet watercourses to mountain streams. It rapidly colonizes streams, upstream and downstream, but also across drainage basins. Its populations spread upstream at a speed of 2 to 4 km *per* year in the main stream (the Brinjame river) and at a speed of 800 m *per* year in the tributaries (Figure 8). In one of the tributaries, it caused the loss of a whole population of white-clawed crayfish in less than a year.

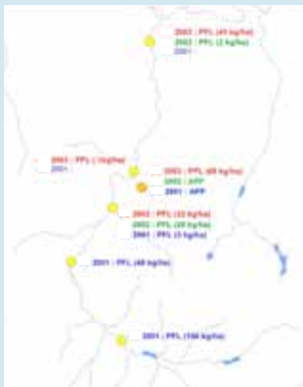

Figure 8. Progress of signal crayfish (PFL), and extinction of white-clawed crayfish (APP) in the Brinjame basin (PNR Morvan) between the summers of 2001 and 2003 (Source: L. Paris, PNR Morvan).

1.5 – Other exotic species, present or to come

In addition to the three invasive species presented above, three other exotic crayfish species are inventoried in France. The **narrow-clawed crayfish** (Figure 9) or Turkish crayfish (Astacus leptodactylus) is the only non-American species: its original distribution area covers central and eastern Europe. It was imported into France for human consumption and also introduced into farms from the 1950s. It was recorded in 53 counties in 2013. It lives in quiet waters, where its presence usually remains inconspicuous. Barely invasive or not invasive at all, rarely abundant and sensitive to aphanomycosis, it is considered as 'naturalised" in France.

The two latest comers are the **Kentucky river crayfish** (*Orconectes juvenilis*) and the **calico crayfish** (*Orconectes immunis*). They both originally came from the East of the United States (Figure 9). The former was first found in a tributary of the Doubs river in 2005 (Collas *et al.*, 2007). The latter was observed for the first time in Alsace in 2010 and is presently colonizing the Rhine tributaries (Collas *et al.*, 2012). The invasive character of these species remains to be confirmed.

More guests could well be added to the list in the near future. Dodging all regulations, tropical fish shopkeepers

Figure 9. From left to right: narrow-clawed crayfish, Kentucky river crayfish and calico crayfish.

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offer many exotic species for sale, among which species from Australia (such as Cherax species) and marbled crayfish, not to mention others. That trend has been gaining new momentum these last years with the development of internet sales and crayfish exchange markets (Figure 10).

Figure 10. Example of a private internet ad offering an exotic crayfish for sale (Source: www.leboncoin.fr).

1.6 – Complex, inadequate regulations

For a long time, crayfish have remained untargeted by any kind of regulation in France. The first bill that provides a regulatory framework for the subject is the 1983 decree "relative to the protection of crayfish in France". It states that the marketing, import and transport of live spiny-cheek, signal and red-swamp crayfish requires official authorisation. The "fishing" law of 1984 introduced new clauses in terms of species introduction: it mentions for the first time the notions of "unrepresented species" and of species "likely to cause biological disturbances". In both cases, it forbids the intentional or inadvertent introduction of these species into natural environments, whether they are open or closed waters. Then, in 2006, the water and aquatic habitat law (WAHL) abrogated the transport ban for live spiny-cheek and signal crayfish in order to facilitate the exploitation and marketing of their stocks, so that only the ban on red swamp crayfish transport still holds true. Consequently, the different exotic species present in France are under heterogeneous regulatory statuses that can even contradict one another depending on the selected criteria (Table 1).

Tableau 1. Regulatory status of exotic crayfish in France.

	Recorded species? (decree of Dec. 17th, 1985)	*Species likely to cause biological disturbances? (article R 432-5 of the environmental code)*	*Introduction? ("Fishing" law of 1984, article L 432-10)*	*Live marketing, import and transport? (decree of July 21st, 1983, then WAHL in 2006)*
Spiny cheek crayfish	*Yes*	*Yes*	*Prohibited*	*Allowed*
Signal crayfish	*Yes*	*Yes*	*Prohibited*	*Allowed*
Red swamp crayfish	*No*	*Yes*	*Prohibited*	*Subject to authorisation*
Narrow-clawed crayfish	*Yes*	*No*	*Allowed*	*Allowed*
Kentucky river crayfish	*No*	*Yes*	*Prohibited*	*Allowed*
Calico crayfish	*No*	*Yes*	*Prohibited*	*Allowed*

In view of the major ecological stakes related to exotic crayfish, such laws and regulations do not appear adapted to the enforcement of environmental regulations. Consensually speaking, their major flaw lies in the fact that it is impossible to enforce the clauses of the environmental code: although the introduction of exotic crayfish is prohibited, and rightly so, except for narrow-clawed crayfish, prohibition is never implemented in the field for lack of decrees specifying how the laws should be enforced. Moreover, characterizing the "offence" was rendered highly improbable when the restrictions on the transport of live crayfish (except red swamp crayfish) were lifted. Besides, a standardization of the law texts and statuses of these crayfish is needed on the European scale. In the absence of any kind of regulation, the exotic fish and pet sectors remain uncontrollable entry points for American and Australian species, and some of them could generate new invasions in the near future.

Flooded grasslands at Grand-Lieu lake: an attractive habitat for red swamp crayfish

2 **Understanding invasions:**

from colonization to impacts

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How do invasive crayfish colonize new habitats? How do they affect local crayfish populations, and, on a larger scale, the functioning of the ponds and rivers they colonize? What are the consequences of invasions on local socio-economic activities?

Along with the global awakening to the scope of the effects of exotic crayfish invasions, these questions stand out as major research stakes in aquatic biology and conservation biology.

This second section provides a set of new data on the impacts of invasive crayfish that was presented during the Saint-Lyphard seminar. The data mostly result from research studies led about red swamp crayfish in the Brière and the Camargue, and they give it a preponderant place. This state of facts highlights the crucial need for more knowledge about the effects of other invasive crayfish present in France - signal crayfish to start with.

2.1 – The ways red swamp crayfish colonize aquatic habitats: *in situ* studies

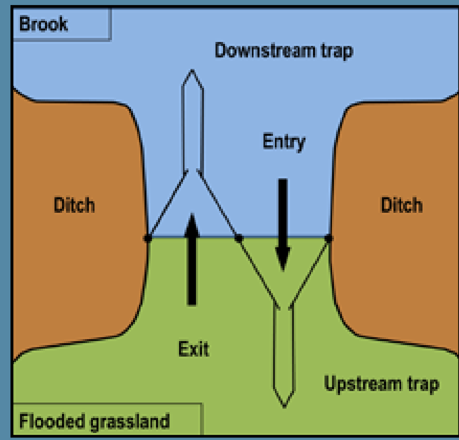
Intentional introductions undoubtedly played a role in the red swamp crayfish colonization processes observed in France. But the specific behaviour of this species shows a natural propensity toward colonizing new habitats. It alternates long stationary phases, during which it hides in its burrows in the daytime and only gets out at night to feed, with dispersion phases during which it can sometimes cover important distances (up to more than 3 km in one day, Gerhardi *et al.*, 2000). Such mobility enables the species to settle into new environments step by step, overland movements included. In spring, *i.e.* the season when their activity is at the top of its intensity, they can more particularly reach flooded zones: at the interface between water and earth, these habitats where food is plentiful can also create a temporary continuum between two separate environments – a sand pit and the neighbouring stream, for example.

What uses for flooded grasslands?

An *in situ* study (*F. Fonteneau, Rennes 1 University*) aimed to decipher red swamp crayfish movements between flooded zones (grasslands) and Grand-Lieu lake (Loire-Atlantique). That large, shallow lake is strongly colonized by red swamp crayfish, and its water surface can vary from 5,000 hectares in winter to 2,000 hectares in summer. Its floodplain grasslands, which spread on very large surfaces, constitute a key environment for the breeding, feeding and growth of many animal species, ranging from fish to birds. More specifically, the study focused on characterizing the movements of red swamp crayfish, but also of fish, between the so-called «Bonhommes» flooded grasslands (28 ha) and the lake, in order to assess the response of these animals to the degradation of their living conditions that goes along with the progressive decrease in water levels. To that end, from April 14th to May 18th, 2012, when grasslands had run almost completely dry, fine-mesh funnel traps were placed at the inlets and outlets of each connexion between the grasslands and a brook that leads to the lake (Figure 11).

Funnel traps were lifted daily throughout the study period. Crayfish and fish were identified, measured and counted in each trapping direction (fish were released in the same direction as they had been caught while crayfish were destroyed, following regulations). Crayfish cohorts were analysed according to two size classes.

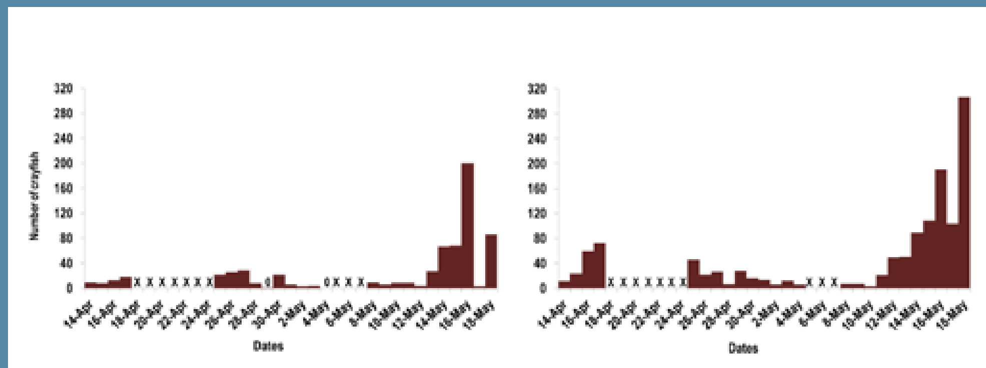
Figure 11. Schematic diagram of the trapping device set up between the stream and the flooded grasslands of Grand Lieu lake (Source: F. Fonteneau, Rennes 1 University).



Over the study period (while the water level was dropping), 647 crayfish were caught while getting into the grasslands, and 1,278 while getting out of them. Crayfish were the only species that moved in or out while the water level was dropping (Figure 12). Fish (691 young pike, but also roach, rudd and topmouth gudgeon) were only caught on the way out, which indicates that they got into the grassland earlier during the flooding phase.

The time-course of crayfish movements, on the way in as well

Figure 12. Red swamp crayfish movements between Grand Lieu lake and a flooded grassland. Left: on the way in (from the lake to the grassland), right: on the way out (from the grassland to the lake). X: no trapping performed while 0: zero catch (Source: F. Fonteneau, Rennes 1 University).



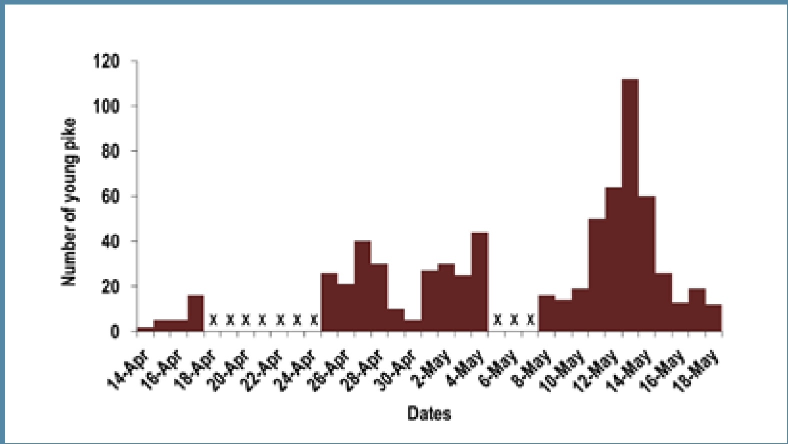


Figure 13. Young pike movements on the way out of the flooded grassland, toward Grand Lieu lake. X: no trapping performed (Source: F. Fonteneau, Rennes 1 University).

as on the way out, was particularly important in the last five days before the grasslands ran completely dry. Small-size individuals were those that migrated the latest, whichever the direction. The catch "record number" was reached the day before the grasslands were completely dry, with 320 individuals caught on their way out. Thus, when the water level drops, crayfish movements take place rather later than fish movements out of the grasslands – as in the case of young pike (Esox lucius), whose catches peaked one week before trapping came to an end (Figure 13 above).

These specific crayfish movement profiles can be related to the relatively good tolerance of the species to water warming, up to 32°C and more. They evidence the capacity of the invasive to exploit the food resources of flooded grasslands down to the last moment, and confirm that flooded grasslands play the role of nurseries for juveniles.

Possible overland colonization: from pond to pond in the Parc naturel régional de Brière

The unconnected marshes of Mès and Brière are both located within the boundaries of the PNR Brière. They offer an ideal field for studying *in situ* the modes of colonization of more or less isolated ecosystems (pond networks). Crayfish population density, expressed in CPUE (catch *per* unit effort), was higher in the Brière marsh (CPUE values ranging from 5 to 7 crayfish/trap/24hrs) than in the Mès marsh (CPUE value around 2.5 crayfish/trap/24hrs). A study (*A. Tréguier, Inra and Rennes 1 University*) aimed to characterize the propagation of the invasive through the two pond networks nearby these marshes as related to diverse topographic variables.

The first step consisted in recording crayfish presence in each of the two networks - 158 ponds in total. The survey was carried out using two complementary methods (see section 3.4): trapping using funnel traps lifted daily, to provide an indication on crayfish abundance in ponds, and environmental DNA, to increase the chance to detect the species, especially when it is present in small numbers. In the Mès pond network, 37% of the ponds were occupied by the species, and 60% in the Brière pond network (Figure 14).

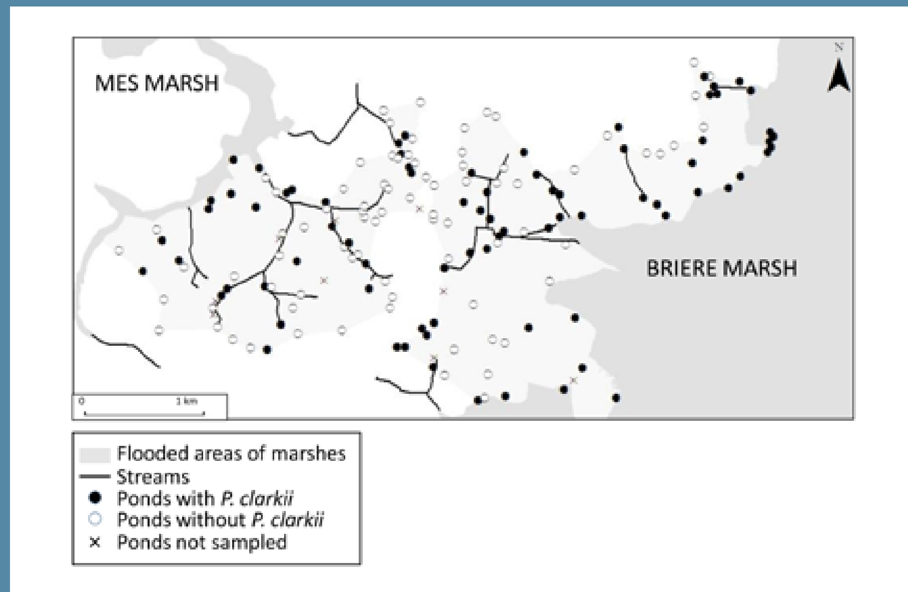


Figure 14. Distribution of red swamp crayfish across the pond networks close to the Mès and Brière marshes (Source: A. Tréguier, Inra and Rennes 1 University).

Using these distribution data, a set of statistical models was built to characterize the effect of different variables on the presence or the absence of red swamp crayfish in any given pond: the distance from the source environment (Brière or Mès marshes) ; "propagule pressure" (comparable to crayfish density in source environments or to the number of neighbouring ponds occupied by crayfish) ; pond perimeter, and other physical descriptors for ponds.

Results are clear-cut in the case of the Brière marsh pond network, where propagule pressure was the highest. The preponderant factor turned out to be the source environment: a pond stands all the more chances of being colonized as it is closer to the marsh. The probability for a pond to be invaded also increases along with its perimeter, and with the number of colonized ponds within a 50-meter radius. However, correlations were far less high for the Mès pond network, where the distance from the source environment did not appear to have such a marked effect. Several hypotheses can account for this fact, among which weaker propagule pressure, or dispersal caused by other animals, especially predators, or by humans. The effect of landscape variables (for example the presence of hedges that limit connectivity or the nature of terrestrial habitats) remains hard to grasp. Monitoring the evolution of their distribution in the years to come could allow for finer analysis.

2.2 – "Crayfish plague": a lethal danger for native species

In 1860, very high crayfish mortality rates were reported in the Po basin, in Italy. The crayfish were seen wandering in full daytime, very weak and then dying, they had dark blotches on their carapace. Dead individuals displayed whitish outgrowths on the ventral face of their exoskeleton and on their eyes. The mysterious ailment, soon called "crayfish plague", rapidly spread across Europe, from the Netherlands to the Ukraine and to the shores of the Baltic Sea. In 1876, the first epizootics (diseases that affect all individuals) were reported in the Alsace and Lorraine regions: in 1895, two-thirds of the French territory had been hit.

The disease was associated to a parasitic "fungus", *Aphanomyces astaci* (Schikora, 1903), that belongs to the Oomycete class. Since then, it has caused huge numbers of population extinctions in Europe: also called aphanomycosis, it is still a major cause for the decline of native crayfish nowadays. Yet, it still remains poorly documented, and since 2010 it has been the target of an epidemiological research programme funded by the Onema. Present European knowledge and research approaches about the subject were presented during the Saint-Lyphard seminar (*F. Grandjean, Poitiers University*).

Crayfish plague is fatal for native crayfish

Invasive species as latent carriers and vectors

Aphanomycosis first appeared in Europe before the first crayfish species from America (*Orconectes limosus*, see section 1.2) was introduced. But exotic crayfish invasions play an important role in its propagation, especially upstream, and in its transmission to native crayfish. North American crayfish are "healthy" carriers of the parasite: when they are infected, a very quick immune response is triggered, with increased melanin production, this allows for their cuticle to heal around the encysted spores of the parasite. The immune response of European species is comparatively much slower: the plague degrades their chitin and their tissues in-depth until death occurs, usually in 100% of the cases.

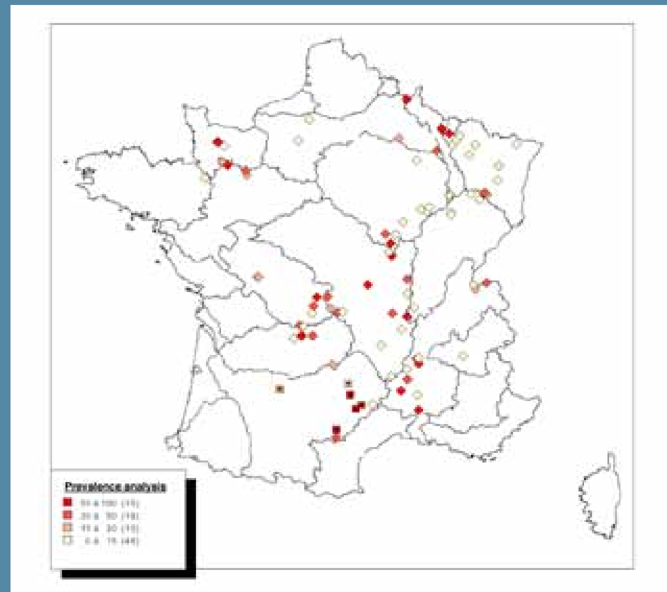
Infection, which reaches only crayfish, is transmitted by diseased individuals and bodies, which remain carriers of the pathogen for a long time. However, infected crayfish lose their contagious character at each moult. This feature explains why females, which moult less often when they are gravid, are generally struck more than males. The pathogen can also be transmitted by fish or other animals coming from contaminated zones, or by objects (gloves, boots…) that were once in contact with the spores. That last point implies systematic disinfection of materials for the field staff who have to deal with an epizootics.

A growing research effort

Although aphanomycosis has been the subject of a growing monitoring and research effort for a decade, it still wreaks havoc among crayfish populations. In the Czech Republic, ten epizootics were recorded between 2004 and 2010, causing the loss of eight noble crayfish populations and two white-clawed crayfish populations (Kozubíková *et al.*, 2008). In Norway, nine mass mortality events have occurred in different regions since 1998. The signal crayfish appears to be particularly implicated in the transmission of the pathogen to native species due to its pronounced invasiveness and its capacity to colonize streams by moving upstream. In France, a survey was led by the Onema and the University of Poitiers to characterize the infestation rate of the species, based on a sampling of 89 populations distributed across the territory: 55 of them turned out to be infested at various degrees (Figure 15).

Similar studies revealed variable infestation rates for signal crayfish again across Europe: 0% in Denmark (Skov *et al.*, 2010), 15% in Hungary (Kozubikova *et al.*,

Figure 15. Rates of signal crayfish infestation by aphanomycosis in France – 89 populations sampled for a total of 1,030 individuals (Source: adapted from the Onema).



2009), 22% in the Czech Republic (40% for spiny-cheek crayfish) (Kozubikova *et al.*, 2009), and up to 86% in Norway (Vrålstad, unpublished).

Detecting the parasite in its early stages is possible by extracting and amplifying its DNA from a piece of the ventral cuticle of a crayfish. Thanks to the development of new molecular tools, recent studies identified several pathogen strains from a given host crayfish. These approaches open new paths for studying aphanomycosis, and especially the role of invasive crayfish in its propagation. Noticeably, they show that the pathogen strains hosted by exotic species are more virulent than the “ancestral” strains present in Europe before the first mortality episodes (Makkonen *et al.*, 2012). In parallel, various observations suggest that resistance to the pathogen is found in some populations of native crayfish (Kušar *et al.*, 2013). Studies are being carried out in Europe to confirm and provide more details about that point.

2.3 – Red swamp crayfish, a major disturbance of foodwebs

Beyond the damage caused to native species by direct competition or by the transmission of aphanomycosis, invasive crayfish are likely to cause disturbances, sometimes on a large scale, on the whole functioning of the ecosystems they colonize. The Saint-Lyphard seminar provided the opportunity to present a number of studies led in the Camargue and the Brière dealing with the place taken by red swamp crayfish in foodwebs.

One crayfish in the pond: fewer plants, fewer invertebrates

A study performed with mesocosms (small-size experimental devices functioning like simplified ecosystems) focused on the impact of red swamp crayfish on the ecological functioning of temporary ponds (H. Rodriguez, La Tour du Valat). Thirty open-air tanks, one square meter in surface and about 300 litres in capacity, were lined with sediment, and then filled with water in December 2010. A full protocol for monitoring the faunistic and floristic composition of each tank was defined: biomass and macrophyte composition were assessed ; micro-invertebrates were sampled and identified ; macro-invertebrates were caught using landing nets, identified and placed back into the same tank. At the beginning of April 2011, the survey evidenced that biological communities similar to the ones that develop in the temporary ponds of the Camargue had settled in the tanks: five macrophyte species and 56 invertebrate species were found on average. One week later, the operator introduced red swamp crayfish into the mesocosms, distributed into three groups: one individual *per* tank in the first group, three individuals *per* tank in the second group – the third group of tanks was left crayfish-free and was used as a control. The study was led from the beginning of April to mid-July. It consisted in monitoring the evolution of biological communities in the three groups of tanks, with one sampling every two to four weeks.

The results are unequivocal. Eleven weeks after only one crayfish was introduced, average macrophyte biomass had dropped by 30% compared to the control tanks (Figure 16). In the tanks that hosted three crayfish, the difference was even more significant.

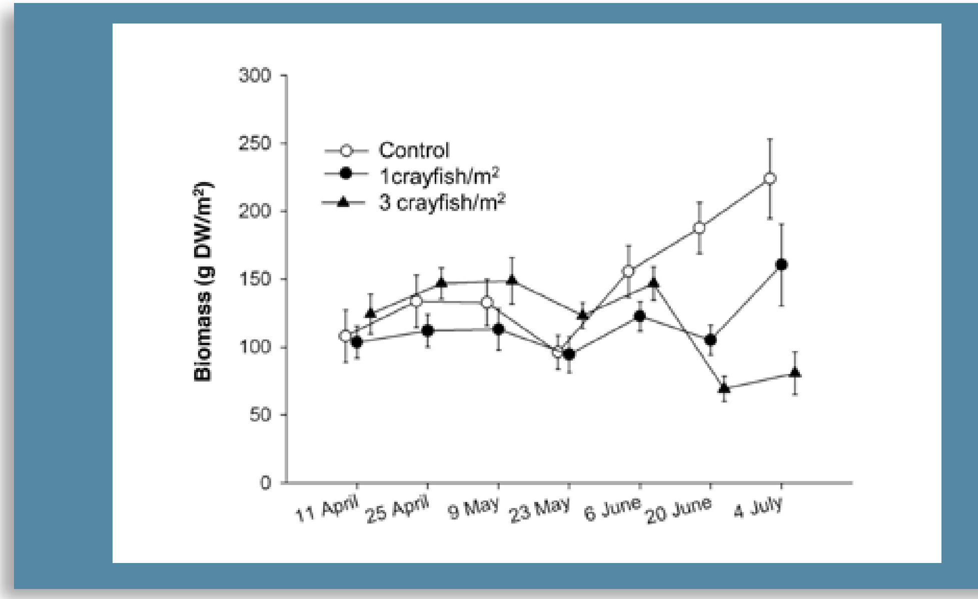


Figure 16. Evolution over time of average macrophyte biomass after the introduction of one or three red swamp crayfish, as compared to control mesocosms (Source: H. Rodriguez,Tour du Valat).

Using mesocosms to study the effects of invasive crayfish on biodiversity

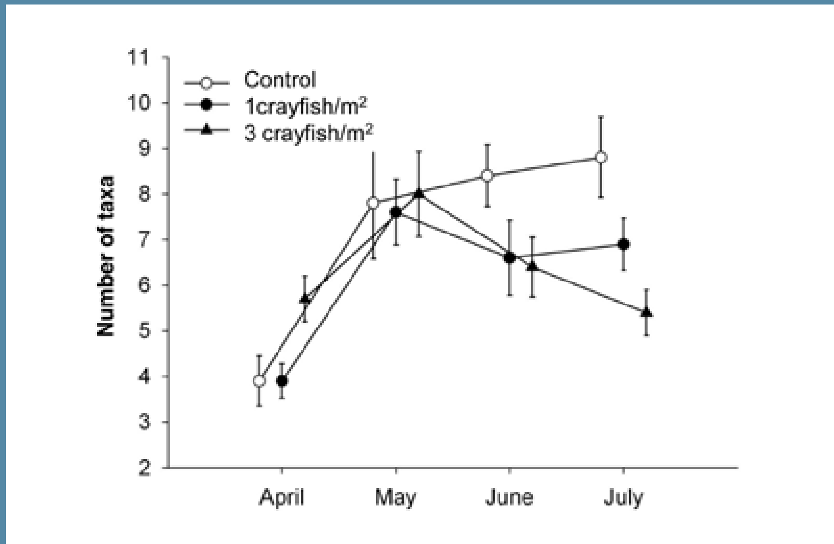


Figure 17. Evolution over time of the average numbers of invertebrate species after the introduction of one or three red swamp crayfish, as compared to control mesocosms (Source: H. Rodriguez,Tour du Valat).

Red swamp crayfish also had a deep impact on the diversity of macro-invertebrate communities (Figure 17 above).

Altogether, this study confirms that red swamp crayfish alter the composition and the structure of plant communities in ponds. They also affect the macro-invertebrate community by reducing its taxonomic richness and the total number of individuals. These impacts appear to be all the deeper as the number of crayfish within the ecosystem is higher.

A central place in the colonized ecosystem

In the Brière, a study focused on the effects of the invasion of aquatic ecosystems by red swamp crayfish (J-M. Roussel, Inra). To that purpose, an analysis of the stable isotopes of carbon (C) and nitrogen (N) in foodwebs was developed. This tool is very often used in ecology studies for it provides synthetic data on trophic organization and ecosystem functioning. Thanks to carbon isotopes, it is possible to track the pathways of the energy used by the different trophic compartments. In aquatic foodwebs, carbon can originate from the fixation of dissolved carbon by primary producers such as phytoplankton (the algal pathway), or from the benthic organic matter that results from the degradation of organisms such as phragmites (the detritus pathway). As for nitrogen isotopes, they provide clues about the trophic level of the different species in the foodweb, *i.e.* primary producers, primary consumers, secondary consumers, up to upper predators.

Three types of aquatic environments were sampled: canals (waterways, permanent water), phragmite reed beds and temporarily flooded grasslands. Samplings were performed exhaustively from aquatic foodwebs, including zooplankton samples, various fish and benthic invertebrate species, and of course red swamp crayfish.

Invertebrates collected to study foodwebs using stable isotope analysis

Nitrogen isotope analysis in these samples first of all evidenced the formidable trophic plasticity of red swamp crayfish (Figure 18). Depending on habitats, it can indeed eat detrital organic matter (primary consumer) or invertebrate preys (secondary consumer). Juveniles mainly depend on phytoplankton and zooplankton production (the algal pathway), more particularly in flooded grasslands, whereas adults rather depend on detrital organic matter.

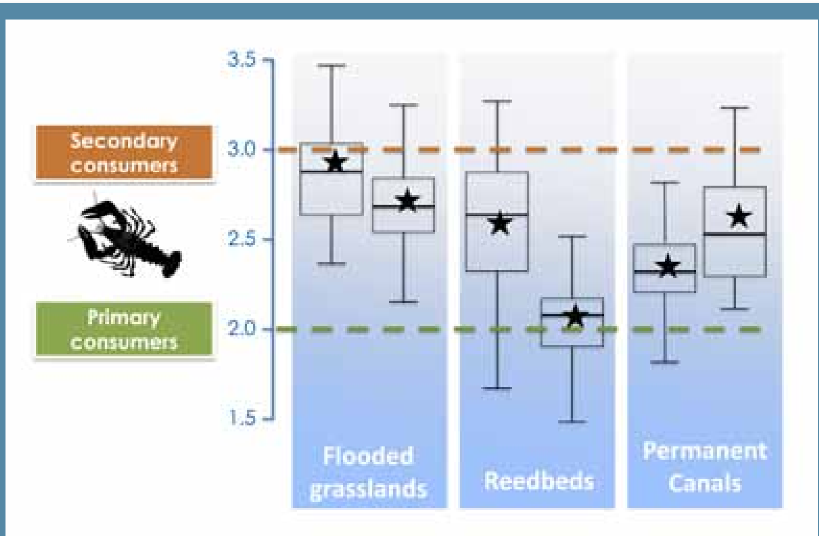


Figure 18. Trophic position of red swamp crayfish according to invaded habitats as revealed by the analysis of nitrogen stable isotopes in the foodwebs of the Brière marshes. Stars indicate mean values, rectangles and horizontal lines represent dispersion of values (Source: J-M. Roussel, Inra).

More generally, the red swamp crayfish has become a central actor in the foodwebs of invaded ecosystems, and has imposed itself as the main link for energy transmission to fish (Figure 19).

The analysis of nitrogen stable isotopes was also applied to the fish of the same marshes. It yielded a stunning observation, *i.e.* nearly all species, from roach to pike, displayed very close trophic levels, just above the level of crayfish (Figure 20). This means that crayfish has become the main food resource for the fish of the Brière ecosystem.

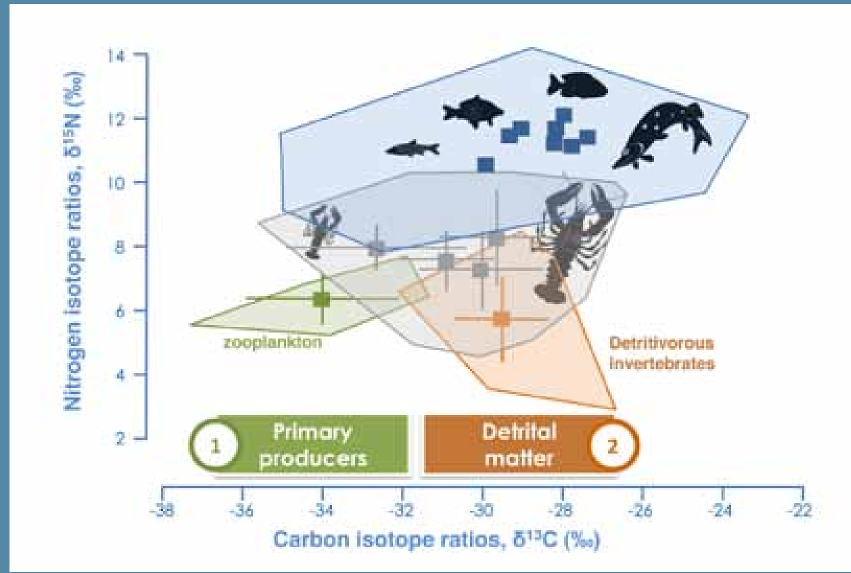


Figure 19. Analysis of carbon and nitrogen stable isotopes in the Brière. Crayfish imposes itself as a key player in the energy transfer from primary producers and detrital organic matter to fish (Source: J-M. Roussel, Inra).

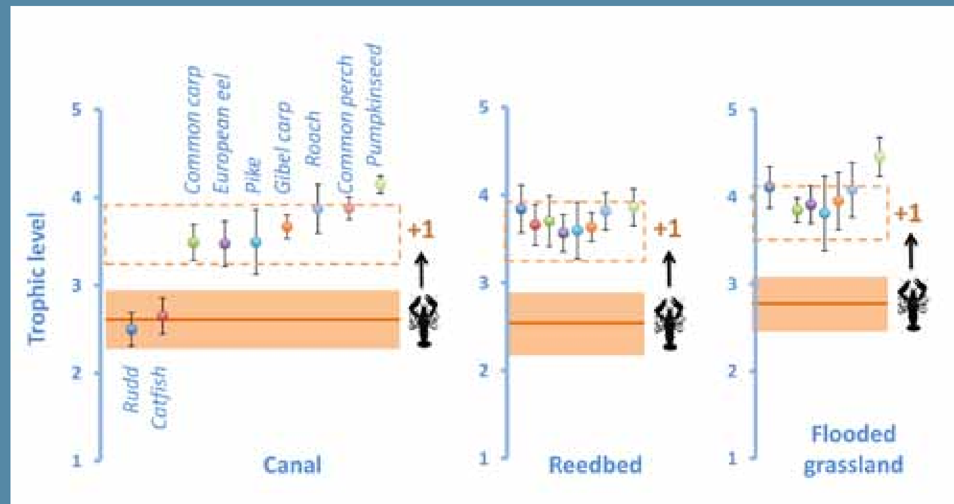


Figure 20. Trophic positions of fish according to the habitats sampled in the Brière, as revealed by the analysis of nitrogen stable isotopes. We can note that in most cases, the different species are placed at a trophic level above crayfish (Source: J-M. Roussel, Inra).

Another study attempted to characterize the role of red swamp crayfish in the Camargue foodwebs (H. Rodriguez, Tour du Valat). Through an analysis of the gut contents of 122 crayfish caught *in situ*, it was possible to get more details about the species' feeding diet. The observations confirmed that the invasive is outstandingly omnivorous and opportunistic, and also confirmed the place taken by detrital organic matter in its food: 84% of the analysed crayfish had eaten debris, and these represented no less than 60%, on average, of total gut content volumes (Figure 21).

The study also assessed the place taken by the invasive in the food of avian predators. Through the analysis of stomach contents again, it revealed that, in the Camargue, red swamp crayfish represented 45% of the fresh-weight feeding diet of white spoonbills (*Platalea leucorodia*) (mean value of 28 individuals) ; 45% for squacco herons (*Ardeola ralloides*) (22 individuals) ; 80% for glossy Ibises (Plegadis falcinellus) (34 individuals) and 85% for cattle egrets (*Bubulcus ibis*) (10 individuals). Previous observations confirm that range of values: in the Camargue again, crayfish represented around 90% of the feeding diet (fresh weight) of Eurasian bitterns (Botaurus stellaris) (Poulin *et al.*, 2007) ; it was also found in the faeces of 68% of European pond turtles (Emys orbicularis) (Ottonello *et al.*, 2005) and in the stomachs of 79% of European catfish (Silurus glanis) (Martino *et al.*, 2011).

Thus, red swamp crayfish have become, in the Brière or in the Camargue, a preponderant resource for numerous bird and fish species. This change has led to an unstable trophic balance, characterized by receding of plant and macro-invertebrate communities and simplified links between organisms within ecosystems.

Populations of predators such as waders, fish or otter sometimes spectacularly increase. The phenomenon is observed with enthusiasm by managers of crayfish-invaded environments, but it can only be a side-effect of invasion. It is a seeming sign of the good health of ecosystems, but it actually relies on a deeply disturbed ecological situation whose long-term evolutionary paths remain hard to foresee.

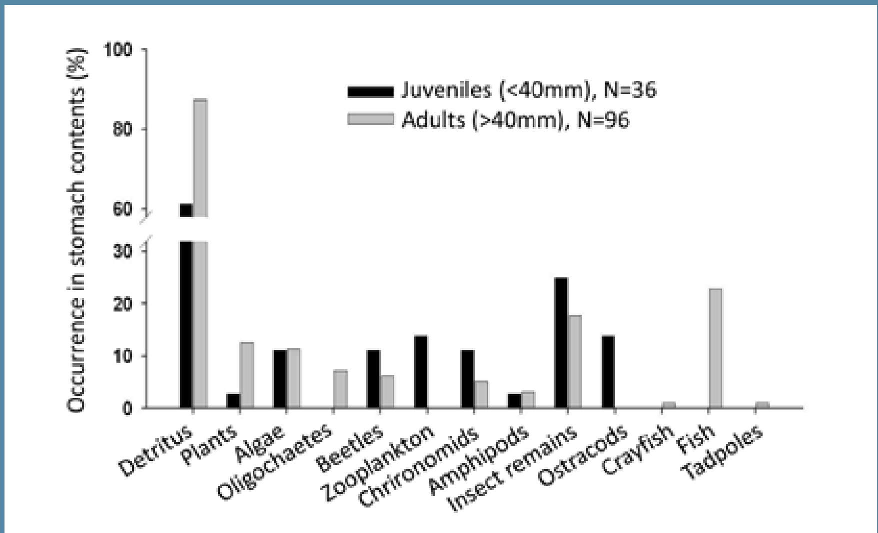


Figure 21. Gut contents of juvenile and adult red swamp crayfish in the Camargue (36 juveniles and 96 adults) (Source: H. Rodriguez, Tour du Valat).

Red swamp crayfish: a prey which is now frequent in the diet of numerous bird species

Eclairage

Are crayfish contaminant bioaccumulators?

Invasive crayfish, due to the central part they tend to take within the foodwebs of invaded ecosystems (see section 2.3), are also likely to play a major role in the transfer of chemical contaminants to the fish, birds and mammals that eat them. Quantitative data about their bioaccumulation potential were brought by a recent study at the scale of the Loire basin (*C. Lemarchand, VetAgro Sup*). The contamination levels of a set of “marker” species (fish, birds, invertebrates…) for environmental conditions, but also of invasive crayfish (spiny-cheek, signal and red swamp crayfish) were measured in nine sites distributed over the whole basin. The author analysed the tissues of nearly 500 crayfish for their content in about fifty compounds, among which the main pesticides present in the river, 16 PCB congeners and heavy metals (lead, mercury, cadmium, copper, arsenic).
The results highlighted that crayfish were systematically contaminated by pesticides (mainly DDT and lindane, at relatively low levels), PCBs (about 9 mg/kg for the three species), and above all heavy metals – copper, cadmium and mercury ahead (Figure 22).

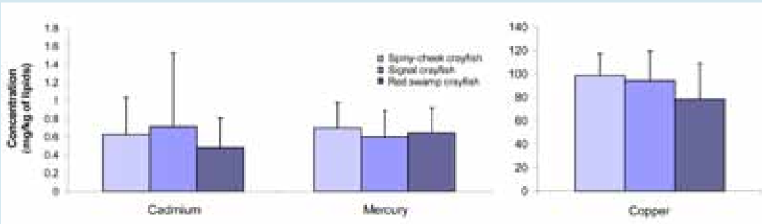


Figure 22. Mean heavy metal concentrations in the invasive crayfish of the Loire basin (mg/kg of lipids) (Source: C. Lemarchand, VetAgro Sup).

For a given compound, the average concentrations observed in crayfish displayed limited variations across species or across sites: the results suggest a global contamination, of the “background noise” type. Conversely, high variations occurred across individuals, suggesting a capacity to get rid of pollutants via moulting. Altogether, invasive crayfish appear likely to promote the transfer of metals to predators – while molluscs (*e.g.* the Asian clam cited below) are comparatively “better” PCB bioaccumulators (Figure 23).

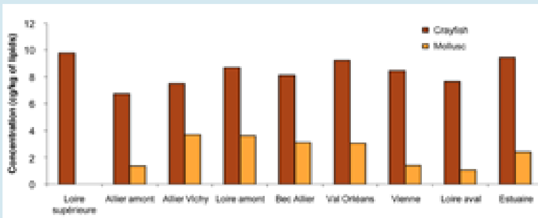
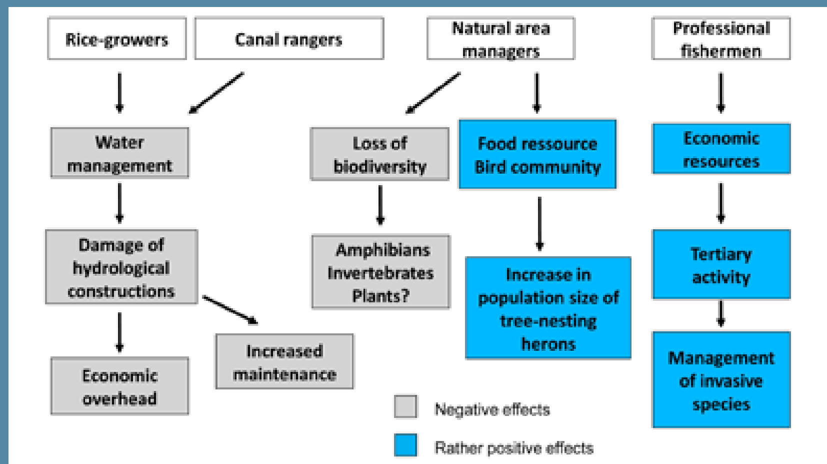


Figure 23. Copper accumulation (cg/kg of lipids) by invasive crayfish as compared to an invasive mollusc, the Asian clam, in the Loire basin (Source: C. Lemarchand, VetAgro Sup).

2.4 – Socio-economic impacts: a first approach in the Camargue

Invasive crayfish are at the origin of important ecological disturbances in certain invaded environments. They are also likely to locally affect socio-economic activities in various ways: impacts on halieutic resources, aquaculture and agriculture, loss of attractiveness related to the degradation of the faunistic and floristic heritage… In order to tackle these effects, a growing need for transdisciplinary approaches linking together ecological and societal impacts, costs and potential benefits of invasions, is being expressed by managers: they are concerned about getting a more global understanding of ecological issues, and also about having access to the “naturalist’s” knowledge developed by various field stakeholders when faced with invasion. Very few studies of that type have been led so far. Among them, a sociological study (*T. Prola, Tour du Valat*) aimed to detail the ways different Camargue stakeholders perceived red swamp crayfish. The methodology was a classical one in social sciences: it relied on semi-directive interviews completed by transdisplinary bibliographical references. The survey, although non exhaustive, implied 19 professionals concerned by crayfish invasion: six rice-growers, four canal rangers, six natural area managers and three professional fishermen. The interviews made it possible to get an insight into the impacts induced by the invasion – whether positive or negative – as perceived by each professional (Figure 24).

Figure 24. Effects of red swamp crayfish invasion as perceived by different stakeholders in the Camargue (Source: T. Prola,Tour du Valat).



These results yield a few surprising lessons. Thus, exotic crayfish are globally perceived as causing few problems or no problem at all by professional fishermen and, to a lesser extent, by natural area managers. The former see crayfish as a new economic resource and a potential diversification source for their activity ; the latter are keen observers of the demographic development of certain species (especially bird species) induced by crayfish proliferation, after the paradox already mentioned in 2.3.

At the opposite end, the sectors in which crayfish arouse most concern are those that strongly depend on water management, *i.e.* canal rangers and above all rice-growers, who see the burrows bored by the invasive as likely to dry up rice fields, and therefore as a threat to their professional activity. Such specific water management is indeed likely to generate favourable conditions for the development of crayfish populations. This fact highlights the whole complexity of the socio-economic effects of biological invasions.

Finally, the results of that study were crossed with a survey led in parallel (Tour du Valat and MAVA Foundation) to assess red swamp crayfish abundance in the different Camargue habitats: rice fields, canals, temporary ponds, semi-permanent marshes and permanent marshes.

The results evidence that the zones most invaded by crayfish – marshes and temporary ponds – are those that arouse the least concern from stakeholders, *i.e.* fishermen and managers. On the contrary, rice-fields are environments where crayfish density is the lowest, and yet where they arouse the deepest concern. This observation shows how difficult it is to grasp the consequences of the development of red swamp crayfish in a given territory along with their socio-economic complexity.

Bank of a canal in the Brière damaged by many burrows of red swamp crayfish

3 **Invasion management:**

which operational approaches?

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The issue of exotic crayfish invasions has imposed itself as a major research topic in Europe for more than a decade. The biology of these species, the mechanisms whereby they colonize natural environments and their combined effects on ecosystems and biodiversity are the subjects of a growing number of scientific studies. The results provide details about the extent of their impacts, often sensed by managers on the field, and confirm the need to develop tools and operational strategies for the preservation of aquatic environments under the threat of or faced with invasion. Yet, paradoxically, the field of invasive crayfish management remains comparatively little investigated at the European scale. To bridge that gap, the research studies led in the Brière, as well as others elsewhere in France, attempted to devote a larger place to the exploration of operational approaches for crayfish invasion management. During the Saint-Lyphard seminar, nearly 50% of oral presentations were definitely focused on management. The data presented on the occasion, which are the topic of this third section, contribute to ranking France among the leading countries in Europe for exotic crayfish invasion management.

3.1 – European overview: numerous approaches investigated

Since 1990, an average 140 scientific articles devoted to crayfish have been published every year in the European literature. However, an analysis of these sources reveals that only a small number of articles is devoted to their impacts, except for red swamp crayfish (48 references in total) and signal crayfish (20 references), and an even smaller number of articles addresses the control and/or the eradication of their populations. This observation can partly be explained by the fact that the importance of the overall issue of biological invasions was only tardily taken into account by European authorities. Thus, only at the end of 2008 did the European Commission publish a communication entitled "Towards an EU strategy on invasive species" (COM (2008) 789 final). In order to make progress about the subject, in 2011 the EU set up three workgroups in charge of (i) prevention, (ii) the monitoring and rapid response system, and (iii) eradication, control, management and restoration.

This last group reaffirmed the necessity to adopt a hierarchical approach for the management of any kind of invasive species, following four generic principles:

- prevention of new introductions
- early detection and rapid response
- eradication when possible
- permanent control of populations when eradication is not possible.

To open the session of the Saint-Lyphard seminar devoted to the means to eradicate or control invasive crayfish populations, a review of the works implemented about that theme was presented (*C. Souty-Grosset, Poitiers University*).

That non-exhaustive overview of current tools (see below) provides general information about their advantages and limitations. It also shows the diversity of the approaches investigated to eradicate or control invasive crayfish.

None of these options can be a "cure-all": altogether, it appears that the best results will be obtained, on a case-by-case basis, by combining several strategies with a view to so-called "integrated control". In all cases, choices should be made as regards the cost induced for the natural environment. Following the overview, several studies and feedbacks at the French scale provided clues for determining the respective efficiency levels of the strategies available for managers.

Trapping

Trapping constitutes a simple strategy that generates limited impacts on natural environments, provided that funnel traps are selective (trap "only" crayfish) and that operators avoid enhancing the bioturbation phenomenon and conveying pathogens as much as possible. However, it is not drawback-free. It is costly in time and manpower, and its efficiency depends on the time of the day and the season (see section 3.3). Besides, it tends to preferentially catch large males, and this lowers the pressure of competition on younger individuals. In the case of signal crayfish, it was thus established that removal by way of trapping finally induces a higher growth rate of individuals within the population, as well as more important migration distances (and therefore a more important dispersal of the species) (Moorhouse and Macdonald, 2011a,b).

Various options have been tested (more particularly Stebbing *et al.* (2004), for signal crayfish, or Aquiloni and Gherardi (2010) for red swamp crayfish) to improve trapping efficiency. The use of **baited** traps makes it possible to significantly increase the number of catch *per* unit effort (CPUE) – especially when combined to a **vibratory signal**. The use of female **sexual pheromones**, or the introduction of a receptive female in the funnel trap, permits to catch mainly males – without allowing for much higher catch efficiency than baited traps. Nevertheless, it is efficient even in the case of low crayfish densities, and can therefore be used as an early response to invasion.

Biocontrol

Associated to trapping, **biocontrol by predator fish** is an interesting option. Eel (*Anguilla anguilla*) appears to give the best results on red swamp crayfish (see section 3.3). It was introduced in a small lake near Zurich (Switzerland), and within a few years the crayfish stock, estimated from trapping, was divided by four (Frutiger and Müller, 2002). However, crayfish are admittedly less active (and therefore less likely to get trapped) in the presence of the predator, for they detect its odour (Hazlett *et al.*, 2002).

Sterilisation

Combined to a systematic catch effort, the sterilisation of large males, by **X-ray irradiation** (Aquiloni *et al.*, 2009) or using a **mechanical protocol** (see section 3.4), aims to lower the reproductive potential of a population. Trapped females and juveniles are destroyed, while sterilised males are released and thus limit the fecundity of the remaining females. These approaches are validated in laboratory, but their efficiency still needs to be confirmed in natural environments.

Electrocution

Electrocution was tested (Peay *et al.*, unpublished) in a small shallow brook on signal crayfish, using a specific device that delivered repeated shocks at low (20 kW) or high (96 kW) power. Mortality, assessed by manual counting, ranged between 86 and 97% for the high power value. All crayfish sizes were affected; the authors discussed the issue of mortality induced on a few non-targeted species. That first test suggests a potential use as a control method rather than for eradication.

Physical control

Various **physical control systems** have also been tried in streams: fish passes that stop crayfish passing through (especially Frings *et al.*, 2013), creating obstacles (Dana *et al.*, 2011) or modifying existing thresholds to prevent invasive species going upstream. These options are costly and hard to implement on large sections, but they can be contemplated locally in the case of high ecological stakes upstream (see section 3.4). In closed waters, resorting to a prolonged drying-up period can prove necessary and efficient (see section 3.2).

Biocides

The use of selective **biocides** is a topic of on-going research. Natural pyrethrin has been tested at 0.15 mg/l on signal crayfish in several Scottish ponds (Peay *et al.*, 2006), after removing the fish, stopping the outflow and first spraying the banks. Important mortality rates were noted (100% after five days on caged specimens). In Norway, tests in cages, in funnel traps or in a drained pond also demonstrated a high sensitivity of the species to Betamax, a synthetic veterinary pyrethroïd (Sandodden *et al.*, 2010). Laboratory trials (Morolli *et al.*, 2006) on red swamp crayfish, using three synthetic pyrethroïds (cypermethrin, deltamethrin and cyfluthrin) also showed high sensitivity of the species – concentrations leading to the death of 50% of the crayfish in 24hrs did not exceed 0.22 µg/l. A one-hundred fold higher deltamethrin concentration did not induce any mortality on common carp after 24 days of exposure. These tests suggest, provided that in-depth toxicity studies are performed on other animals, that synthetic pyrethroïds could be used to control or suppress harmful red swamp crayfish populations in limited and localised areas.

Biological agents

The use of **biological agents** is also under study. In the United States, a team (Davidson *et al.*, 2010) tested bacteria (21 Bacillus species, among which six Bacillus thuringiensis strains), nematodes and a virus as possible biological controls of virile crayfish Orconectes virilis. Only the virus responsible for the white spot syndrome in shrimp (White Spot Syndrome Virus) turned out to be highly pathogenic and transmissible by cannibalism, but not by water. It was also pathogenic for other species, including red swamp crayfish. The virus was also tested on a few non-targeted species – water fleas, amphipods, mosquitoes or dragonfly larvae – that did not get infected.

3.2 – Drying-up, a tried solution in closed waters

The occurrence of invasive crayfish in a stream is linked in most cases to the presence of one or several colonized stretches of water in the same drainage basin: they constitute major sources for subsequent invasion of neighbouring environments – via natural ways or human transport. Within spared drainage basins, eradicating these sources of invaders is of prime importance. In the Vosges county, the survey led by the Onema in 2006 identified a limited number of sites colonized by invasive crayfish: eight ponds harboured signal crayfish, and thirteen others were invaded by red swamp crayfish. Following their detection, State authorities reacted determinedly and used currently applicable county regulations that call for pond drying-up by prefectoral decree (Figure 25, next page).

An assessment of these operations was presented (M. Collas, Onema) during the seminar. At the Bellefontaine site, two dam ponds invaded by red swamp crayfish, with respective surfaces of 2,000 and 6,000 m2, were dried up in November 2009. A physical barrier (a wired enclosure) had previously been fixed around the banks. Quicklime treatment was applied the following week. Active hunts operations

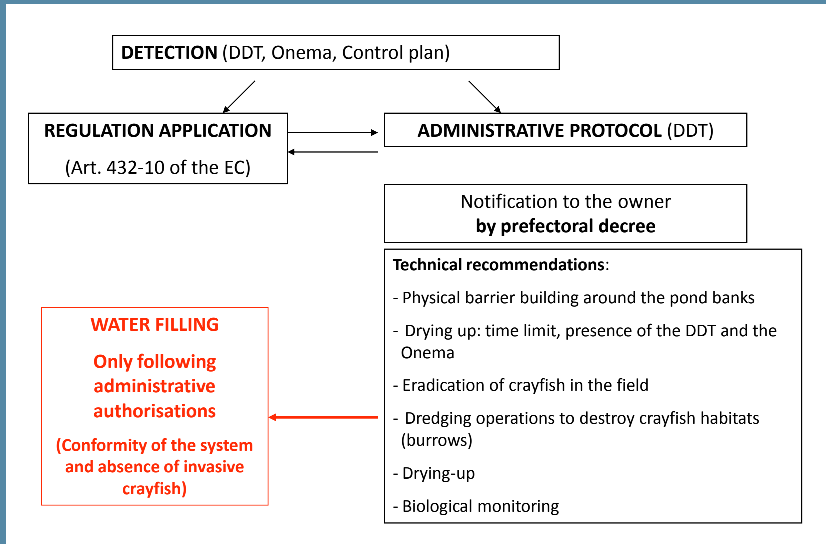


Figure 25. Management of invasive crayfish in the Vosges county – draining can be chosen if stakes are high enough (Source: M. Collas, Onema).

coupled to crayfish removal from the site were carried out during the winter and summer drying-up operations that followed (Figure 26). Most catches were performed in the days following draining, but live specimens were found until July. Taking into account the capacity of the invasive to survive for a long time in the burrows it bores, a population can be considered as eradicated after three years' drying. The ponds were filled again in 2013.

Below is another example, at the Noires Feignes brook site. That zone of about thirty hectares is classified as a sensitive natural area (SNA). It was composed of two dam ponds of 1.5 and 0.21 hectares, both invaded by signal crayfish – moreover healthy carriers of aphanomycosis. Prefectoral authorities gave the owners notice to sort out the state of their ponds in 2009. Then the Conseil Général of the Vosges county stepped into the process by developing a restoration and long-term management project for the site. In November 2009, the ponds were drained and then left to dry up in winter and summer. As a result, signal crayfish populations were eradicated. Dredging operations were carried out, and then the larger pond was filled with water again in

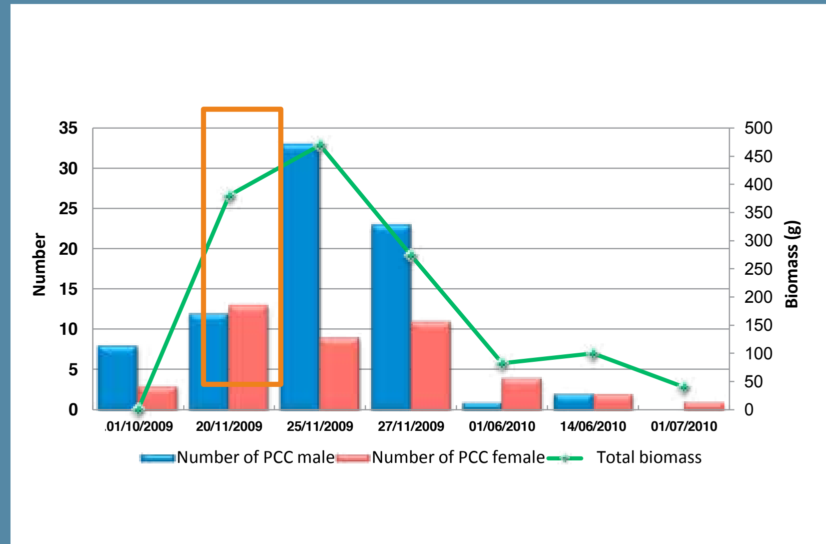


Figure 26. Temporal evolution of red swamp crayfish (PCC) catches in a small pond in the Vosges, before and after drying-up (Source: M. Collas, Onema).

autumn 2012. The smaller pond was purchased by the local authorities and its management entrusted to the "CREN de Lorraine". It was filled with soil material in order to restore the ecological continuity of the basin. Hydromorphological restoration operations were led: a minor river bed was recreated, the dyke was partially levelled out, and the former banks were recreated.

Erasing of a pond

Measures of the same kind were applied to virtually all known sources of invaders in the county: as a result, five signal crayfish populations and one red swamp crayfish population were eliminated. One site, composed of 12 ballast pits invaded by red swamp crayfish, was impossible to "treat" due to technical difficulties and to strong resistance from the owners. For lack of such a solution, another strategy was chosen, whose success remains to be assessed: predator fish (perch, pike, European catfish) were re-introduced, and crayfish were trapped by the owners.

Several lessons can be learnt from these globally conclusive experiments. First, their success with a view to preserving neighbouring environments depends on how early invaders are detected. It also requires very good collaboration between the State authorities, the Onema and the Direction Départementale des Territoires, as well as with courts – and this in the first place implies informing public prosecutors about the issue of crayfish… In all cases, such operations remain highly time-consuming for the services that implement and monitor them. And last of all, they can turn out to be costly for pond owners, who presently support their cost alone.

3.3 – Trapping efficiency limited by numbers

Drying-up operations are tried but costly solutions, so they are only adapted to stretches of water that can be drained and constitute localised invasion sources. For other types of invaded environments, marshes, natural lakes, canals…, managers can choose systematic trapping. That option was implemented on a large scale in the Parc naturel régional de Brenne (Indre), whose 182,700 hectares distributed over 51 towns and villages harbour a remarkable network of more than 4,000 ponds.

In the Brenne, 230,000 crayfish caught were not enough

As soon as red swamp crayfish were identified in one of the ponds in July 2007, the Parc naturel régional reacted by setting up a dedicated steering committee, followed by a study trip in the Brière with local representatives in 2008, to get a better knowledge of the problems induced by the species. After the presence of crayfish was confirmed in many Brenne ponds, a "crayfish brigade" was created in 2009 to implement a management strategy: the team was composed of five agents, and funded by the EFRD (European fund for regional development), the regional DREAL and the Centre region. It was granted an annual budget of 88,840 euros in 2011 (including wages, field equipment, fuel and telephony). In close link with pond owners (ponds are mainly privately owned), town/village councils and fishing guards, it led a campaign aimed at catching and systematically destroying crayfish, using a fleet of 400 funnel traps. Trapping was performed by the agents with the owners' prior agreement, or directly by owners or by fishing guards after a convention was signed with the Parc naturel régional for funnel trap loans.

Two highly colonized sites were also dried up in 2011.

Catches were recorded into a database so that it was possible to monitor the evolution of populations and colonization of new sites. In four years, nearly 230,000 crayfish were thus removed from the 104 colonized ponds (578 ha in total) known so far (Table 2).

The results (*A. Coignet, PNR Brenne*) of that volontarist operation are contrasted. The noticeable drop in total catch numbers after a peak in 2010 suggests that a sustained trapping effort caused the invasive to retreat. At the pond scale, an efficient

Tableau 2. Evolution of the numbers of crayfish caught by the "brigade" and its partners in the PNR Brenne (Source: A. Coignet, PNR Brenne).

Number of crayfish captured						
Operators	2009	2010	2011	2012	2013 (1er half year)	Total
Brigade PNR Brenne	11 085	43 113	7 817*	20 183	9 033	91 231
Owners and managers	3 233	40 262	44 459	11 170	15 842	114 966
Chérine Reserve	11 506	2 870	3 552	4 060	901	22 889
Total	**25 824**	**86 245**	**55 828***	**35 413**	**25 776**	**229 086**

* Decrease linked to complete drying up of two major sites colonized by crayfish

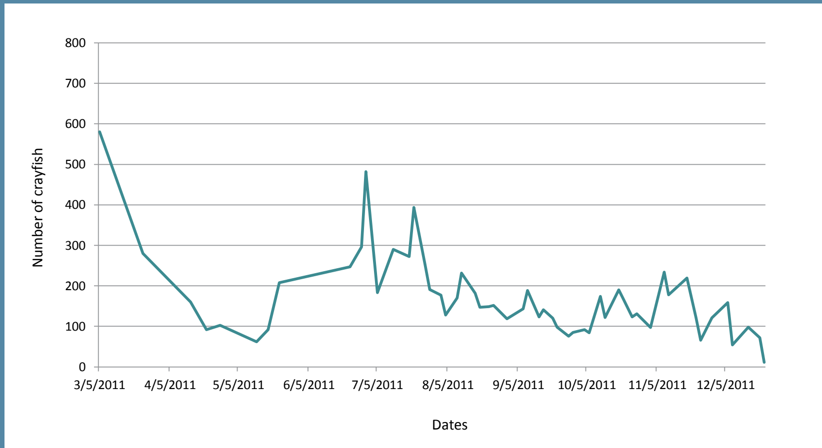


Figure 27. Evolution of the numbers of crayfish caught in La Roche Chevreux pond (Source: A. Coignet PNR Brenne).

control of crayfish numbers appeared possible. Thus, in la Roche Chevreux pond (13 ha), drained in 2009 and subjected to continuous trapping (15-20 funnel traps lifted twice a week), crayfish catches noticeably dropped in 2011 (Figure 27 above).

But comparatively to species dynamics, the strategy did not prove sufficient to solve the problem in the long term. At the Brenne scale, many ponds are connected to one another. That network functioning dampens the successful control of the species reached locally: draining operations can cause the species to spread downstream, while juvenile fish transfers to refurbish fish stocks are likely to bring crayfish eggs and larvae into new environments. Finally, the "brigade" cannot intervene everywhere, notably for lack of an agreement with some owners.

For the future, the PNR Brenne is seeking a complementary method to trapping. Among possible approaches, the use of predator fish is on the list once again. Prior to repopulation measures, a study is scheduled, in partnership with the INRA of Rennes, to characterize the prey-predator relationships between red swamp crayfish and different candidate species – pike, zander, perch, eel or carp.

Depleting a crayfish stock via trapping: "mission: impossible"?

The limits of trapping were also evidenced within the framework of a study (*J-P. Damien, PNR Brière*) led in the Brière between 2009 and 2012. In response to managers' requests, the operation was aimed at assessing red swamp crayfish stock depletion in several experimental ponds, including two (225 and 775 m²) isolated by a fine-mesh enclosure to prevent peripheral recolonizations.

The first objective was to select the trap that offered the best compromise between crayfish catch efficiency and selectivity, in order to limit the impacts on other species – fish, batrachians, dragonfly larvae… The experiments were made on different systems (several ponds, an open canal at the heart of marshes, as well as ponds in hedgerow

A pond in the Brière enclosed using wire mesh fencing to conduct an intensive trapping of red swamp crayfish

landscape) following a standardized protocol (see section 3.5). They enabled the team to select a semi-cylindrical wired funnel trap with a 5.5-mm mesh size, with one entry point on each side, to lead the stock depletion experiment. Nevertheless, a second, much more selective trap prototype was identified: it is recommended when the risks of catching non-targeted species are high.

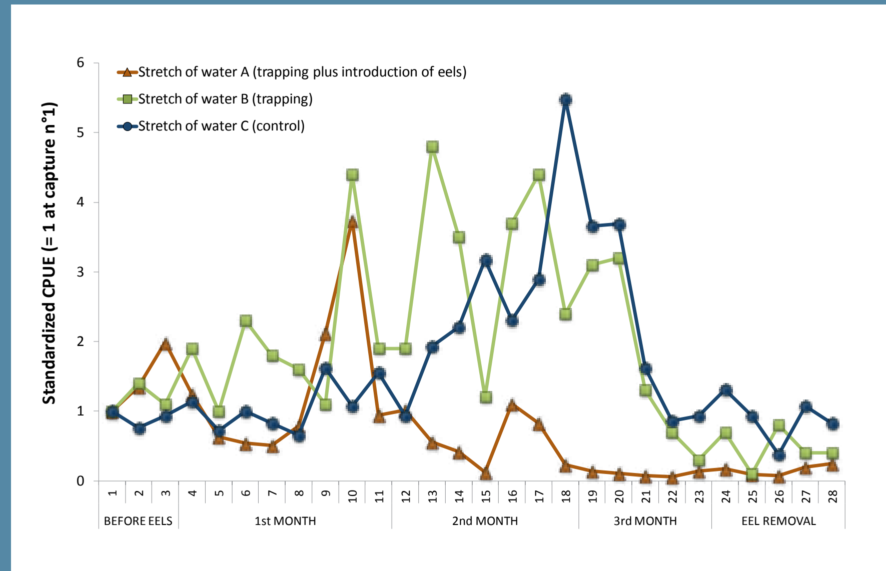
Continuous trapping was performed in the two enclosed ponds for three years, from April to the summer, when ponds totally dried out (in July) – the time when crayfish activity is at its highest. In one of the ponds (A), 15 funnel traps were used (*i.e.* one for 15 m^2). In the other (B), 70 traps of different types were set (i.e one for 10 m^2). The funnel traps were lifted every 24 to 72 hours, and each time crayfish numbers and biomass were recorded. Over the whole study period, 38,000 individuals were thus removed from these two tightly enclosed ponds, with a cumulated surface of only 0.1 hectare, but the operation did not succeed in depleting the stock! After a total of 10,371 funnel trap lifts, catches had decreased by 90% in pond (B) and by only 46% in pond (A).

In parallel, the team studied *in situ* the effect of the introduction of eels on the crayfish population. In pond (A), 31 eels, 55 cm long on average, were introduced mid-April 2012 for a three-month period. The trapping effort continued on an identical basis, during and after their stay in the basin, allowing for comparative monitoring of catch numbers. As for pond (B), it remained eel-free, but continuous crayfish trapping continued. Finally, a third pond (C), where traps were set for 24 hrs twice a week, was used as a control: catch conveyed above all the trend in crayfish activity throughout the season.

The results show a sharp decrease of the numbers of trapped crayfish in the pond containing eels, starting the second month after their introduction (Figure 28). Catch numbers remained close to zero several weeks after the predators were removed.

To sum up, this study illustrates how hard it is to thoroughly get rid of a red swamp crayfish population by using trapping alone, even after a prolonged, sustained catch effort, in small ponds isolated from their surrounding environment by an impassable wire fence. It confirms the potential interest of biocontrol,

Figure 28. Effect of the introduction of eels on red swamp crayfish catches, expressed in catch *per* unit effort (CPUE: crayfish/trap/24 hrs) in a pond (A), as compared to two other ponds: one with continuous trapping (B) and the other without continuous trapping (C) (Source: J-M. Paillisson, CNRS).



via the introduction of eels in the present case, as a complementary strategy to trapping. However, the true efficiency of that option needs to be specified by further studies. The presence of eels has an inhibiting effect on crayfish behaviour (see section 3.1). The observed decrease in catch numbers does not necessarily reflect a proportional drop in the stock of crayfish present in the pond, as evidenced by the decreasing activity of crayfish throughout the season in the control pond (C).

Beware though: eel is considered as "endangered" by the IUCN and benefited from several conservation measures. Its use to control red swamp crayfish populations can only considered after taking into account the conservation issues of the species (notably the fact that we should not move individuals in closed areas making not possible migration to the sea).

Focus

Professional fishermen: actors of invasive crayfish regulation?

In situations where the eradication of an exotic crayfish population seems out of reach (vast, open and/or highly colonised areas), the exploitation of that abundant food source for commercial trade appears to be, for some stakeholders, a conceivable option for controlling the demography of the invasive while generating socio-economic benefits. The Comité national des pêcheurs professionnels en eau douce (CONAPPED) took a stand in favour of that point of view during the Saint-Lyphard seminar. Faced with the decrease in halieutic stocks, specific preservation measures (the "eel" plan) or sanitary consumption restrictions (the "PCB" plan), the sector has experienced a sharp decrease in its numbers in the last 30 years – today the CONAPPED gathers 435 continental professional fishermen, 21 journeymen and 55 sailing fishermen who operate in the estuarine zone. The recess of the national market goes along with an increase in imports and illegal trade. In that very hard context, the companies of the fishing sector see the soaring invasive crayfish populations as a lever for diversification or career change.

The CONAPPED led a reflection (*N. Stolzenberg*) to refine the outlines of a fishing and integrated transformation sector for red swamp crayfish, which is already exploited locally, in Grand-Lieu lake or in the Camargue, for example. A good practices charter was proposed to provide a framework for these activities, with the aim to "*ensure the securing and traceability of the sector as regards red swamp crayfish, between production sites and transformation sites, in strict abidance of the law*". Professional fishermen, taught by managers and scientists, could bring their technical know-how and important catching means (around 300 kg of crayfish *per* day and *per* fisherman) to the service of a transparent, mastered and responsible management of the stocks of invasive species. The CONAPPED underlined the benefits – socio-economic but also ecological – of such as sector, compared to the financial cost of inaction in the face of invasions. However, that viewpoint was greeted cautiously by many participants of the Saint-Lyphard seminar. The main objection mentioned the risk of progressively seeing invasive species associated to economic interests (and thereby leading to their faster dispersal) considered as part of our natural heritage, at the expense of the need to control invasions and to preserve the health of aquatic environments.

3.4 – Little streams and high ecological stakes: which management strategies?

The means implemented against invasive crayfish should of course be considered in the light of a cost-benefit analysis: cash and time-costliest options have to be saved for the sites with the highest ecological stakes. This is especially the case when an identified population of native crayfish (see 2.2), or of another sensitive species, is directly under the threat of an invasive's progress. Thus, in the Natura 2000 site of the Sarthon basin, the Parc naturel régional de Normandie-Maine implemented a volontarist strategy whose results were presented (*M. Scelles, PNR Normandie-Maine*) at the Saint-Lyphard seminar. This Sarthe tributary had one-third of its course historically inhabited by white-clawed crayfish, but its basin was colonized by signal crayfish. In 2007, only six localized stretches still harboured the native species, *i.e.* 2.45% of the whole river length.

To preserve these populations, managers led a whole set of operations first focused on the infestation hotspots of the basin. In 2007, the cleaning up of a wash house allowed for the removal of nearly 300 invasive crayfish. About 270 more catches were made by hand fishing and electrofishing, and by nocturnal investigations. In 2008, an invaded pond was drained and then quicklimed, while hand fishing in the stream allowed for the destruction of 500 crayfish. But these operations turned out to be insufficient to hold back the progress of signal crayfish.

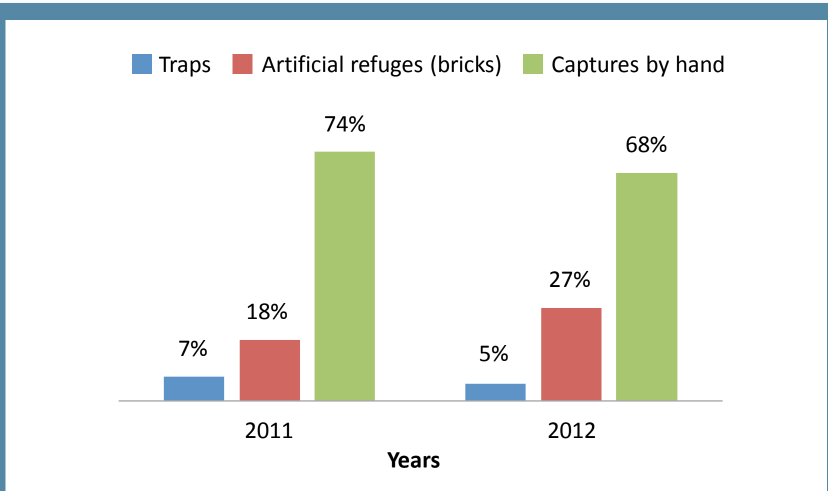
The Saules et Eaux company (*T. Duperray*) was missioned to define a strategy to continue the fight. Constructions (an underground pipe and a threshold under a bridge) were modified to stop the invasive having access to the upstream sectors, without impeding fish movements. Then, in 2009, the effort shifted to the implementation of a protocol for the mechanical sterilisation of signal crayfish males[1], developed by Saules et Eaux and successfully tested in laboratory. The operation consists in trapping as many crayfish as possible. Females and juveniles are destroyed while large, dominant males are sterilised and then released into the environment[2]: then they mate with the remaining females, and this results in non-viable eggs. After

[1] *The protocol has not been made public yet; it is in press.*
[2] *All the authorisations required for such an operation were obtained from the relevant authorities. Altogether, all the studies presented in this issue required authorisation from the State authorities.*

the geographical boundaries of the invasive's populations were marked, artificial refuges (bricks) were set in the stream to improve population monitoring and catch efficiency. In 2011 and 2012, intensive campaigns were led: crayfish were caught by hand, at night, for two consecutive weeks, at a rate of eight hunts *per* site ; artificial refuges (bricks) were lifted in the daytime and funnel traps were also used. Thanks to a rigorous monitoring of these operations, it was possible to assess the true efficiency of the different catching methods, *per* stream stretch and *per* size class (Figure 29).

Night catches by hand remained the most efficient way, ahead of artificial refuges; the use of funnel traps only allowed for a limited number of catches. In total, 3,300 specimens were caught in 2011, and more than 1,500 in 2012. But large males that had been sterilised and then released only represented 2 to 3% of these numbers, mostly composed of juvenile specimens. Egg-laying was monitored in 2011 and 2012. The results turned out to be disappointing: the first year, out of 80 sexually mature females observed, 37 (*i.e.* 46.3%) produced viable eggs; the second year, out of 31 sexually mature females,

Figure 29. Compared efficiency of the trapping modes implemented in the Sarthon, expressed as percentages of catch numbers. Night catch, by hand, was by far the most efficient method (Source: T. Duperray, Saules et Eaux).



25 produced viable eggs. This relative failure can be explained by the fact that it is hard to catch a sufficient number of large males: a line of research to improve these results will therefore consist in setting up more efficient and more selective catching methods. Other operations of targeted physical compartmentalisation could also allow for a better control of the colonization front.

In the Dunière, juvenile catch rate divided by 10

More convincing results were obtained (*T. Duperray, Saules et Eaux*) in the Dunière torrent (Ardèche), where a population of signal crayfish contaminated by aphanomycosis was discovered in 2007. Thanks to the field effort, 1,059, 749 and 519 crayfish were caught in 2009, 2010 and 2011, respectively, among which between 20 and 30% of males that were sterilised and then released. Juveniles represented nearly 20% of catches the first year, but that rate later dropped to stabilize around 2% the next two years: such a trend suggests a significant effect of sterilisation operations on the fecundity of the local crayfish population. The deployed efforts undoubtedly slowed down colonization.

Similar results were obtained in the small la Foux brook, in the Parc national des Cévennes: in 2002, the discovery of a well-established population of signal crayfish that posed a threat to the white-clawed crayfish still present on the site resulted in a determined action of the Parc national des Cévennes and the Onema, including depletion fishing campaigns and then the resort to sterilisation in 2010. Upstream colonization nevertheless continued, but at a much slower speed than those noted in the absence of management measures: over nine years, the invasive only gained 550 m upstream (vs. 800 m *per* year, for example, in the Brinjame tributaries, in the Morvan – see Focus at the end of Chapter 1). Marking operations (via the insertion of transponders) led on the same stream also supplied valuable data about the movements and the growth rate of signal crayfish in that local context.

Despite these contrasting results, male sterilisation methods, associated to a sustained catch effort, and depending on cases to physical compartmentalisation of populations, currently remain the combination most likely to yield positive results in small-size sites

New tools to capture crayfish (forceps and sucker)

© Théo Duperray – Saules et Eaux

© Matthieu Scelles – PNR Normandie Maine

with high ecological stakes – where the use of more aggressive methods such as biocides is out of the question. The impact of sterilisation on the dynamics of a population depends first of all on the regularity and the length of the action plans that are implemented, and also on the efficiency of the associated catch effort. Levers still exist for improving the latter. To that purpose, field operators will gain in efficiency if they get properly equipped. A range of original tools has been developed (*T. Duperray, Saules et Eaux*): an aquascope and a light periscope to make night hunts easier, an optic fibre endoscope for inspecting burrows, olfaction-led funnel traps, catching forceps and even a "crayfish sucker"… These tools, along with others to come, reflect all the cleverness and the tenaciousness managers will have to exhibit to preserve the natural balance of the most sensitive streams in the long term.

3.5 – Population monitoring: toward a standardized method for red swamp crayfish

For managers faced with a biological invasion in the long term, it is indispensable to have at their disposal operational, standardized methods that they can transpose across environments, to allow for the monitoring of populations over time and assess the efficiency of the implemented regulation actions. Such tools exist or are being developed for many invasive species, especially plant species. Yet, they are still missing as regards exotic crayfish. Such is the case for red swamp crayfish in the first place, which are presently sampled in quite diverse ways depending on sites (Paillisson *et al.*, 2011). One of the aims of the programme led in the Brière was to elaborate, especially for that species, a reproducible method based on passive trapping, easy to implement for field staff and yielding as complete and robust as possible data on population composition. Its development was the subject of a complete study, whose conclusions (*J-M. Paillisson, CNRS*), were presented at the Saint-Lyphard seminar.

Which trap should be used?

The team tested a broad range of traps in three experimental sites of the Brière marshes representative of different environments (pond, reed-pond and flooded grassland): traps of different geometric shapes, fish funnel traps made of different materials, with variable mesh sizes and numbers of entries… For nine days, in April and June 2011, these different devices were lifted daily. Catches were analysed in detail, following a set of criteria: crayfish presence (occurrence) rate, number of crayfish caught *per* trap and *per* day, size-structure and sex ratio, as well as non-targeted species count.

At the end of the experiment, a semi-cylindrical wired funnel trap (SCW), with two lateral rigid entry points and 5.5 mm mesh size, proved to be by far the most efficient one, with crayfish occurrence values close to 100%, and higher numbers of specimens than the other devices (Figure 30, next page) – for all size classes – for each habitat. Consequently, this type of trap was chosen for the next steps of the study.

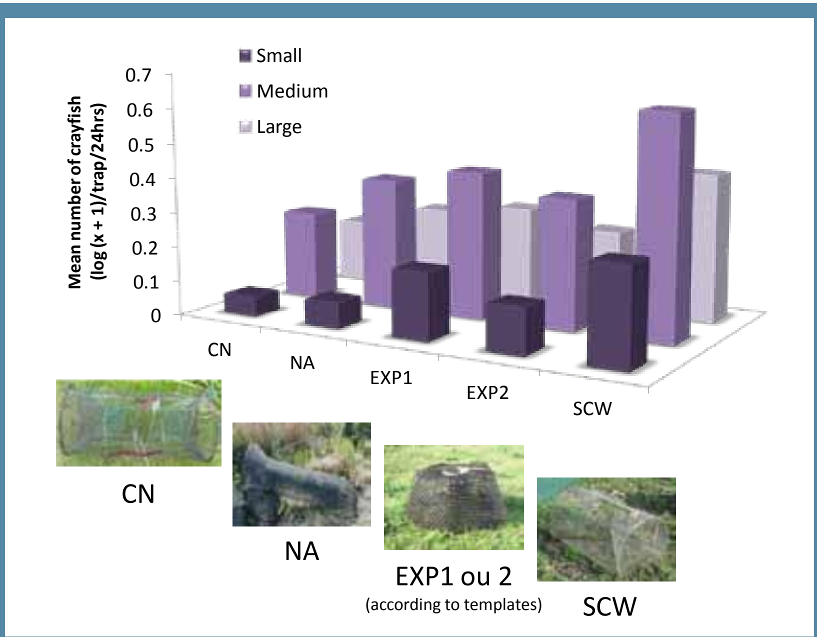


Figure 30. SCW traps (see the text for more explanations) offer the best catch efficiency for all crayfish size classes (Source : J-M. Paillisson, CNRS).

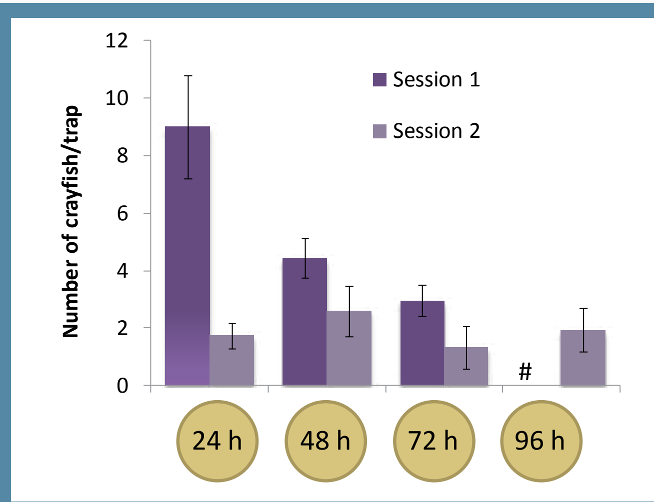
How long should trapping last?

The question of trapping duration is essential to define a standardized protocol for monitoring populations. It is indeed well known that the presence of crayfish in a trap can have a negative effect on the next catches (Kozac and Policar, 2003). Another potential pitfall related to a prolonged stay of traps in water is the risk of crayfish escape.

In order to characterize these effects, experimenters set 15 SCW traps in a small pond for two sessions of 72 and 93 hours altogether. Each trap was lifted out daily; trapped crayfish were counted, measured, marked (one colour *per* day) and then placed back into the trap for the rest of the session. Using this operating mode, it was possible to evidence the repelling or limiting effect of crayfish density within a trap on future catches (Figure 31).

The experiment also confirmed a high "disappearance" rate for long trapping durations, due to escapes or to cannibalism. These losses mainly concerned small-size individuals: long trapping durations, of 48 hrs or more, therefore appear to provide a not-so-true indication on the size structure of the crayfish population under study. These data indicate that **lifting out traps every 24 hrs** appears as the best option for the monitoring protocol. The data provided by this procedure are **catch *per* unit effort (CPUE)**, expressed as numbers of crayfish *per* trap after 24 hours fishing.

Figure 31. Average numbers of unmarked crayfish trapped over time: the observed decrease (session 1) highlights the limiting effect of a high density of previously trapped crayfish on the subsequent trapping rates. (Source: J-M. Paillisson, CNRS).



A crayfish marked for a scientific study

Should bait be used?

The study aimed to better define the effect of the presence of bait in the funnel traps on crayfish catches. Fishing sessions were led, using SCW traps and conical traps, and using dry dog food as bait. That kind of bait was chosen because it is a standard one and it is easy to use in numerous sampling situations. The results thus obtained were compared with catches performed in the same conditions, but in the absence of bait. No difference at all was observed in catch numbers; however, baited funnel traps contained more medium-size crayfish and fewer large-size or small-size specimens. Anyhow, the authors recommend to **rule out the use of bait within the framework of a sampling method**, whose prime aim is to convey the composition of crayfish populations as truly as possible.

Which trapping effort?

The number of traps to be used to accurately characterize a crayfish population present in a site should be chosen on the basis of the best compromise between induced field effort and data quality. Therefore the team aimed to determine the minimum number of traps needed to obtain "reliable" CPUE (catch *per* unit effort) values at the site scale, *i.e.* displaying a low enough coefficient of variation from a statistical point of view.

To that end, digital modelling was performed from a large field data set: 30 sites, representative of various habitats, were sampled for 24 hours using 30 SCW traps located about 10 meters from one another in each site. Catches were subdivided into three age classes. CPUE mean and standard deviation values were calculated for each site. After that, the modelling task consisted in generating by random draw the results that would have been obtained with numbers of traps ranging between 5 and 30, for each site and each size class. Using CPUE mean and standard deviation values corresponding to these data sets, it was possible to characterize result accuracy (in other words to calculate coefficients of variation of CPUE values) as a function of the catch effort.

In the light of these modelling results (Figure 32), the team proposed a number of **25 traps *per* site** as a good compromise between the effort to be deployed on the field and the quality of the data thus obtained. On a given site, traps should be spaced out at least every 10 meters. In certain conditions, the number of traps could be reduced, but considering the diversity of the environmental conditions in which red swamp crayfish are found, it is suitable to note a number of 25 traps; beyond that number, catch data do not bring any more information on the state of crayfish populations.

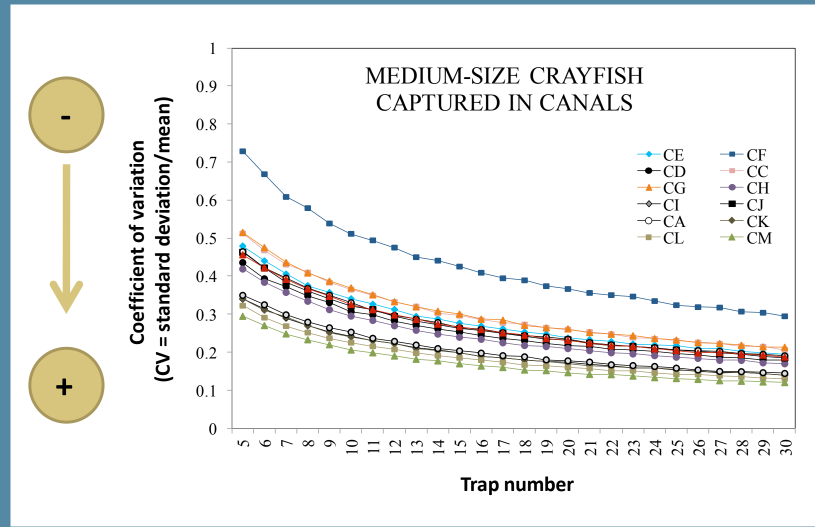


Figure 32. Modelling of the coefficient of variation of CPUE (catch per unit effort) values as a function of the number of traps per site, for medium-sized crayfish trapped in various Brière canals. Each curve corresponds to one site (Source: J-M. Paillisson, CNRS).

Which monitoring protocol?

The protocol for monitoring red swamp crayfish populations consists in distributing 25 SCW-type bait-less traps *per* site spaced out roughly every ten meters, for a 24-hour trapping duration. The results thus obtained are expressed, for a given site, in numbers of average catch *per* trap, or numbers of catch *per* unit effort (CPUE). They can be further detailed according to crayfish size groups, and compared across sites or within a same site after multiple trapping sessions (cross-year sessions, for example).

From a practical point of view, the authors recommend to pack the crayfish from each trap in labelled plastic bags and gather them by site, with a view to the crayfish size measurement step that it is preferable to perform in laboratory in order not to burden the field phase. Two people equipped with a van can set and lift traps at a rate of 4 sites *per* day. For red swamp crayfish, prior authorisation is required for live transport. This protocol is currently in press. It will be the subject of a methodological guide aimed at managers, consultants for environment and other field operators. The recommendations resulting from the study led in the Brière will of course probably be refined once monitoring operations in other sites have been implemented and have supplied new feedbacks.

Environmental DNA, a complementary method?

In addition to passive trapping, alternative methods can be envisaged to contribute to the monitoring of invasive crayfish populations. Such is the case of environmental DNA (eDNA), which is regarded with increasing interest from aquatic environment managers. The approach is derived from molecular biology and bioinformatics technologies. It consists in taking a water sample from the site under study, and then "amplifying" the DNA fragments that are in it, using specific primers (one primer type *per* "group" of species). Then the molecules are identified using a genetic reference database and provide indications about the species present within the environment.

Within the framework of the research programme led in the Brière, a study (*A. Tréguier, Inra and Rennes 1 University*) led in partnership with Spygen Company aimed to specify the interest of the method, as compared to trapping, within the framework of a large-scale survey of the presence of red swamp crayfish. The two methods were studied in parallel in 158 ponds in the Brière. It was one of the very first studies that used the method for invertebrate detection. For each pond, eDNA detection was performed from six water sub-samples taken across the whole perimeter; each sub-sample was composed of 20 samplings of 40 ml of water each. Trapping was performed after water sampling in order to limit contamination risks, following the protocol presented above. Trapped crayfish were distributed into two size classes.

The results are contrasting. Out of the 158 ponds studied, 80 were found crayfish-free by trapping and eDNA; 30 others were found "positive" by the two methods. But for the 48 remaining ponds, contradictory results were found: in 21 ponds, crayfish were caught but their DNA was not detected. For the remaining ponds, it was the reverse: DNA was detected, but no crayfish were trapped. A further analysis revealed that the ponds where crayfish DNA was detected but no individuals were caught were small ponds that displayed high silting-in rates. Additionally, the ponds where DNA from the species was detected were environments that harboured higher average numbers of specimens, and mostly young crayfish.

As it is, this first study suggests that the two methods could be complementary. The environmental DNA technique, which is still being developed for crayfish, could in the long run offer better efficiency for the early detection of crayfish, on condition that targeted technical improvements are made. As for trapping, when it is implemented within the framework of a standardized protocol, it provides data about the abundance and age structure of crayfish populations in colonized environments; presently these data seem hard to obtain from molecular tools.

Assessing crayfish abundance using traps

Conclusion

For two days, the first French meetings on exotic invasive crayfish gathered nearly 120 participants from diverse backgrounds at Saint Lyphard: research organisations, consultants for environment, associations, professional fishermen, devolved State services, local and conurbation authorities. Through thirty-odd oral presentations, and often passionate ensuing debates, the seminar provided the opportunity to share the latest pieces of knowledge about the subject, as it has become a major concern for numerous managers and aquatic environment stakeholders in the last few years.

Starting from a description of present knowledge on invasive crayfish species in France, their biology and recent changes in their distribution, a whole set of new data was brought in for a better understanding of the ways they colonize natural environments and the resulting impacts. These data mainly result from studies led in the Brière and the Camargue on red swamp crayfish; they confirm and more particularly provide details about the extent of the consequences of these biological invasions on colonized environments.

First, on native crayfish, which are the victims of invasive species' competition and of their propagating the lethal disease aphanomycosis. But also on the natural balance of colonized environments altogether: red swamp crayfish actually disrupt foodwebs to the detriment of numerous species – plants and benthic invertebrates in the first place. These different impacts are likely to place France in an awkward position as to the enactment of the European directives on water and on the fauna and flora, which respectively aim to bring surface waters back to a good condition (or keep them so) and to preserve habitats and natural heritage species (including native crayfish). Finally, socio-economic activities are potentially affected by these proliferations, as suggested by one of the very first sociological surveys about the subject, led in the Camargue.

A growing need for tools and methods for eradicating or regulating invasive crayfish populations is currently emerging in response to the severity of their impacts. Definitely operational, the Saint-Lyphard seminar granted much time to the possible modes of management in the face of these invasions. After an overview of the scientific studies led across Europe, various feedbacks from experiments in France were presented – systematic trapping, pond drying, mechanical sterilisation of males, biological control using predators or modification of construction works to prevent colonization… From Vosges ponds to Ardèche brooks, from Brenne ponds to Brière or Camargue marshes, the diversity of the management approaches investigated testifies for a volontarist consideration of the issue, and for the dynamism of the scientific stakeholders and managers involved: thus France imposes itself as a leading country in Europe for the management of exotic crayfish invasions.

As for the results of these field actions, they are rather mitigated and underline how hard it is to control invasive exotic crayfish. Eradicating localised populations seems possible, at the cost of a joint and determined action, in closed ponds or little streams, especially at the early stages of invasion. But in large, open (canals, marshes, long streams) and/or highly colonized habitats, eliminating invasive species appears to be out of reach. Regulation or control measures fail due to the strong dynamics of these populations – particularly in the case of red swamp crayfish. The research effort is being pursued. New tools are created and there is still room for improvement. But judging from experience, no “cure-all” method is available: the best results will be obtained, depending on the local parameters of the invaded site and the ecological stakes it implies, by combining several strategies implemented in the light of a cost-benefit analysis.

In all cases, preventing new introductions of invasive crayfish into so far crayfish-free basins is obviously a key management stake for the preservation of aquatic biodiversity. Efficient regulations are of course an indispensable tool to contribute to prevention. The present regulatory framework was the subject of numerous discussions during the seminar and was consensually considered as inadequate. The scientific and naturalist community is presently assessing the modalities for calling to public authorities on a national scale in order to have true water police measures implemented regarding exotic crayfish. Besides, preventing new invasions implies pursuing with the still recent effort undertaken in France to make the different stakeholders aware of the problem: managers, anglers, aquarists, but also the general public. In that respect, the public conference that was given at Saint-Lyphard the day before the seminar started, in front of a large audience composed of teenagers, pensioners, inhabitants of the Brière marsh and citizens who felt concerned, was an encouraging success. In the near future, other events of the same type, but also a broad distribution of popularisation documents, identification guides or articles in the non-specialised press, could help the civil society become more aware of the stakes related to these biological invasions.

Red swamp crayfish, signal crayfish, and other species, present or to come, are going to inhabit our freshwaters for a long time. The research and management effort they demand will also have to be maintained over time. In June 2013, the Saint-Lyphard seminar was the starting point of a reflection and action network on a national scale, about the complex issue of invading exotic crayfish. This young community will have to establish strong, sustainable and constructive links in order to successfully work for the preservation and restoration of aquatic environments.

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Acknowledgements
The authors wish to thank all the speakers and participants for their contribution to the seminar. We also thank all the people who kindly provided photos and illustrations.

ISBN: 979-10-91047-26-5

Layout design: Inzemoon (06 75 24 19 30)
Production: Bluelife (09 66 82 33 55)

Printed on paper from sustainably managed forests by:
Panoply (+33 1 46 94 33 22)

October 2013
Printed in France

PEFC 10-31-1093
Certifié PEFC
pefc-france.org

ONEMA
French national agency for water
and aquatic environments

Front-cover photo © Jean-Marc Paillisson – CNRS