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A National Inventory of Invasive Freshwater crayfish in the Netherlands in 2010

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EIS european invertebrate survey
nederland

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ROYAL HASKONING

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• cover: a screendump of the website www.kreeftenonderzoek.nl halfway the sampling period. Red thumbtacks reflect sites reserved by volunteers. Green thumbtacks are still open for reservation.

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Summary

In late summer 2010, about 200 volunteers from all over the Netherlands contributed to an unusual project: a national survey of invasive crayfish. The goal of this project was to map the distribution of invasive crayfish in the Netherlands. The applied method was not selective for invasive crayfish. However, since the only native crayfish (the Noble crayfish *Astacus astacus*) is on the verge of extincion in the Netherlands, only exotic species were caught in practice. After mapping the distribution, an attempt was made to define the ecological niche of a long established crayfish species: the spiny cheek crayfish *Orconectes limosus*. Finally, we investigated the effect on the water quality and physical structure of the habitat by crayfish species that entered the Netherlands more recently, in particular the red swamp crayfish (*Procambarus clarkii*).

In order to relate crayfish data with environmental data, already existing sampling sites of the waterboards (regional water authorities) are used, provided by the Limnodata Neerlandica. The Limnodata Neerlandica is a national database, administered by the company Royal Haskoning upon instructions from the Foundation for Applied Water Research (STOWA). The database contains quality measurements of all waterboards of the last three decennia. Through a website (www.kreeftenonderzoek.nl), volunteers could apply for one of the preselected sampling sites from the database. Hereby, the project differs from regular inventory projects, where volunteers usually select a sampling site by themselves. After registration, a package with traps and other equipment was send to the volunteers which had to be used according to a standardized protocol. The protocol was designed to determine absence or presence of crayfish at a particular site with a 95% capture probability, if present. The effort needed for this consisted of the use of three unbaited traps that had to be examined at four subsequent mornings.

Over 200 volunteers applied for the project and sampled altogether a total of 294 sampling sites which were distributed throughout the Netherlands. Crayfish were caught at 30% of the sites (n=89), divided among four species: the spiny cheek crayfish (*Orconectes limosus*) at 71 sites, the red swamp crayfish (*Procambarus clarkii*) at 16 sites, the virile crayfish (*Orconectes virilis*) at 4 sites and the narrow clawed crayfish (*Astacus leptodactylus*) at 1 site. Although many new sites with crayfish were found, the overall distribution hasn't changed for any of the four species detected during the survey. However, the study provided the first overview of sites of where we can assume that crayfish do *not* occur with a high level of certainty.

Based on a matrix with linear correlations and other available ecological data, a couple of parameters that appear to be most important explaining the presence of crayfish were investigated in higher detail. The spiny cheek crayfish is not found in waters with a pH<6.4 and hardly any crayfish were found in waters with a salinity (chloride content) higher than 300 mg/l. Other factors that seem to determine the presence of spiny cheek crayfish are a relatively high oxygen content (at least 6.6 mgl/l on average), relatively high temperatures (waters where the maximum temperature exceeds 20° C) and current. No significant relations were found between recently established crayfish species and changes in water quality parameters.

Nederlandse samenvatting

Ongeveer 200 vrijwilligers uit heel Nederland droegen in het najaar van 2010 bij aan een bijzonder inventarisatieproject: het verspreidingsonderzoek uitheemse rivierkreeften. Het doel van dit project was het in kaart brengen van de landelijke verspreiding van deze soortgroep. De gebruikte methode was weliswaar niet selectief voor uitheemse soorten rivierkreeften, maar omdat de enige inheemse kreeft (de Europese rivierkreeft *Astacus astacus*) in Nederland vrijwel is uitgestorven, werden in praktijk alleen uitheemse rivierkreeften gevangen (naast de bijvangsten). Daarnaast is geprobeerd om de habitatvoorkeur te bepalen van een reeds lang gevestigde exoot, de gevlekte Amerikaanse rivierkreeft *Orconectes limosus*. Tenslotte is van recentere nieuwkomers (in het bijzonder de rode Amerikaanse rivierkreeft *Procambarus clarkii*) onderzocht of de soorten effect kunnen hebben op de kwalititeit en structuur van het ecosysteem.

Om relaties tussen kreeften en omgeving te kunnen leggen is gebruik gemaakt van bestaande meetpunten van de waterschappen afkomstig uit de Limnodata Neerlandica. De Limnodata Neerlandica is een nationale database in eigendom van de Stichting Toegepast Onderzoek Waterbeheer (STOWA) en beheerd door Royal Haskoning, waarin waterkwaliteitsgegevens van de waterschappen van de afgelopen drie decennia gebundeld zijn. Vrijwilligers konden zich via een website (www.kreeftenonderzoek.nl) opgeven voor één van de voorgeselecteerde meetpunten uit de database. Hiermee verschilt het project van 'gebruikelijke' inventarisatieprojecten, waarbij vrijwilligers meestal zelf de meetlocatie bepalen. Na aanmelding werd een 'inventarisatiepakket' thuisgestuurd waarmee de inventarisatie uitgevoerd diende te worden volgens een gestandaardiseerd (en voor dit project ontwikkeld) protocol. Het protocol was ontworpen om de aan- of afwezigheid van rivierkreeften vast te kunnen stellen met een zekerheid van 95%, indien aanwezig. De inspanning die hier voor nodig was betrof drie kreeftenfuiken die op vier achtereenvolgende ochtenden gecontroleerd moesten worden door een vrijwilliger.

Ruim 200 vrijwilligers meldden zich aan en bemonsterden in totaal 294 meetpunten in heel Nederland. Op ruim 30% van de meetpunten (n=89) werden invasieve kreeften aangetroffen verdeeld over vier soorten: de gevlekte Amerikaanse rivierkreeft (*Orconectes limosus*) op 71 meetpunten, de rode Amerikaanse rivierkreeft (*Procambarus clarkii*) op 16 meetpunten, de geknobbelde Amerikaanse rivierkreeft (*Orconectes virilis*) op 4 meetpunten en de Turkse rivierkreeft (*Astacus leptodactylus*) op 1 meetpunt. Hoewel van sommige meetpunten nog geen rivierkreeften bekend waren, zijn de reeds bekende verspreidingspatronen van de verschillende soorten op hoofdlijnen niet veranderd. Wel is door dit project voor het eerst een overzicht beschikbaar gekomen van locaties waar invasieve kreeften met hoge mate van zekerheid (nog) *niet* voorkomen.

Aan de hand van een correlatiematrix en reeds beschikbare kennis over de ecologie van de gevlekte Amerikaanse rivierkreeft is een aantal parameters nader onderzocht dat het voorkomen van de soort het beste verklaart. De gevlekte Amerikaanse rivierkreeft is niet gevonden in wateren met een zuurgraad van pH<6,4 en vrijwel geen exemplaren zijn gevonden in wateren met een saliniteit (chloride gehalte) hoger dan 300 mg/l. Andere factoren die het voorkomen van de gevlekte Amerikaanse rivierkreeft lijken te bepalen zijn een relatief hoog zuurstofgehalte (minimaal 6,6 mg/l gemiddeld), relatief hoge temperaturen (wateren waar maxima vanaf 20° C worden gehaald) en enige vorm van stroming.

Bij het onderzoek naar mogelijke beïnvloeding van de waterkwaliteit door rivierkreeften die zich recent gevestigd hebben, zijn geen significant negatieve effecten aan het licht gekomen, hoewel de negatieve associatie met de Ecologische Kwaliteits Ratio (EKR) voor waterplanten *net* niet significant is. Andere factoren die grotendeels afhangen van de ontwikkeling van de onderwatervegetatie zoals doorzicht, zuurstof en macrofauna laten geheel geen associatie met de EKR zien.

Introduction

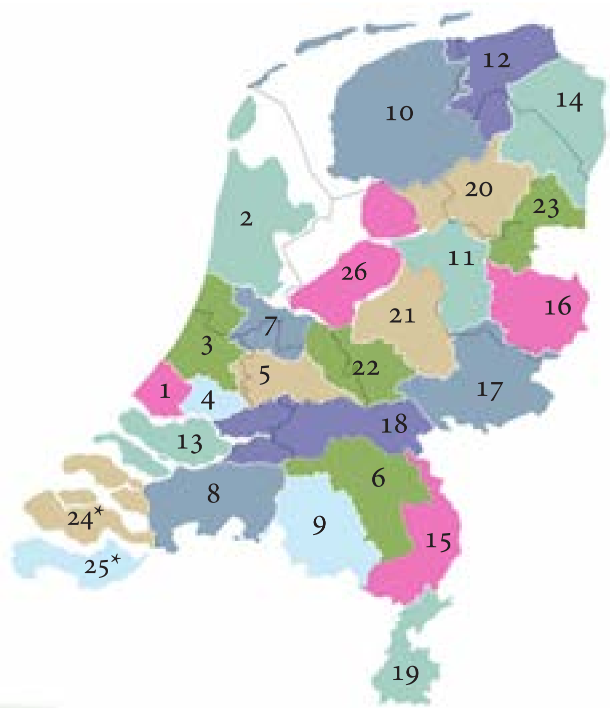
BACKGROUND

During the last ten years, the number of invasive freshwater crayfish species in the Netherlands had nearly doubled due to the release of American species (table 1, appendix 1). The rapid population growth of some of the new invaders raised questions about the impact they might have on freshwater systems. Many of the established species occupy a larger niche than observed in their natural range. Overwhelming evidence of negative economic and ecologic impact has already been observed abroad in four of the established species (red swamp crayfish, white river crayfish, signal crayfish, virile crayfish). In the Netherlands, crayfish are also accused of causing damage, but very little evidence of negative impact is available so far.

The Invasive Alien Species Team of the Ministery of Economic Affairs, Agriculture and Innovation (ELI) commissioned the European Invertebrate Survey - Netherlands (EIS) to organise a national field survey in 2010. EIS is a non-profit organisation whose objective is to collect data on invertebrate species in the Netherlands, and propagate the information for education and conservation. Most data are provided by hundreds of volunteers, guided by more than 50 specialised working groups. Over the years, records of invasive crayfish have also been collected by volunteers. Most data originate from the west of the Netherlands (fig. 2). However, since most volunteers are also operating in the (densely populated) western part of the Netherlands, this could be a result of a biased sampling.

CRAYFISH DISTRIBUTION

The first goal of this project was to collect up to date distribution data on invasive crayfish, at equally distributed sampling sites from all provinces of the Netherlands. To accomplish such a large scale inventory within a limited timespan volunteers were approached. To avoid a geographical sampling bias, all sites are preselected from an existing national network of sites: the 'Limnodata Neerlandica'. This database containes the collected data of the waterboards, which are regional water authorities that regulate and monitor water quality, among other responsibilities (fig. 1). Waterboards are commited to take biological and physical/chemical measurements at fixed sample sites at regular intervals. All data are regularly submitted to the Limnodata Neerlandica. The Limnodata are administered by the company Royal Haskoning under instructions from the Foundation for Applied Water Research (STOWA).The main purpose of STOWA is to coordinate research



1. Hoogheemraadschap van Delfland (HHD)
2. Hoogheemraadschap Hollands Noorderkwartier (HHN)
3. Hoogheemraadschap van Rijnland (HHR)
4. Hoogheemraadschap Schieland en Krimpenerwaard (HHS)
5. Hoogheemraadschap De Stichtse Rijnlanden (HSR)
6. Waterschap Aa en Maas (WAM)
7. Waternet (WAN)
8. Waterschap Brabantse Delta (WBD)
9. Waterschap De Dommel (WD)
10. Wetterskip Fryslân (WF)
11. Waterschap Groot Salland (WGS)
12. Waterschap Hunze en Aa's (WHA)
13. Waterschap Hollandse Delta (WHD)
14. Waterschap Noorderzijlvest (WN)
15. Waterschap Peel en Maasvallei (WPM)
16. Waterschap Regge en Dinkel (WRD)
17. Waterschap Rijn en IJssel (WRIJ)
18. Waterschap Rivierenland (WRL)
19. Waterschap Roer en Overmaas (WRO)
20. Waterschap Reest en Wieden (WRW)
21. Waterschap Veluwe (WV)
22. Waterschap Vallei en Eem (WVE)
23. Waterschap Velt en Vecht (WVV)
24. Waterschap Zeeuwse Eilanden* (WZE)
25. Waterschap Zeeuws-Vlaanderen* (WZV)
26. Waterschap Zuiderzeeland (WZZ)

** Now fused into WS Scheldestromen*

Fig. 1. Waterboards in the Netherlands. Colors indicate the waterboards. Dark lines indicate the provinces.

Table 1. Overview of established crayfish in the Netherlands (see also: Soes & Koese 2010)

	First record	Native range	Source
Noble crayfish - *Astacus astacus* NL: Europese rivierkreeft	Native	Europe	Geelen, 1978
Spiny cheek crayfish - *Orconectes limosus* NL: Gevlekte Amerikaanse rivierkreeft	1968	North-America	Geelen, 1978
Narrow-clawed crayfish - *Astacus leptodactylus* NL: Turkse rivierkreeft	1977	Eastern-Europe	Adema, 1982
Red swamp crayfish - *Procambarus clarkii* NL: Rode Amerikaanse rivierkreeft	1985	North-America	Adema, 1989
Eastern white river crayfish - *Procambarus acutus* NL: Gestreepte Amerikaanse rivierkreeft	2002	North-America	Soes & Koese, 2010
Virile crayfish - *Orconectes virilis* NL: Geknobbelde Amerikaanse rivierkreeft	2004	North-America	Soes & Van Eekelen, 2006
Signal crayfish - *Pacifastacus leniusculus* NL: Californische rivierkreeft	2004	North-America	Knol, 2005

Fig. 2. Records of invasive crayfish (data of all species, recorded between 2000 and June 2010) in the Netherlands collected by volunteers *before* the start of the national survey in 2010 (n=3150 records). Source: EIS.

Fig. 3. Records of invasive crayfish (data of all species, recorded between 2000 and June 2010) in the Netherlands collected by the waterboards *before* the start of the national survey in 2010 (n=323 records). Source: Limnodata Neerlandica.

on behalf of the waterboards.

In theory, waterboards are supposed to have a full picture of crayfish species in their area already, since crayfish are considered to be a part of the macrofauna community (and macrofauna samples are used for the biological assesments). In practice however, it is evident that the sampling method used by the waterboards, by netting a fixed stretch of 5 metres length of the bank, is unsuitable for collecting crayfish. The range of invasive crayfish according to the Limnodata Neerlandica (fig. 3) is considerably smaller compared to the combined records collected by volunteers (fig. 2).

CRAYFISH AND WATER QUALITY

A second goal of this project was to investigate the effects of crayfish on water quality. Crayfish are known to be strong interactors in freshwater systems, which could affect both water quality as well as the physical habitat in various ways (Lodge et al. 2000). In still or slow flowing waters, destruction of the submerge vegetation by crayfish is likely to be the prime source for changes in other (trophic) levels. Crayfish could affect the vegetation by foraging, by non-consumptive cutting, by digging and, indirectly, by causing turbidity which reduces the amount of light in the water and increases siltation.

Box 1. Crayfish in the Netherlands

A total of ten crayfish species have been observed in the Netherlands: one native species (the noble crayfish *Astacus astacus*) and nine invasive species. Six invasive species are established (narrow-clawed crayfish *Astacus leptodactylus*, signal crayfish *Pacifastacus leniusculus*, spiny-cheek crayfish *Orconectes limosus*, virile crayfish *Orconectes virilis*, white river crayfish *Procambarus acutus* and red swamp crayfish *Procambarus clarkii*). The status of one invasive species (marbled crayfish *Procambarus fallax*) is currently unclear. Two invasive species (the stone crayfish *Austropotamobius torrentium* and the redclaw *Cherax quadricarinatus*) have been recorded only once.

Crayfish are imported either by the aquarium trade or the consumption trade. The consumption trade led to many of the early introductions (e.g. narrow clawed crayfish, red swamp crayfish, spiny cheeked crayfish). Nowadays, this trade has declined considerably due to the rise of imported, prepared red swamp crayfish from China. Still, there is a marginal interest in live crayfish for consumption, but this trade is nearly limited to the species that are already established. The aquarium trade has increased significantly since the 1980s and the number of traded species is high and variable. Most of the traded (tropical) specimens have little chance surviving in the wild, but some cold water specimens (for ponds) are also traded and some of these have the potential to become established and invasive. The trade in crayfish for aquaria and ponds must be considered as the prime source of potential new invaders. Source: Soes & Koese 2010.

Crayfish in the Netherlands. Upper row from left to right: noble crayfish, narrow clawed crayfish, spiny cheek crayfish, virile crayfish. Lower row from left to right: signal crayfish, white river crayfish, red swamp crayfish, marbled crayfish. Photos B. Koese, except signal crayfish: R. Lipmann.

Another reason to link the sampling sites with the Limnodata Neerlandica was to relate data on recently introduced crayfish with long term biological, physical and chemical parameters. A couple of species have expanded their range only very recently (since 2000). Therefore, a gradual reduction of the ecological quality since then might be detectable by comparing the conditions before and after introduction.

CRAYFISH AND ECOLOGICAL CONSTRAINS

A third goal of the project was to define the ecological niche of the spiny cheek crayfish *Orconectes limosus*. The spiny cheek crayfish is the only widespread species in the Netherlands, which had been present for over four decades. We assume that this species has since been able to invade all (connected) suitable habitat. Therefore, any possible effects that the species could have had on the waterquality, could not be reconstructed based on the Limnodata Neerlandica because the most reliable data in this database have been collected in the last twenty years. Old data from, for instance, the 80's and 90's were not used because of the varying methods of sampling and water quality compared to the current methods.

Material & methods

SELECTION OF SAMPLING SITES

Sampling sites for this project were selected *a priori* selected from the Limnodata Neerlandica, based on the following steps and criteria:

- Sample sites with less than two full sets of chemical parameters (see table 2) since 2000 were excluded.
- To the remaining subset, macroinvertebrates, macrophytes and physical-chemical samples were added.
- All data were sorted in descending order by water board, based on the number of biological and physical chemical samples.
- Per water board, 18 sample sites with the most available data were published on a website. These sites were open for sampling by volunteers. We aimed for an availability of at least three samples of macroinvertebrates, three samples of macrophytes and three physical-chemical samples per sampling site. This rule was applied with some flexibility, depending on the availability of data for each water board, to guarantee national coverage. Therefore, the data coverage was not 100% for all sampling sites.

RECRUITING VOLUNTEERS

Volunteers were approached through the crayfish newsletter (a digital newsletter to inform volunteers associated with EIS about crayfish) and through advertising on various websites such as www.ravon.nl and www.totalfishing.nl. Prior to the start of the inventory, a website was constructed (www.kreeftenonderzoek.nl). This site contained a link to a Google map which displayed a total of 468 sampling sites (18 per waterboard in a total of 26 waterboards) (fig. 5). To accomplish a good geographical distribution of sampling sites thoughout the Netherlands, we aimed for at least 11 sites to be sampled per waterboard (and thus a total of at least 286 samples). Through the website, volunteers could apply for one or more sampling sites. After registration, the volunteers received a package with equipment (three traps, a permit to use the traps, aluminium pre-printed labels and rope to attach the traps to the bank). Often, volunteers applied for more than one site and by doing so, they spared budget and material (if they were willing to re-use equipment), which enabled other volunteers to apply for more sites than the original minimum target.

Table 2. Availability of data (physical-chemical parameters) for the sampled sites (n=294)

Physical-chemical parameters		
Parameter	**Availability**	**Coverage**
Chloride	288	98%
Total Nitrogen	288	98%
Oxygen	287	98%
Electric Conductivity	285	97%
Temperature	281	96%
pH	279	95%
Sulphate	274	93%
Transparency	270	92%
Total Phosphorus	265	90%
BOD	235	80%
Calcium	226	77%
Chlorophyl-a	206	70%
Magnesium	201	68%
Sodium	192	65%
Potassium	187	64%
Particulate matter	163	55%
Orthophosphorus	155	53%
Bicarbonaat	152	52%
Bicarbonate	131	45%
Ferrum	102	35%
Biological parameters		
Parameter	**Availability**	**Coverage**
Macro-invertebrates 2000-2005	261	89%
Macro-invertebrates 2000-2005	197	67%
Macrophytes 2006-2009	188	64%
Macrophytes 2006-2009	182	62%

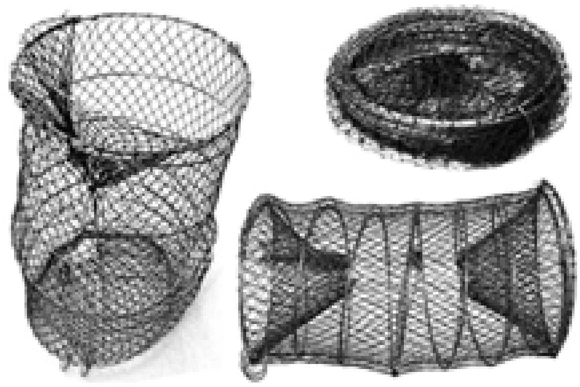


Fig. 4. The LiNi ® trap, used for the inventory.

SAMPLING

In collaboration with Statistics Netherlands (CBS), a sampling protocol was designed for this inventory (see box 3). The protocol consisted of the use of three Swedish LiNi® traps (www.lini.se) (fig. 4), which had to be examined at four consecutive mornings within the prescribed sampling period per site. A list of instructions for the volunteers can be found in appendix 4. As a principle, we designed the protocol for unbaited traps for several reasons:

1) to avoid by-catches as much as possible;
2) to avoid damage to the traps (e.g. by rats, herons or other animals);
3) to standardize the protocol as much as possible

(by avoiding the risk of volunteers using different kinds of bait);

4) to minimise the effort for volunteers.

Before the traps were used in the field, volunteers were asked to soak the traps in fresh water in a bucket for about a week to get rid of the 'new odour'. After sampling, the volunteers were requested to fill in a sampling form (see appendix 5). Besides records of crayfish, the volunteers were asked to fill in a couple of field parameters, mostly by multiple choice options. These were:

- width of the water (<2, 2-5 or >5 metre).
- structure of the bank (natural bank, natural bank and shoring, weathered shoring and shoring).
- coverage of floating and emergent vegetation (%)
- coverage of duckweed (%)
- weather conditions (dry, rain, rain & thunder)
- bycatches (optional).

PERIOD

A sampling period between August 15 and October 15 2010 was prescribed. 95% of all sites were sampled within this period (277 out of 294 sites). A couple of volunteers were unable to sample within this period and sampled slightly earlier or later. All samples were collected between July 15 and October 20. A fixed, short sampling period in late summer was prescribed for two reasons:

- All invasive species with a sufficient number of records in the EIS-database have been shown to be (very) active in this period (e.g spiny-cheek crayfish, red swamp crayfish, eastern white river crayfish, virile crayfish and signal crayfish);
- By sampling in late summer, bycatches of amphibians could largely be avoided.

DATA ANALYSIS: HABITAT PREFERENCE

In order to investigate the habitat preference of the spiny cheek crayfish, all available parameters were linearly correlated with the presence and abundance of the crayfish species. Then, the most promising parameters were investigated for relations with the abundance of the spiny cheek crayfish. In this interactive process, the data of the parameters that explain the occurrence of spiny cheek crayfish are repeatedly removed to reveal the next explaining parameter.

Derived parameters were also used, for instance: minimum pH and percentage of oxygen measurements <5 mg/l. Such derived parameters are expected to

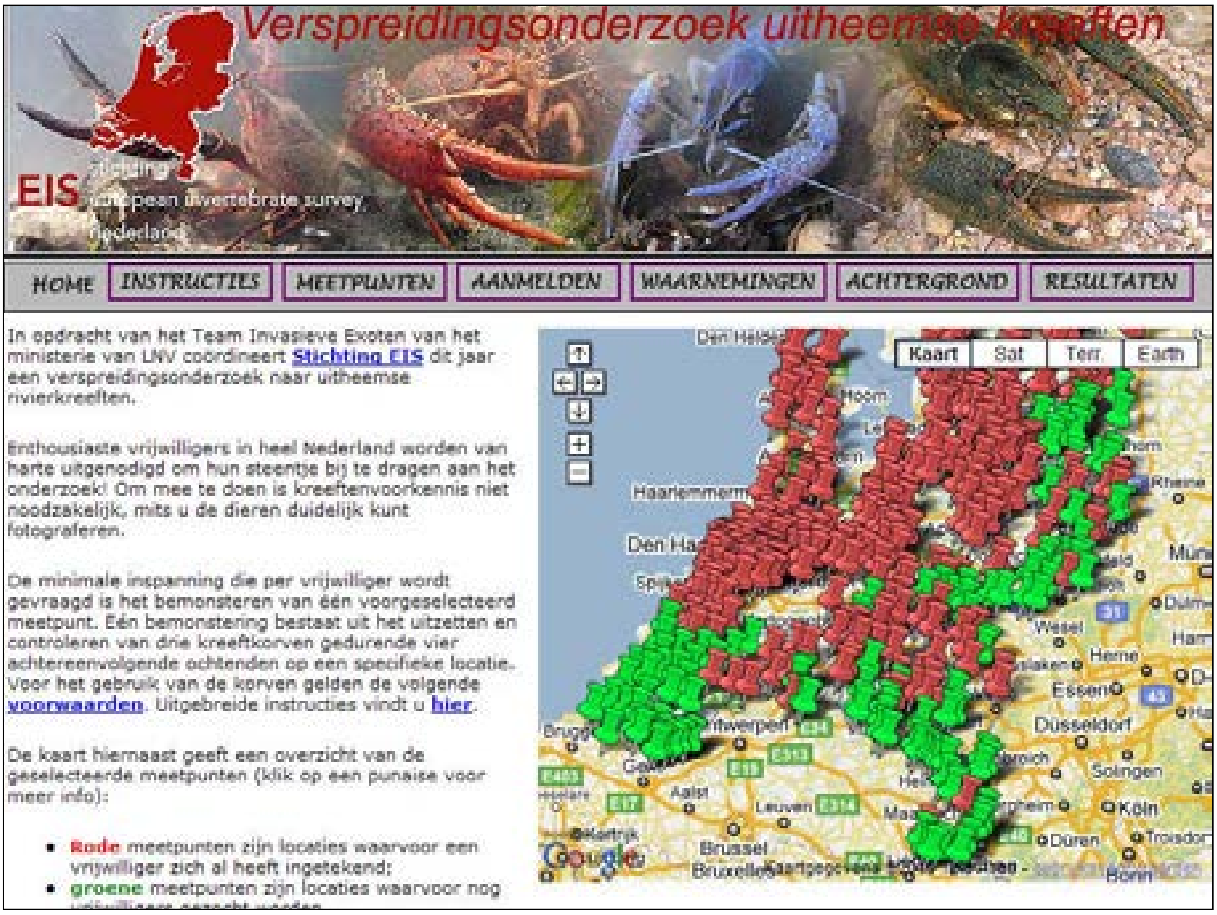


Fig. 5. A screen dump of the website www.kreeftenonderzoek.nl, halfway the sampling period. Red thumbtacks reflect sampling sites reserved by volunteers. Green thumbtacks are still open for reservation.

represent the ecological relation better than just the common parameters (year average). The selection of the most critical factors was not only based upon correlations. Ecological knowledge played an important role as well, since linear correlations do not neccesarily reveal all possible associations in the dataset. A good example of such a parameter is pH. In our data there is hardly any correlation between the pH and the presence of spiny cheek crayfish. However, it is known that the species is not found at a low pH. Therefore, the pH was taken into account in further analysis to identify sites that could be considered to be unsuitable for crayfish.

A principal component analysis (PCA) was conducted with Canoco©. A PCA is a mathematical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of uncorrelated variables called principal components. The number of principal components is equal, or in this case, less than the number of original variables. This transformation is defined in such a way that the first principal component has highest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it will be orthogonal to (uncorrelated with) the preceding components.
With the PCA it is possible to visualize the relative importance of all factors on the presence of spiny cheek crayfish. The description of the habitat preference was derived by 'peeling' of the dataset. The main driving factor describing variation within the dataset was identified and removed from the dataset after which the process was repeated for the next 'most important' relation between the data and the presence of spiny cheek crayfish.

In addition, the dataset was investigated to see whether certain types of waterbody were more or less sensitive to colonization by the spiny cheek crayfish. The different types considered:

- Brackish waters: >300 mg/l chloride;
- Ditches: fresh, stagnant, line shaped waters less than 8 meters wide;
- Canals: fresh, stagnant, line shaped waters less than 8 meters wide;
- (Small) lakes: fresh, stagnant mosaic shaped waters;
- Streams: fresh, running waters.

Box 2. Ecological Quality Ratio.
The Ecological Quality Ratio (EQR) is one of the metrics used within the European Water Framework Directive (WFD) to define water quality.

The EQR is expressed in a value between 0 and 1 and the following classes are defined:

- 0.8 – 1.0: Very good (=the reference)
- 0.6 – 0.8: Good
- 0.4 – 0.6: Moderate
- 0.2 – 0.4: Insufficient
- 0.0 – 0.2: Bad

For artificial waterbodies, four classes are used: a score of 0.6-1.0 is considered as one class. A score of 0.6 is the minimum objective for each waterbody.

The metrics are calculated according to Van der Molen & Pot (ed.) (2007) for natural waters and Evers & Knoben (ed.) (2007) for the ditches and canals. For calculations for this survey, the evaluation module from Dawaco Ecology was used (see www.dawaco.com).

DATA ANALYSIS: ECOLOGICAL IMPACT

First, for all crayfish the dataset was investigated for any relationship between the Ecological Quality Ratios (EQR, see box 2) derived from macroinvertebrates and macrophytes. The spiny cheek crayfish was omitted from further analyses. The species has been present in the Netherlands for several decades. Therefore, it was not possible to reconstruct the effect of colonization for this species based on the available data because the species was already widely distributed prior to 2000. However, a reconstruction of the impacts of the invasion by crayfish might be possible for the remaining three species, especially the red swamp crayfish, which was hardly found before the year 2000, and the virile crayfish, which was discovered for the first time in 2004 (Soes & Koese 2010).
At sampling sites where these species were found, the difference in quality between two periods (2000-2005 and 2006-2009), which is likely to reflect the period before and after colonization, was investigated. A T-test was applied to test for significant quality changes between the two periods.

Additionally, it was investigated as to whether the red swamp crayfish, the virile crayfish and the narrow clawed crayfish reduced the water transparency to a level below the WFD standards.

Finally, the macroinvertebrates samples from the locations where a crayfish species was found, were investigated with a Detrended Correspondance Analysis (DCA). A DCA is a statistical technique widely used by ecologists to find gradients in large datasets of species that typify ecological community data. Unlike the PCA, which uses a large set of explaining variables to define other, derived variables, no underlying explaining variables are involved in a DCA. Instead, gradients are calculated based on a matrix and the resulting variables reflect the distances between the objects in the matrix.
With the DCA, we calculated the position of the crayfish in relation to other species at the same location based on the most recent samples (2004-2009). In the resulting graph, we labelled the taxa with EQR values and a species specific velocity indicator, ranging from 1 (only present in stagnant water) to 5 (only present in running water). The labelling with velocity data yielded a clear pattern. The DCA combined with EQR data resulted in a seemingly unstructured and confusing image and was not included in this report.

ANALYSES WITHOUT RESULTS

Besides the DCA combined with EQR data, other analyses did not reveal clear relationships as well, sometimes due to a lack of parameters. This is the case for the following physical-chemical parameters in relation to the presence of the spiny cheek crayfish:

- ferrum;
- chlorophyll;
- transparency;
- depth;
- magnesium;
- potassium;
- particulate matter.

The correlations between the electrical conductivity, sodium and chloride are so high that the analyses are only conducted with chloride.

Box 3. Design of the sampling protocol

The aim of the sampling protocol was to claim absence or presence of crayfish at a particular site with a 95% capture probability, if present. First, a reference dataset of a year-round monitoring of a large population of white river crayfish (*Procambarus acutus s.l.*) with three unbaited traps was used to determine the minimal capture probability of an individual trap at this particular site. As can be seen in fig. 6, the capture probability varies throughout the season. In summer, crayfish were caught almost every day in every trap, sometimes in great numbers. The individual capture probability is >85% per trap in this period. In winter however, the individual capture probability drops to 25%. Often, only one crayfish per trap in every four days is found. We assumed that the situation in winter of this large population with high densities of crayfish, might reflect a situation with low densities of crayfish in another habitat in summer.

white river crayfish

Thus:

Capture probability per trap if crayfish occur:	0.25
Probability per trap to catch nothing if a crayfish occur	0.75

Based on three traps and four days, this results in the following capture probabilities:

Probability of catching nothing in all traps on all days	0.031
Probability of catching at least one crayfish in all traps on all days	0.968 (> 95%).

Of course, different combinations of days and traps can be used, but all turned out to be less optimal. With two traps, six days are needed to obtain a 95% capture probability. More traps per sampling site would have been too costly.

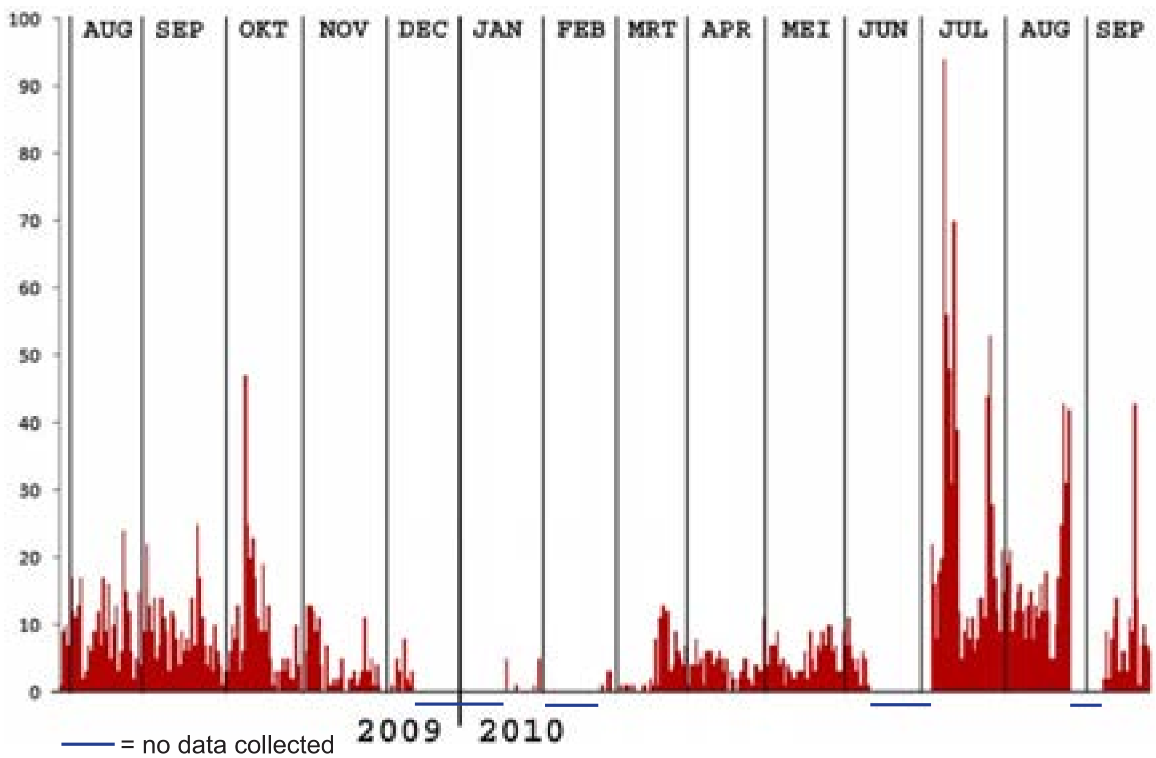


Fig. 6. Total catchments of white river crayfish *Procambarus acutus* s.l. at daily controls of three unbaited traps in a ditch near Giessenburg (province of Zuid-Holland). Source: Joop Verbeeth (www.landschapsmonument.nl)/EIS-Nederland.

Results

VOLUNTEERS AND SAMPLING SITES

A total of 204 volunteers signed up for sampling a total of 347 sites (1,7 site per person on average). Of the reserved sites, 294 were actually sampled (84%) (table 3, appendix 3). Sampling at four sites was impossible because of dry or otherwise unsuitable habitat (fig. 7a). Personal circumstances explain 33 unsampled sites. The result of 14 reserved sites is unknown.

The desired threshold of at least 11 sites per waterboard was achieved for 16 out of 26 waterboards.

However the total amount of sampled sites (294) exceeded the desired minimum of 284, because of a surplus of reservations in some areas. Locally, even a 'shortage' of sites occured (more volunteers than sampling sites available). In those cases, extra sampling sites were picked from the Limnodata Neerlandica and placed on the website. This explains why more than 18 sites could have been sampled in one of the waterboards (WS Aa en Maas).

Table 3. The number of reserved and sampled measurements points per waterboard (see fig. 1 for list of full names of the waterboards)

Waterboard (shortened name)	**Abb.**	**Reserved locations**	**Sampled locations**
Delfland	HHD	23	17
Noorderkwartier	HHN	18	17
Rijnland	HHR	12	11
Schieland & Krimpenerwaard	HHS	12	11
Stichtse Rijnlanden	HSR	13	11
Aa en Maas	WAM	20	19
Waternet	WAN	14	12
Brabantse Delta	WBD	9	7
Dommel	WD	11	11
Frylân	WF	18	13
Groot Salland	WGS	16	16
Hunze en Aa's	WHA	17	16
Hollandse Delta	WHD	13	7
Noorderzijlvest]	WN	17	17
Peel en Maasvallei	WPM	12	12
Regge en Dinkel	WRD	10	7
Rijn en IJssel	WRIJ	6	6
Rivierenland	WRL	12	9
Roer en Overmaas	WRO	10	8
Reest en Wieden	WRW	13	6
Veluwe	WV	16	13
Vallei en Eem	WVE	12	12
Velt en Vecht	WVV	5	4
Zeeuwse Eilanden*	WZE	10	9
Zeeuws-Vlaanderen*	WZV	10	5
Zuiderzeeland	WZZ	18	18
TOTAL		**347**	**294**

* Now fused into WS Scheldestromen

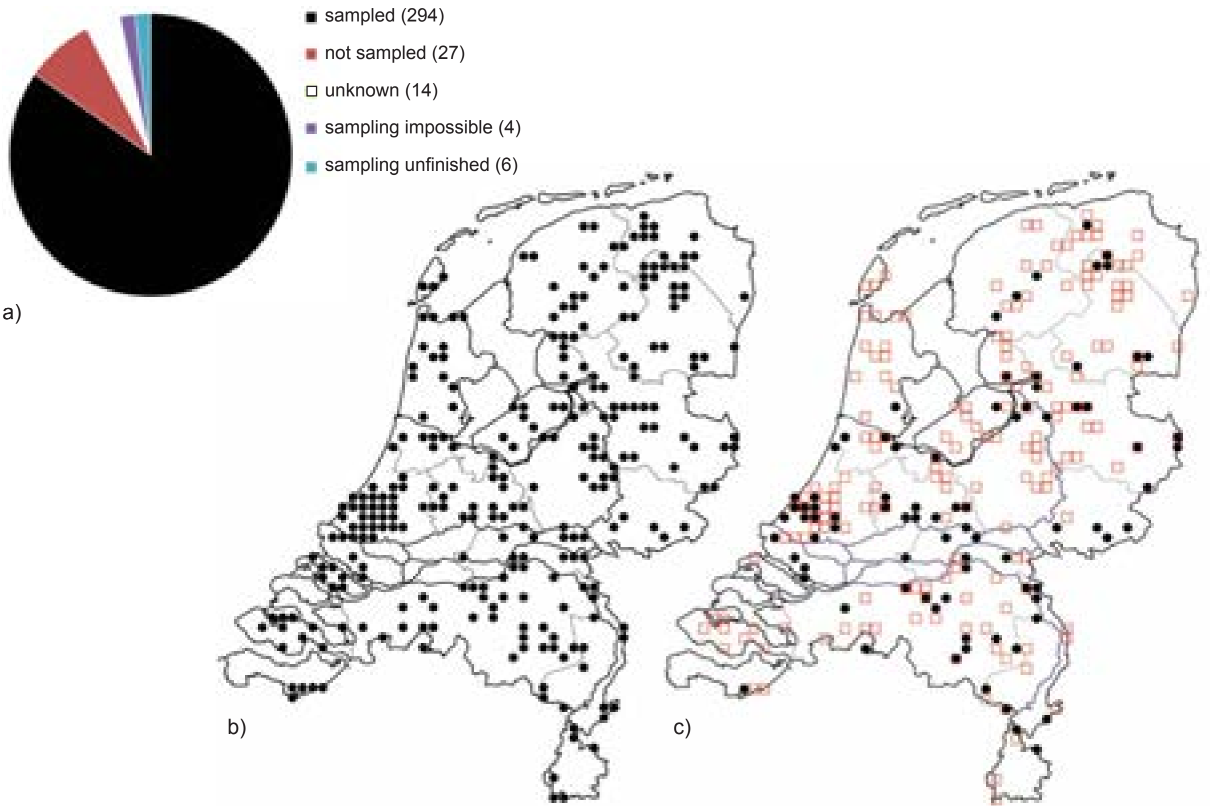


Fig. 7. a) pie plot of the effort of volunteers after reservation of a site (n = 347 reservations); b) distribution of reserved locations; c) distribution of sampled location: black dots: crayfish found, red squares: no crayfish found. A dot or square reflects 5 km^2.

CRAYFISH

One or more sprecies of crayfish have been found at 89 out of 294 sampling sites (over 30% of all sampled locations). Four species were recorded: the spiny cheek crayfish was most commonly found at 71 sites (fig 10a), followed by the red swamp crayfish at 16 sites (fig. 10b), the virile crayfish at 4 sites (fig. 10c) and the narrow-clawed crayfish at 1 site (fig. 10d). Two species of invasive crayfish with a very limited distribution in the Netherlands, the signal crayfish and the white river crayfish (fig. 9) were not detected during the inventory. At several sites, crayfish had not been recorded previously. However, none of the records acquired with the inventory deviate much from previously collected distribution data.

The amount of data acquired through the inventory of the spiny cheek crayfish nearly equals the amount of passively acquired data in 2010 (fig. 10a). For the red swamp crayfish the amount of passively acquired data in 2010 is much larger than the amount of data acquired with the inventory (fig. 10b). The total number of crayfish caught per site varied from one to 25 (fig. 8). The maximum number of species caught at a single site was two, which happened at four sites. On three occasions the spiny cheek crayfish was found sympatrically with the red swamp crayfish. In one occasion the spiny cheek crayfish was recorded together with the virile crayfish.

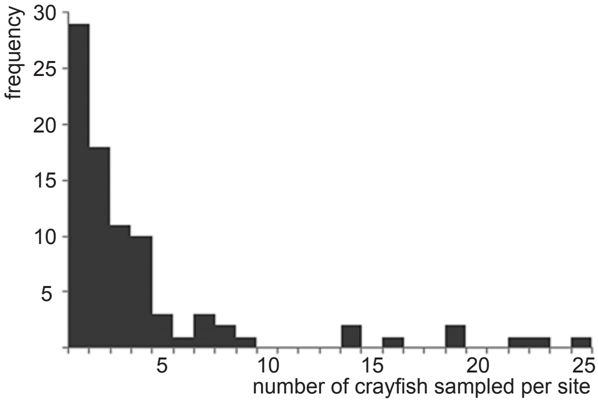


Fig. 8. Frequency of the number of crayfish per sampling site.

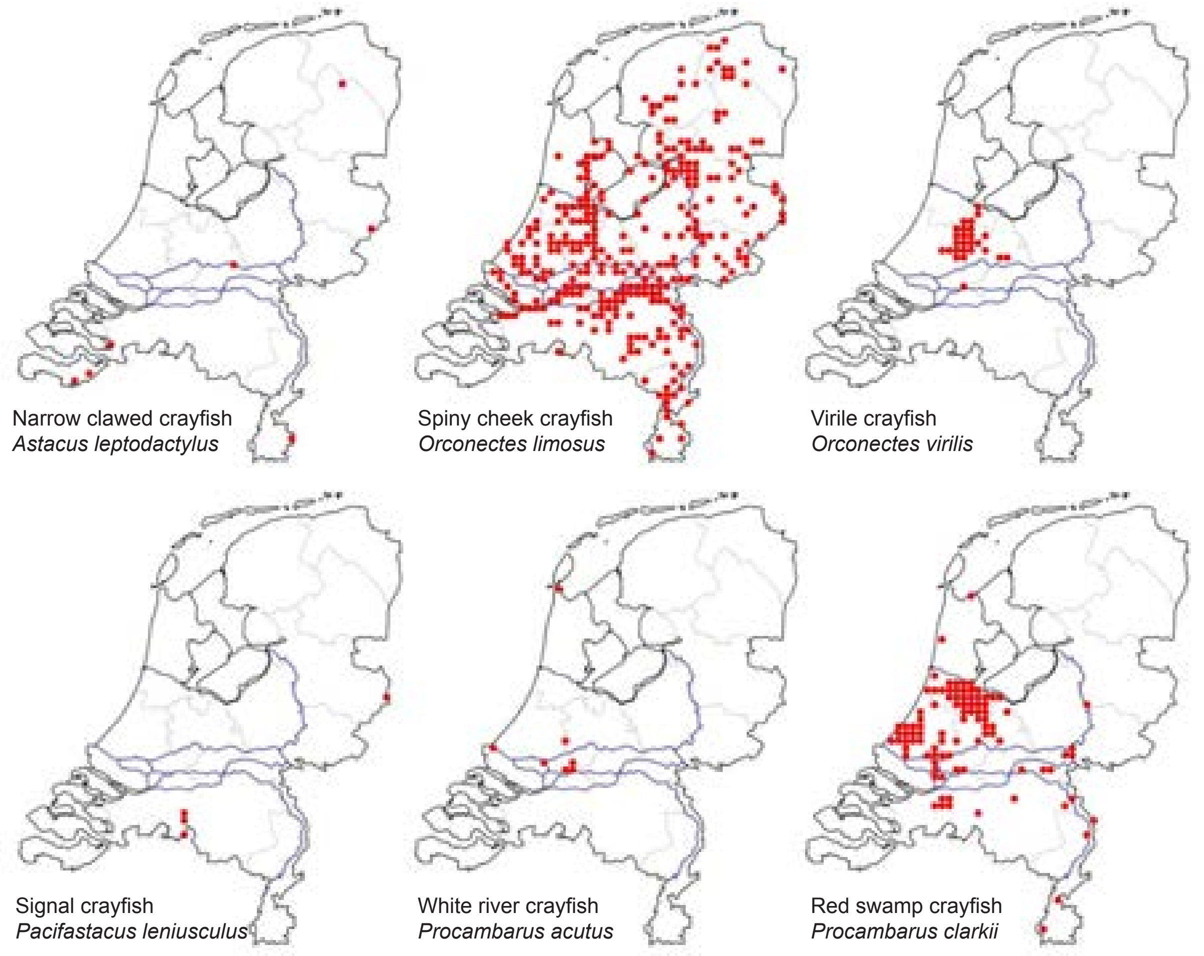


Fig. 9. Distribution maps of crayfish in the Netherlands, based on all available data since 2000. Not included are the native Noble crayfish (*Astacus astacus*), of which only one location is left and invasive crayfish who have been recorded only once, such as the stone crayfish (*Austropotamobius torrentium*), the redclaw (*Cherax quadricarinatus*) and the marbled crayfish (*Procambarus fallax*). Source: EIS.

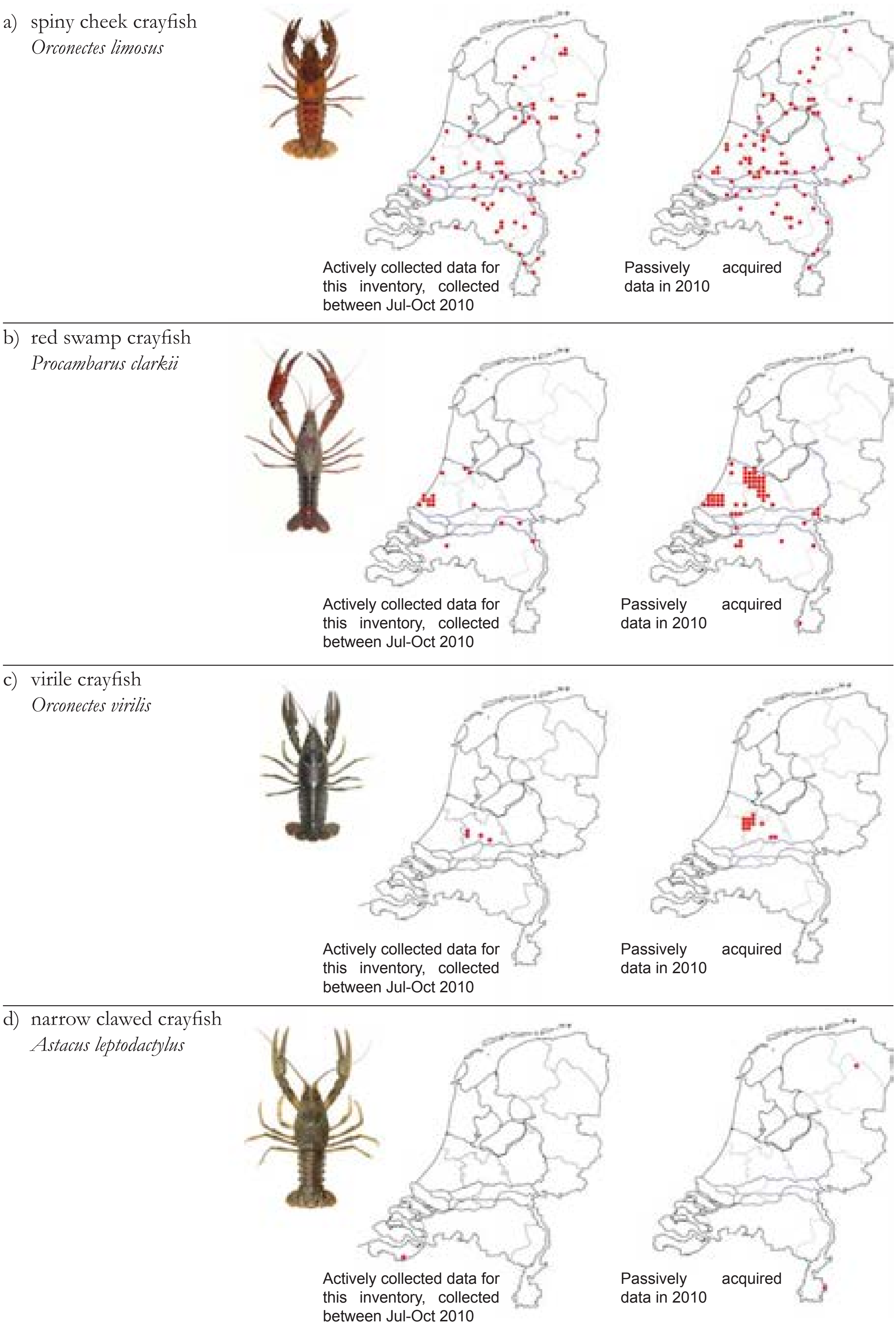


Fig. 10. Distribution of sightings of all four crayfish species caught during the inventory (maps on the left), compared to passively acquired data 2010 (maps on the right). Passively acquired data are records reported at EIS-Nederland and the online portal www.waarneming.nl

HABITAT PREFERENCES OF THE SPINY CHEEK CRAYFISH

There were no strong correlations between the presence and abundance of the spiny cheek crayfish and the different parameters (table 4). This does not imply that the species is insensitive to these parameters. There are clear parameter values beyond which the species does not occur or hardly so. The most promising parameters for elucidating the occurrence of the spiny cheek crayfish were investigated further. The Van Wirdum Diagram (fig. 11) shows that nearly all locations where the spiny cheek crayfish was caught are in the centre area of the graph: brackish waters, weak buffered waters and too a lesser extent seepage waters hardly contain any records of the species. The water of the Meuse and the Rhine however, which flows through a great part of the Netherlands is very suitable.

Table 4. Linear correlations (*r*) of presence and abundance of the spiny cheek crayfish (SCC) with chemical parameters and field characteristics.

	SCC presence
Ortho phosphorus	-0.23
% oxygen measurements <5 mg/l	-0.21
Oxygen concentration	0.17
Minimum oxygen concentration	0.15
Oxygen saturation rate	0.14
Vegetation coverage (excl. submerged)	-0.13
Sulphate	-0.13
Calcium	-0.13
No. of samples with visibility to the bottom	0.12
Biological Oxygen Demand (BOD) (5 day, 20°C)	-0.12
Magnesium	-0.12
Width	0.12
Total Phosphorus	-0.12
Electric Conductivity (EC) (20°C)	-0.12
Maximum temperature	0.11
Chloride	-0.11
Bicarbonate	-0.10
Transparency	0.10
Sodium	-0.09
Chlorophyll-a	-0.08
Bank type	-0.07
Duckweed coverage	-0.06
Particulate matter	0.06
Total Nitrogen	0.06
Potassium	-0.05
Minimum pH	0.04
Maximum of pH	0.03
Depth	-0.01
% of samples with visibility to the bottom	-0.01
Ferrum	0.00

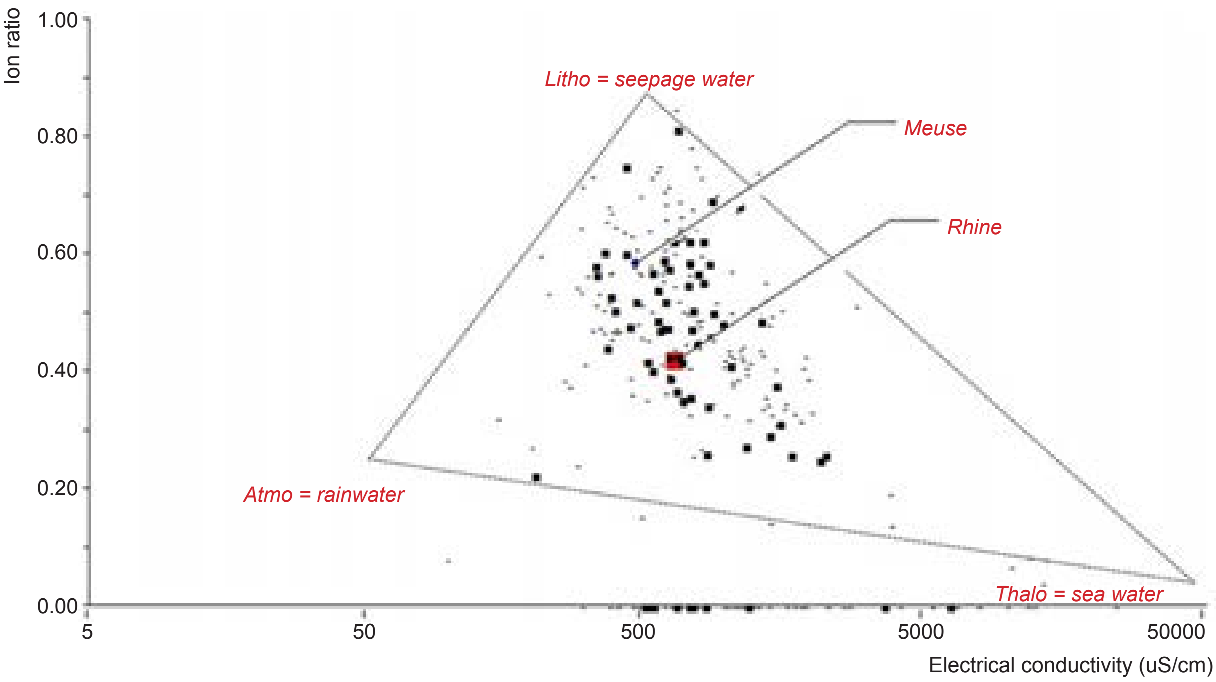


Fig. 11. Diagram of Van Wirdum: occurrence of the spiny cheek crayfish (*Orconectes limosus*). For records that intersect the X-axis, the ion-ratio is unknown. The ion ratio is based on the ratio of calcium and chloride.
■ = *Orconectes limosus* found, **-** = *Orconectes limosus* not found

Salinity and acidity

The Van Wirdum Diagram showed that brackish and weakly buffered waters hardly ever have records of the spiny cheek crayfish. This has been further investigated for chloride content and pH of the water bodies as shown in figure 12. There are clear boundary values below which (pH) and above which (chloride) the species does not occur. Over 95% of the sites with spiny cheek crayfish have a minimum pH of 6.4 and contain a maximum of 300 mg/l of chloride. Higher values of chloride were recorded only at two sites. Additionally, no spiny cheek crayfish are found in the waters with very low chloride concentrations (<20mg/l) either. However, such waters were hardly present in the dataset. Of the other species, the red swamp crayfish was found at sites with slightly lower pH-levels. Four out of sixteen sites had average acidity values of pH<6 (fig. 13).

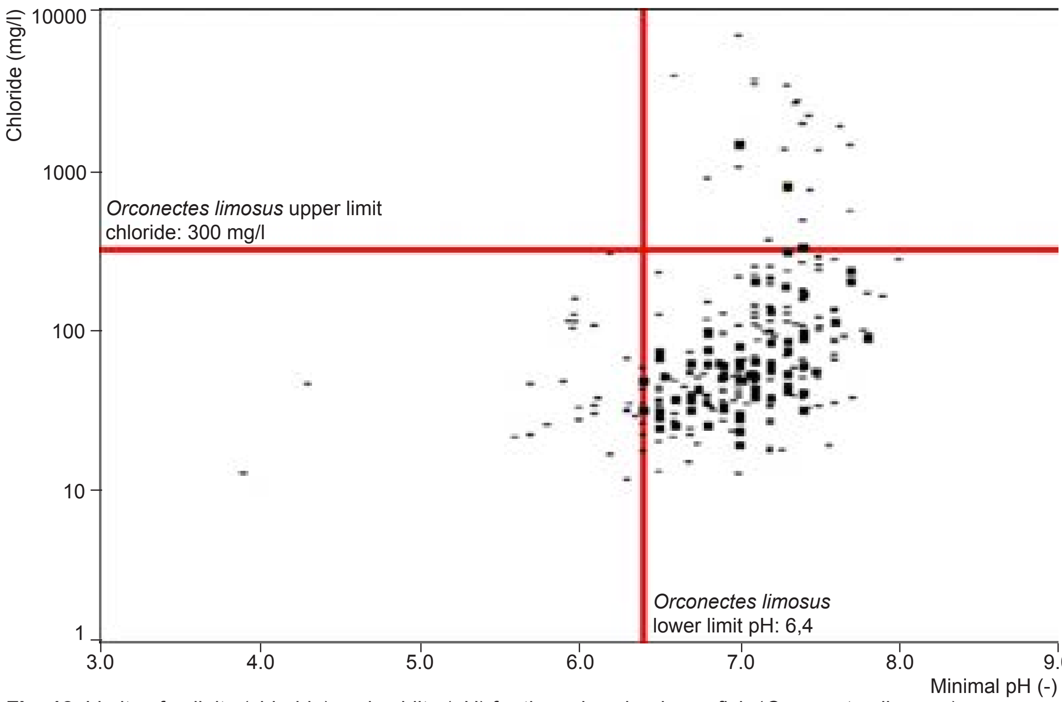


Fig. 12. Limits of salinity (chloride) and acidity (pH) for the spiny cheek crayfish (*Orconectes limosus*).
■ = *Orconectes limosus* found, - = *Orconectes limosus* not found

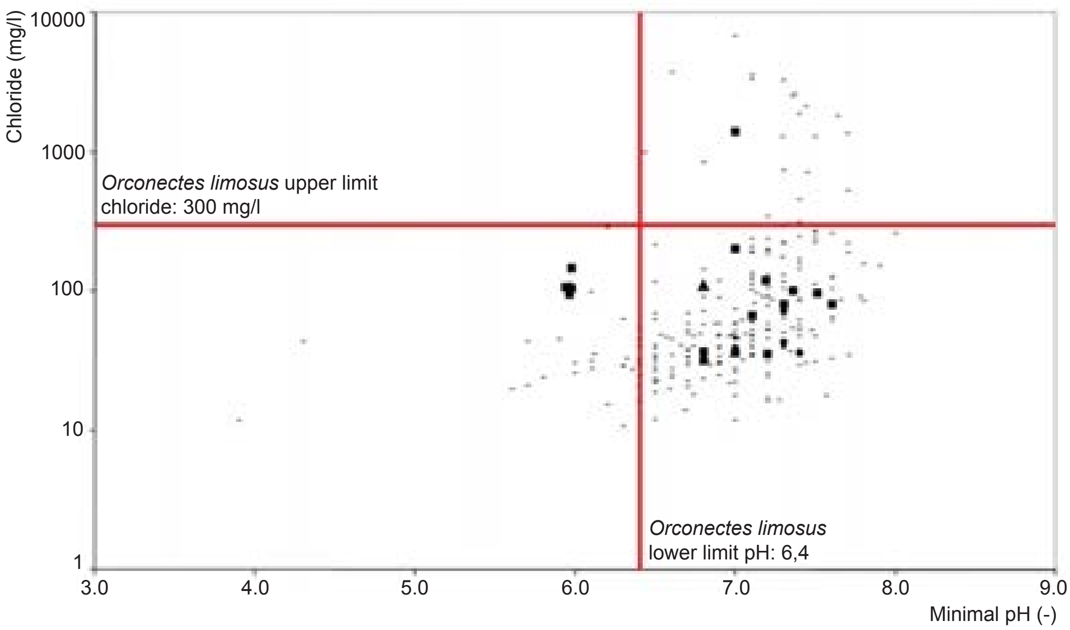


Fig. 13. Limits of salinity (chloride) and acidity (pH) for *Orconectes limosus* (see fig. 12) in relation to other crayfish species. ■ = *Procambarus clarkii* found, ● = *Orconectes virilis* found, ▲ = *Astacus leptodactylus* found, - = no crayfish found.

Oxygen

Almost none of the crayfish were caught at sample sites with average oxygen concentrations lower than 6,6 mg/l (fig. 14, 15). Temporal (sharp) drops of oxygen concentrations also seemed to affect crayfish distribution. Very few crayfish were recorded at sites where more than 20% of the measurements had an oxygen level below 5 mg/l, even though the average concentrations were more than 6,6 mg/l at the particular site. For these analyses, the acidic and brackish waters were left out.

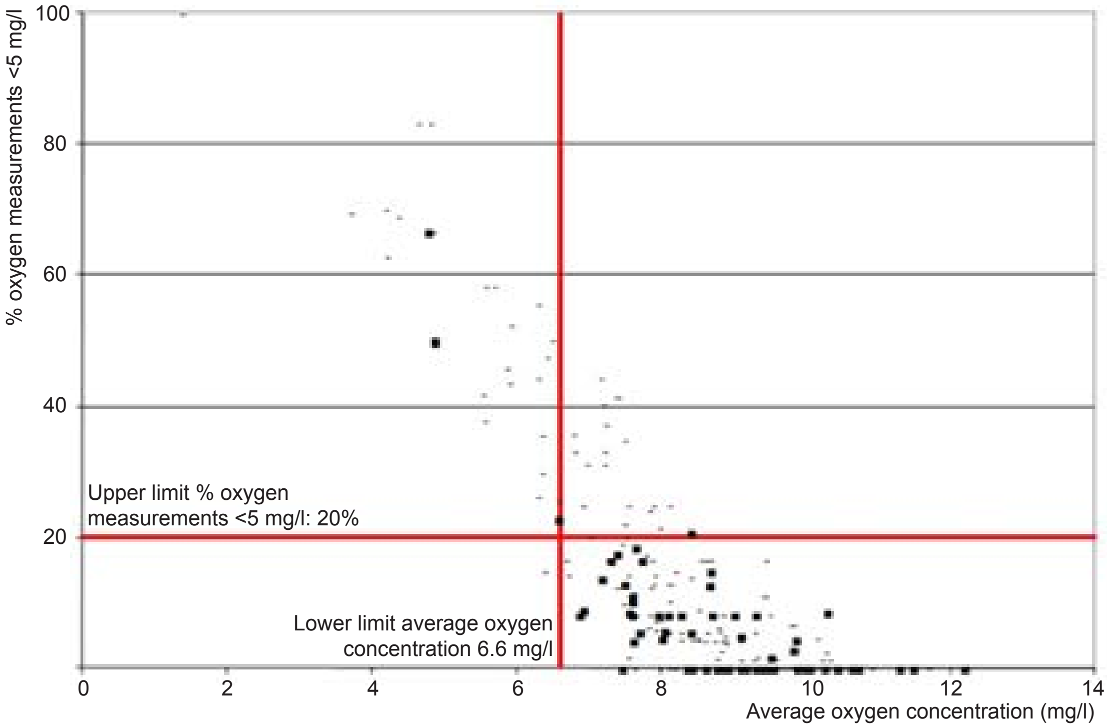


Fig. 14. Limits of oxygen of *Orconectes limosus* (acidic and brackish waters excluded).
■ = *Orconectes limosus* found, - = *Orconectes limosus* not found

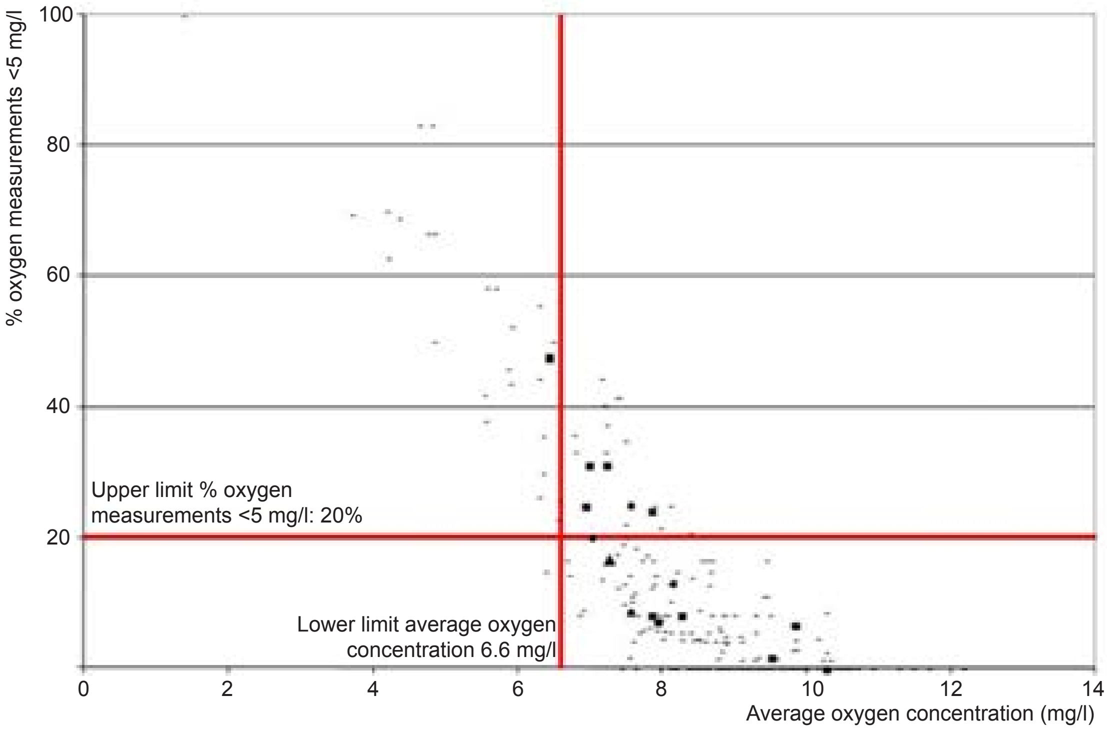


Fig. 15. Limits of oxygen for *Orconectes limosus* in relation to other species.
■ = *Procambarus clarkii* found, ● = *Orconectes virilis* found, ▲ = *Astacus leptodactylus* found, - = no crayfish found.

Temperature

The spiny cheek crayfish was not found in cooler waters, e.g. waters that do not exceed a maximum temperature of 19° celsius (fig. 16). The spiny cheek crayfish was also rare in warmer but very narrow water bodies (width <2 metres). These were almost all running waters (and one ditch).

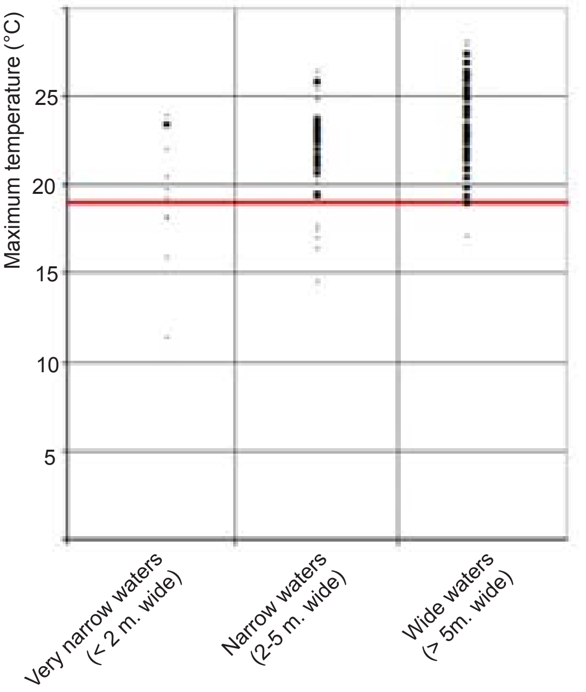


Fig. 16. Temperature limits of *Orconectes limosus* in relation to width classes of the waterbodies (dataset without brackish, acidic and oxygen-poor waters).
■ = *Orconectes limosus* found, **-** = *Orconectes limosus* not found.

Despite the absence of the species in smaller running waters, the overall occurrence of the species in habitats that are classified as streaming waters is high: in around a third of all sampled streaming waters the species was found (fig. 17). For these analyses, the acidic and low oxygen containing waters were excluded.

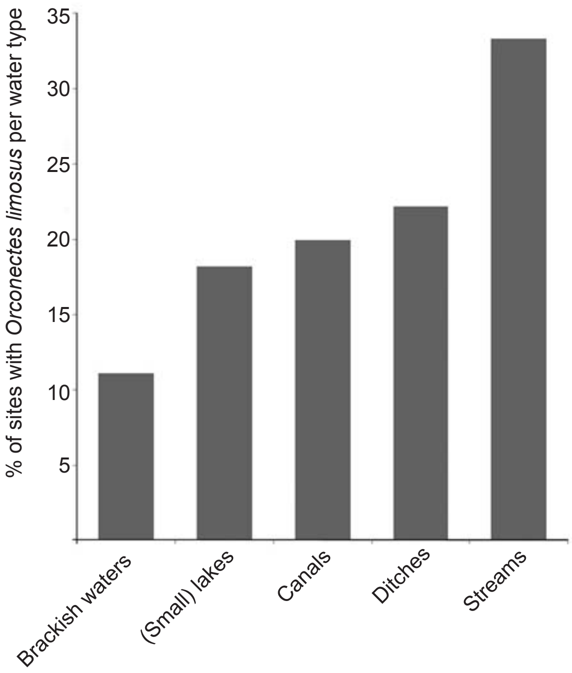


Fig. 17. Percentage of sites per water type with *Orconectes limosus* (whole dataset).

Sulphate and calciumthreshold

Fig. 18 shows the values of sulphate and calcium. The spiny cheek crayfish was not recorded at sites with calcium values above 135 mg/l or with sulphate values above 230 mg/l. However, the proportion of sites with records of the spiny cheek crayfish declined markedly with increasing concentrations of calcium and sulphate above 100 mg/l of both calcium and sulphate. Thus, high values of sulphate and calcium seem to limit the presence of the spiny cheek crayfish, but due to a limited number of samples, it is difficult to make firm statements about the upper boundary. For this analysis, brackish, acidic, low oxygen and cold waters were removed.

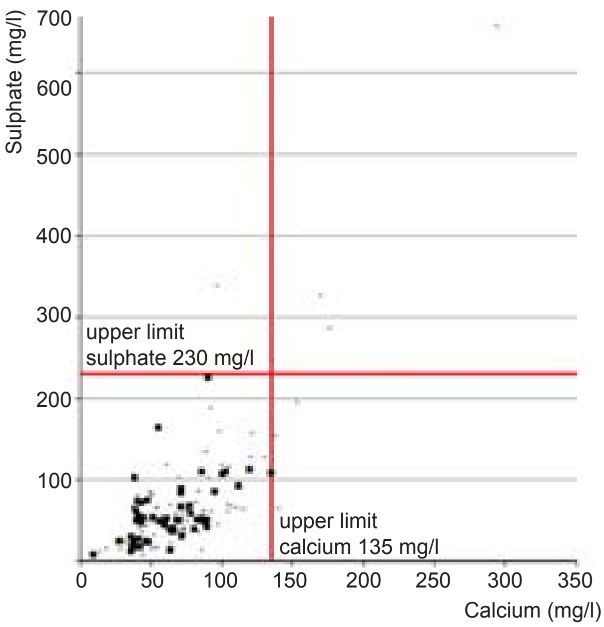


Fig. 18. Possible limits for sulphate and calcium for *Orconectes limosus* (dataset without brackish, acidic, oxygen-poor and cold waters).

Habitat parameters

No clear associations were found between the presence of the spiny cheek crayfish and the habitat parameters (structure of the bank and the coverage of floating and submerged vegetation) collected by the volunteers (fig. 19). The species was found in habitats with completely artificial banks ranging from those to entirely natural banks. As a logical consequence, habitats with a high vegetation coverage were mostly found in classes with (partly) natural banks. Overall, the coverage of vegetation seemed generally low in habitats containing spiny cheek crayfish, but this might also be a sampling effect. Overall, a very low number of sites with a high cover of vegetation coverage were sampled. For this analysis the brackish, acidic, low oxygen and cold waters were removed.

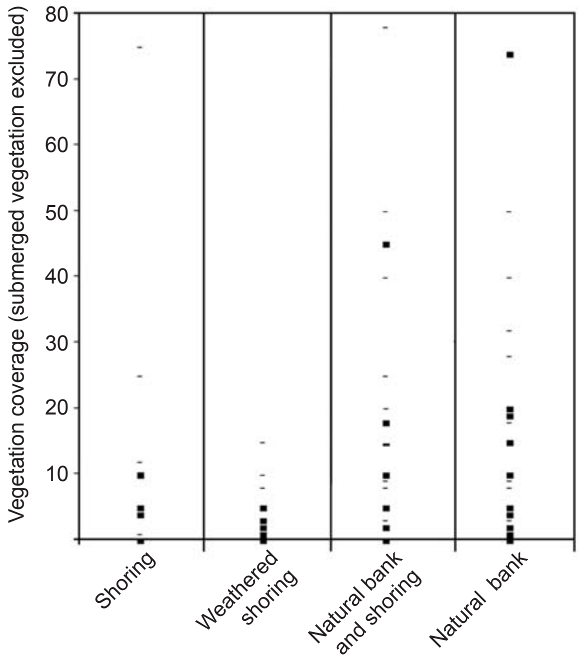


Fig. 19. Occurence of *Orconectes limosus* in relation to vegetation cover and bank type (dataset without brackish, acidic, oxygen-poor and cold waters).

Nutrients

Finally, the relation with nutrients was investigated. The species mostly inhabits waters with a relatively high nutrient content. The optimum was found in waters classified as 'poor' (fig. 20). The species was almost absent in waters with a very low nutrient status. The brackish, acidic, low oxygen and cold waters were removed for this analysis.

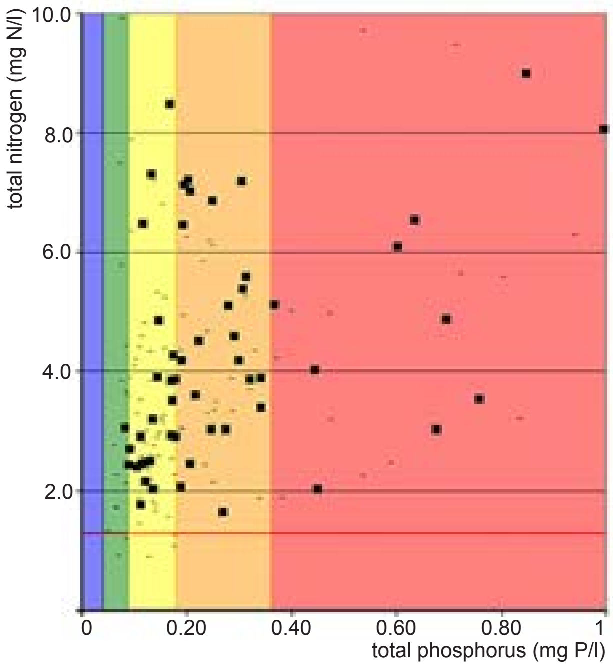


Fig. 20. Relation between nutrients (total phosphorus and total nitrogen) and the occurence of the spiny cheek crayfish. The proportion of samples per class are: high: no records, good: 11%, moderate: 32%, poor: 51%, bad: 6 %. Nutrient standards after: Van der Molen & Pot 2007, Evers & Knoben 2007 and Heinis & Evers 2007.

Principal Componant Analysis (PCA) of best parameters

Figure 21 shows the investigated parameters that gave the best results, depicted in a PCA diagram. Factors parallel to the x-axis (such as minimal pH) have a very weak linear correlation with the occurence of crayfish. Factors parallel to the y-axis have a stronger correlation and support the previous findings. That is:

- Sites with higher values for maximum temperature and width are more likely to have crayfish present.
- Sites with higher nutrient concentrations, low oxygen contents and high calcium and chloride values are less likely to have crayfish. Note that for nutrients, crayfish dissociate only with very high levels (e.g. >0.4 mg P/l). Fig. 20. demonstrated that crayfish actually *do* associate with relatively high nutrient levels.

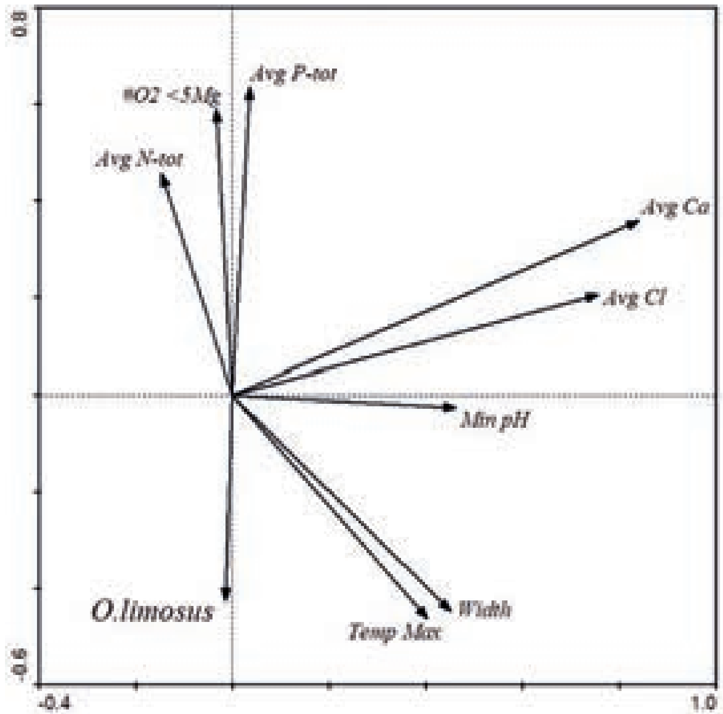


Fig. 21. Results of the PCA

Detrended Correspondance Analysis (DCA)

The DCA of the macroinvertebrates samples at locations where at least one of the exotic crayfish species was found shows that the current velocity is an important differentiating factor (fig. 22). Species of running waters are clearly positioned more to the right in comparison to the species of stagnant waters. The spiny cheek crayfish belongs to the species with a preference for flowing waters. This was already clear from analysis on the occurrence by water type (fig. 17). By contrast, the narrow clawed crayfish, virile crayfish and especially the red swamp crayfish were clearly associated with macrofauna assemblages typical of stagnant waters.

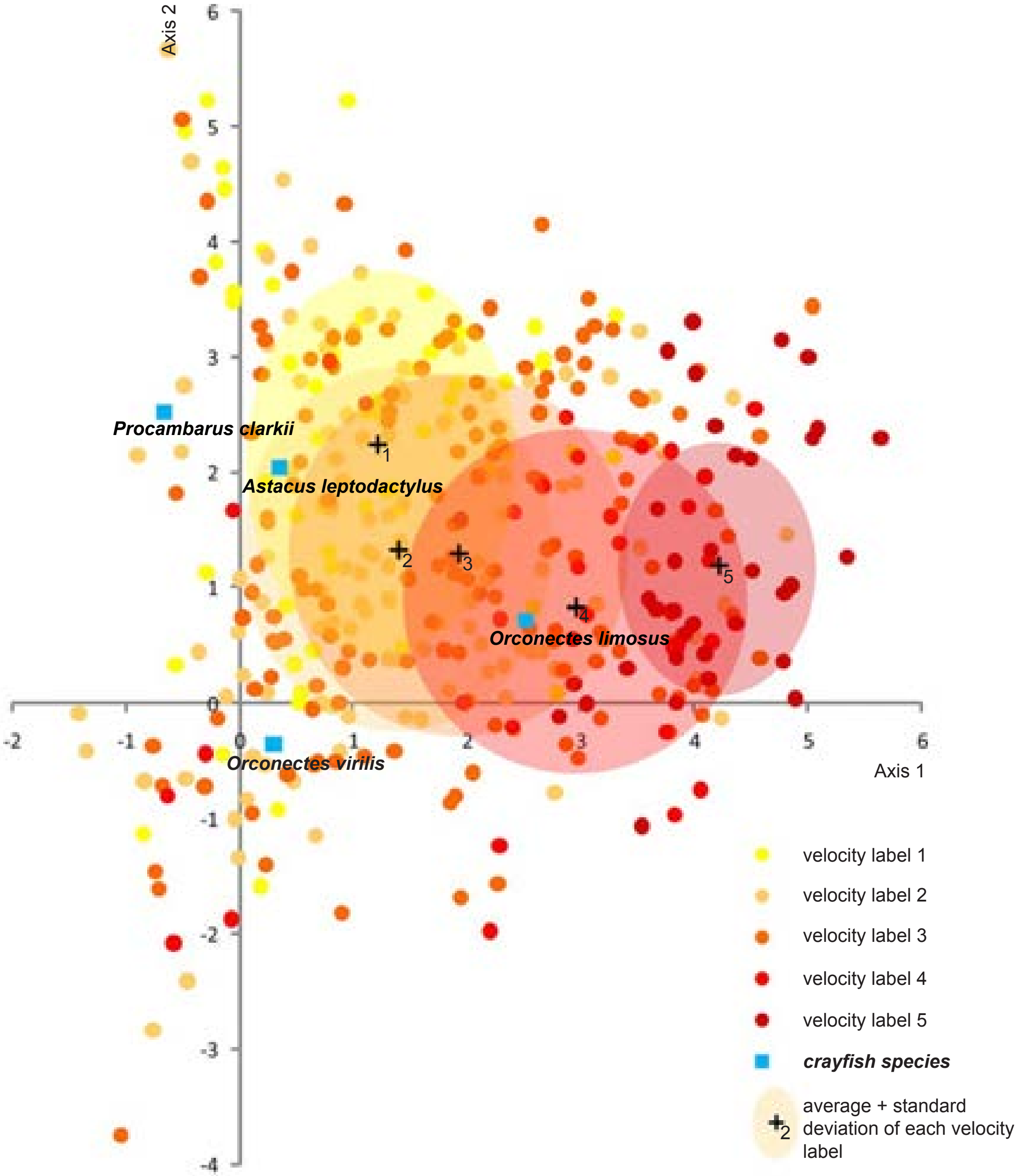


Fig. 22. Results of the DCA with macro-invertebrate samples of locations where at least one crayfish was found, labeled with a stream indication per taxon:
1= only in still waters
2= mainly in still waters or waters with a low velocity
3= both in still and running waters
4= mainly in running waters, sometimes still waters
5= only in running waters

EFFECTS OF INVASIVE FRESHWATER CRAYFISH ON WATER QUALITY

Relations with Ecological Quality Ratio's (EQR)
In waters that scored badly (EQR <0.2) on the macroinvertebrates metric, no crayfish species were found (fig. 23). However, on the macrophyte metric, species were still found in habitats with a very poor species composition (EQR <0.2). Crayfish hardly occured in waters that were categorized as 'good condition' (a minimum EQR of 0.6) on both the macrophyte and macroinvertebrate metric. For macroinvertebrates, on two sites that were categorized as 'good condition', one specimen of the spiny cheek crayfish was found.

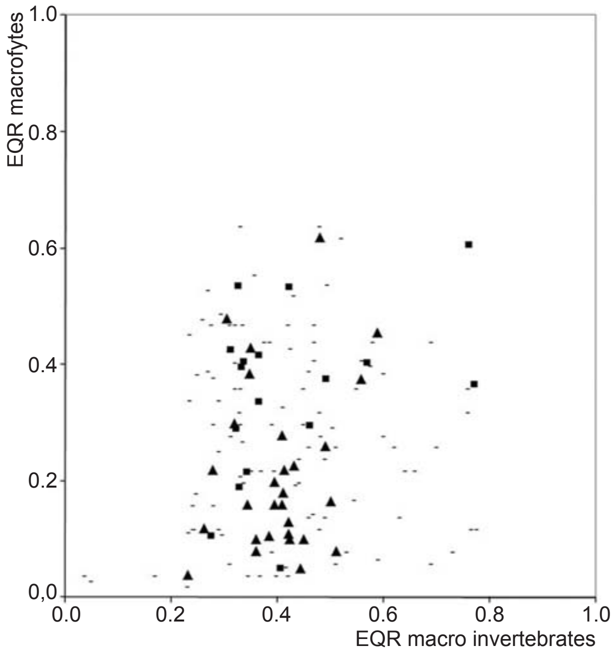
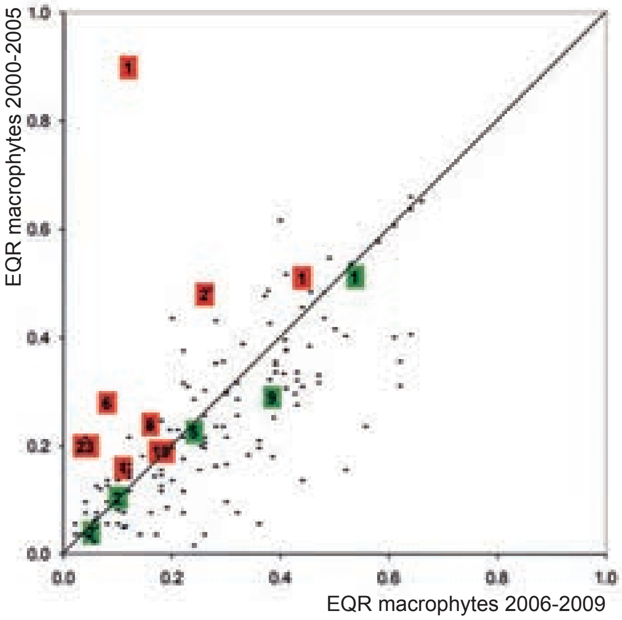


Fig. 23. Relationship of exotic crayfish (all four species found) with Ecological Quality Ratio's (EQR) of macrofytes (metric species composition) and macroinvertebrates

EQR macrophytes: before and after 2005
Fig. 24a shows a clear decrease in quality for the EQR at four sites. At one location (HHR-04) the quality even shifted from 'high' to 'bad' (although the old score might be too high due to sampling differences). Eight sampling sites remained more or less unchanged. At one site, the water quality clearly increased. Overall, the differences in the Ecological Quality Ratio (EQR) in the period 2006-2009 relative to the period 2000-2005 based on macrophytes are just not significant (t-test for equality of means, p=0.053) See also fig. 24b.

EQR macro-invertebrates: before and after 2005
Overall, the differences in the Ecological Quality Ratio (EQR) in the period 2006-2009 relative to the period 2000-2005 based on macroinvertebrates (fig 25a) are not significant (t-test for equality of means, p=0.15). See also fig. 25b. An exceptional change in quality was found at a location where 23 individuals of the red swamp crayfish species were caught (Haagse Beek). Since 2000 three macroinvertebrates samplings have been conducted in the Haagse Beek. In 2000 and 2004 a score of 0.50 and 0.52 respectively was attained on the macroinvertebrate metric for freshwater, buffered ditches (M01a). In 2007, this was only 0.23. This decline was mainly caused by the decrease in the number of 'positive taxa': from 70 in 2000 to 109 in 2004 to 37 in 2007. Whether this decrease is caused by colonization of the red swamp crayfish can not be proven with the current data. Apart from that, the red swamp crayfish was not found in any of the three samples from the Limnodata. This might suggest that this species was not present there until 2007. On the other hand it is possible that crayfish were not registered during regular monitoring.

Transparency
The presence of crayfish did not seem to be correlated with the transparency of the water (fig. 26). In more than 50% of the locations where the other crayfish were found, the values were within the WFD standards for canals.



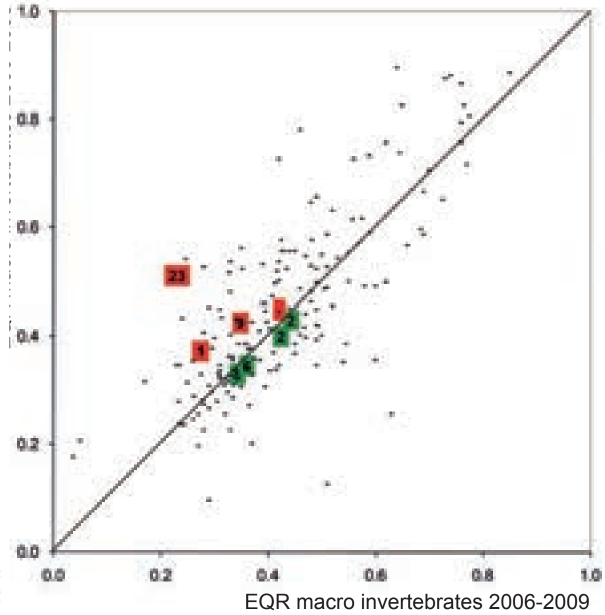


Fig. 24a▲. Effect of crayfish (the spiny cheek crayfish excluded) on the EQR of macrophytes. Green locations: the EQR had improved in 2006-2009 compared to 2000-2005. Red locations: the EQR decreased.
1-23 = number of crayfish found
- = no crayfish found

Fig. 24b▶. Boxplots of the ratio: EQR 2000-2005/EQR 2006-2009 for macrophytes. A large decrease in waterquality results in a higher value.

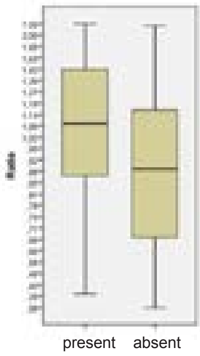
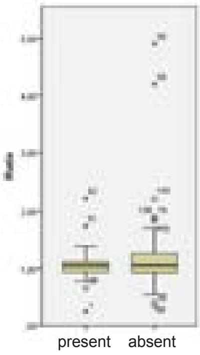


Fig. 25a▲. Effect of crayfish (the spiny cheek crayfish excluded) on the EQR of macroinvertebrates. Green locations: the EQR had improved in 2006-2009 compared to 2000-2005. Red locations: the EQR decreased.
1-23 = number of crayfish found
- = no crayfish found

Fig. 25b▶. Boxplots of the ratio: EQR 2000-2005/EQR 2006-2009 for macro-invertebrates. A large decrease in waterquality results in a higher value.



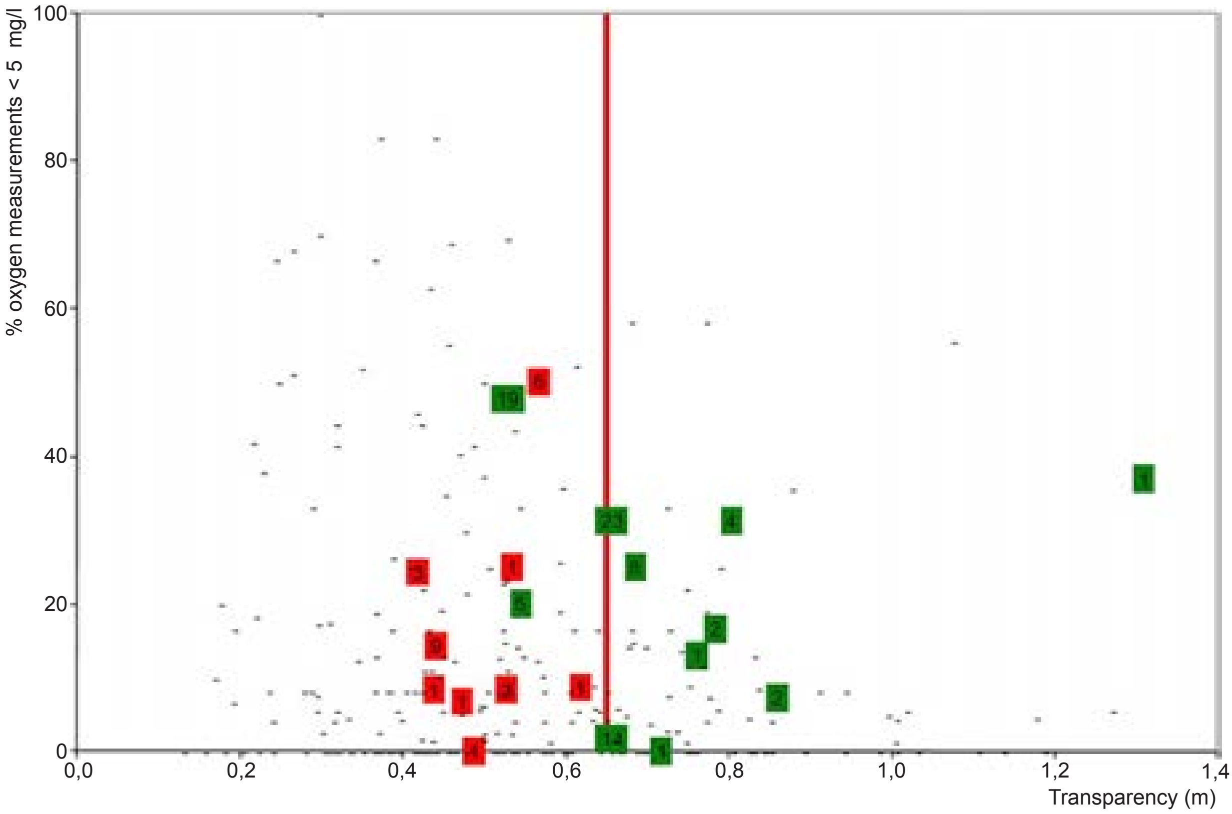


Fig. 26. Relationships between transparency and proportion of low oxygen concentrations. Green sampling sites meet the standard for transparency in canals (0.65 m of transparency or transparency to the sediment). Red sampling sites do not meet the standard. Sites with a bottom shallower than the threshold (such as two green sites in this graph), automatically fulfill the standard.

Trends

Neither the EQR for macroinvertebrates nor the oxygen concentrations show downward trends related to the presence of crayfish (figure 27-28). However, it is notable that both the red swamp crayfish and the virile crayfish appear to be more tolerant of oxygen limitations than the spiny cheek crayfish. The spiny cheek crayfish was rarely found at locations with more than 20% of the oxygen measurements <5 mg/l. The red swamp crayfish and the virile crayfish were found around these values.

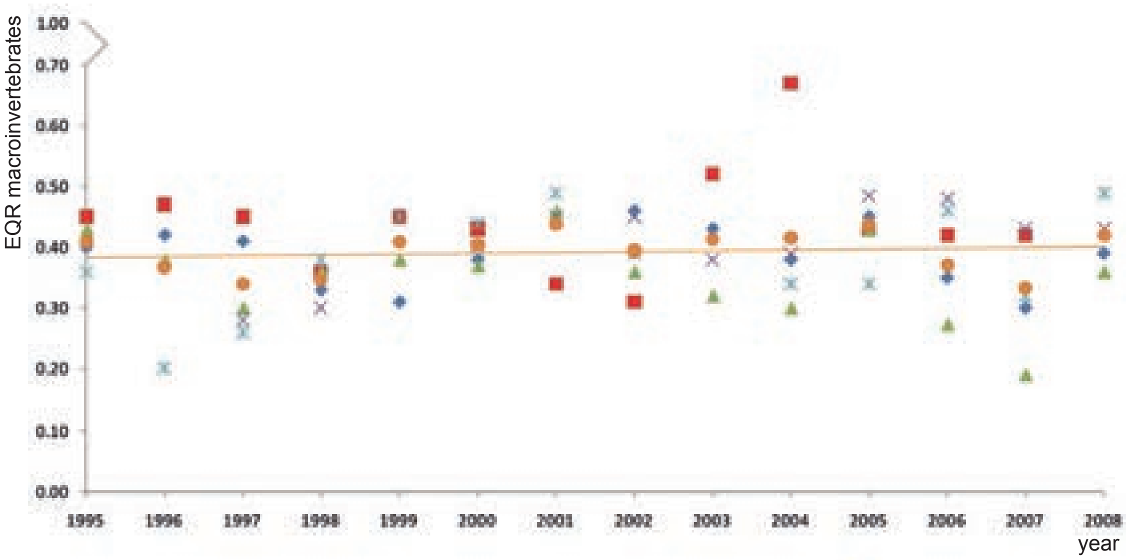


Fig. 27: Macroinvertebrates EQR trends for the 5 measurement points with the most macroinvertebrates data where specimens of the red swamp crayfish, narrow clawed crayfish or virile crayfish were found.

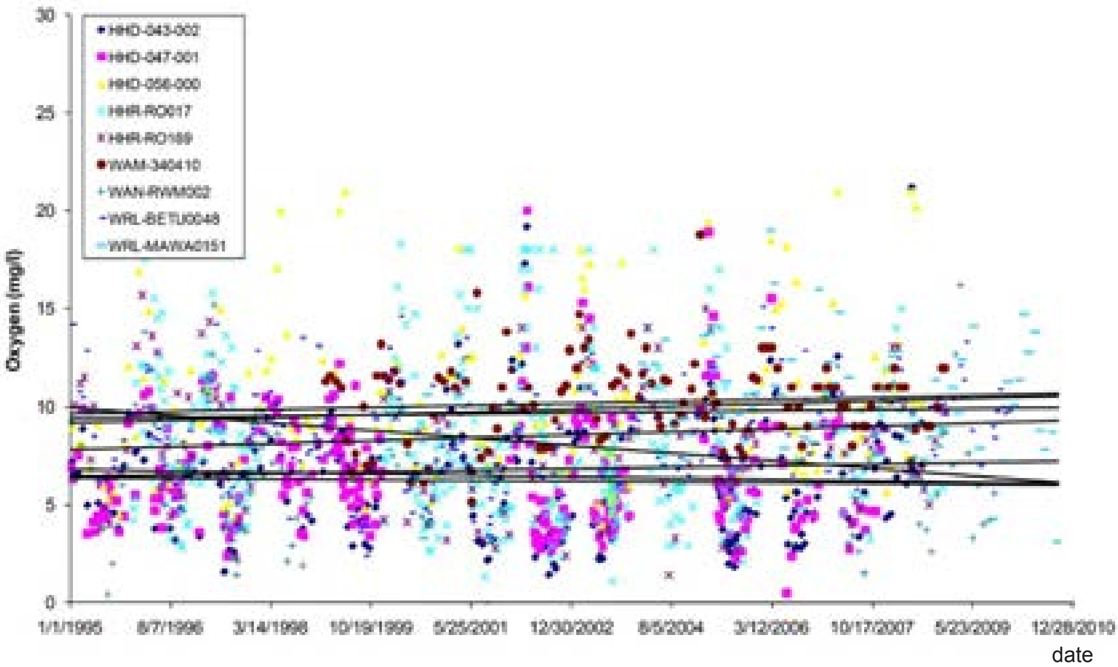


Fig. 28: Oxygen trends for 9 sampling sites with most available oxygen for localities where specimens of *Astacus leptodactylus*, *Orconectes virilis* or *Procambarus clarkii* were recorded.

BY-CATCHES

Fig 29 shows that over 80% of all captures were of crayfish. The total fraction might be slightly overestimated, since volunteers were not obliged to report their by-catches. A total of five water voles (*Arvicola amphibius*), four brown rats (*Rattus norvegicus*), two green water frogs (*Rana esculenta* synklepton) and one juvenile of an Eurasion coot (*Fulica atra*) drowned in traps. This accounts for 0,003 deaths per trap per night (1 kill per 300 controls), based on a total of 3538 'trap nights'. As far as we know, all fish and the common toads were released alive.

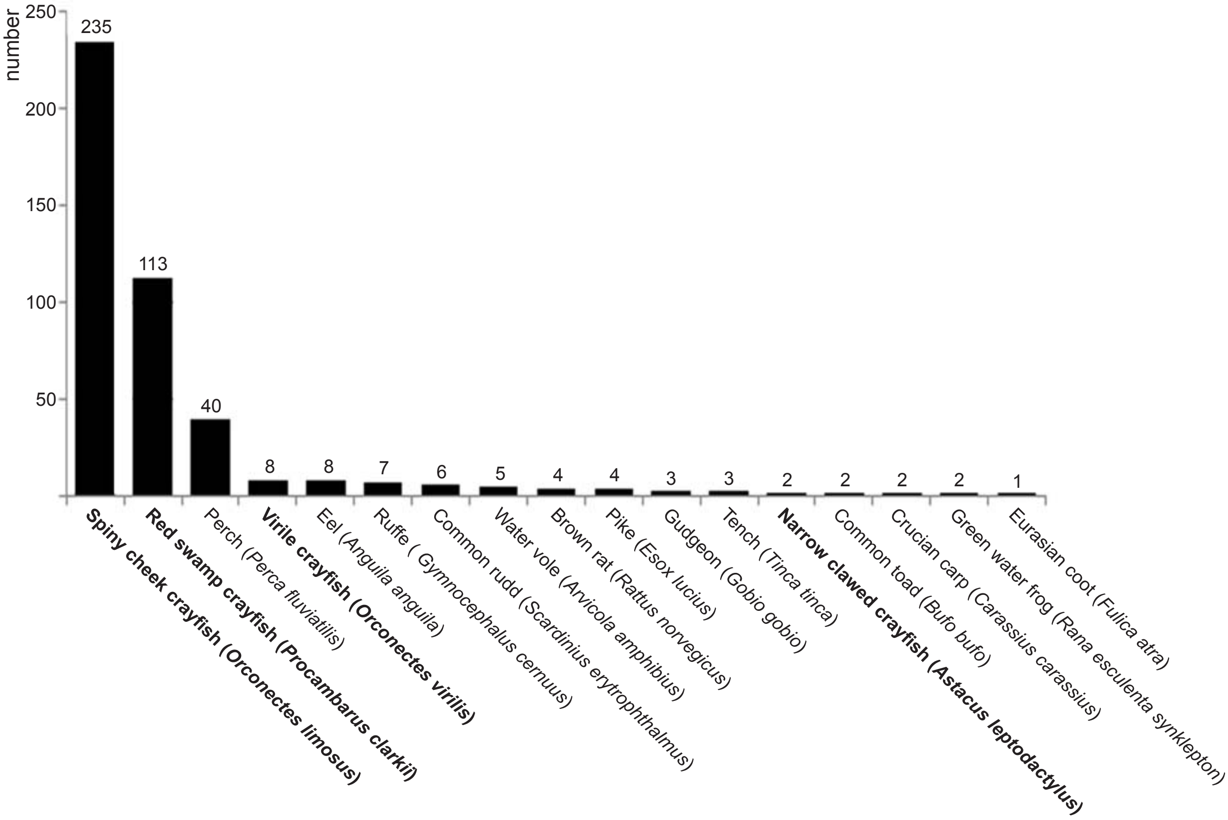


Fig. 29. Catches of crayfish and bycatch

Box 4. Comments from the field


An intact spiny cheek crayfish of about 5 cm, found in the stomach of a perch (*Perca fluviatilis*) near sampling site WRW-16. Photo: F. Boonstra

"I haven't caught any crayfish. However, I did catch a perch of 35 cm [on a fishing rod, ed.] in the Meppeler Diep in Meppel (...). I wanted to eat this fish, since they say it's very tasty. During the cleaning, I suddenly saw a claw of crayfich, coming out of the stomach of the fish (...). It had eaten a crayfish of about 5 cm! Although the traps had been placed in the same water, the Meppeler Diep, a dam could have blocked the crayfish for coming up the sampling site upstream." [Frans Boonstra]

"Ik heb geen kreeften gevangen. Wel ving ik toevallig gisteren een baars van 35 cm [aan de hengel, red.] in het Meppeler Diep aan het Westeinde in Meppel (…). Ik wilde nu eindelijk wel eens baars proeven want dat schijnt heel lekker te zijn. Bij het schoonmaken van de baars zag ik ineens een kreeftenpootje uit de smurrie/maag steken (…) De baars had een kreeft van circa 5 centimeter in zijn maag! Het water waar ik de fuiken had staan, is eveneens het Meppeler Diep. Door een stuw worden de kreeften waarschijnlijk tegengehouden op de meetlocatie." [Frans Boonstra]

"By the way, the fact that I haven't caught any crayfish is not discouraging. I've been involved in head louse inspections at the primary school of my daughters for many years. Here, I experienced that *not* finding what you're looking for can be very satisfactory..." [Caroline Elfferich]

"O ja, ik vind nulwaarnemingen helemaal niet ontmoedigend! Ik ben jarenlang betrokken geweest bij de hoofdluiscontrole op de basisschool van mijn dochters en daar heb ik een grote waardering opgebouwd voor het *niet* vinden waar je naar zoekt..." [Caroline Elfferich]

"I caught two crayfish at day three. During the night of day two to three, it rained a lot. The water level raised from 40 to 60 cm and the width of the stream increased from 2,5 to 3 metres. Also, the water turned considerably turbid." [Hans Moonen]

"Op dag drie twee kreeften gevangen. In de nacht van dag twee naar drie had het veel geregend, het waterpeil in dit beekje steeg van ongeveer 40 naar 60 cm, de breedte van ongeveer 2 naar 3,5 meter en het water werd behoorlijk troebel." [Hans Moonen]

"After the required sampling, I continued using baited traps at another location. In the Piccardthofplas near Groningen, I caught a spiny cheek crayfish in one of the traps within a day. Then, I continued the sampling with unbaited traps for four subsequent mornings, to verify the capture probability. On the last of the four days, I caught one female. See picture attached". [Maarten Loonen]

"Na de verplichte vangsten ben ik elders doorgegaan met beaasde korven. In de Piccardthofplas in Groningen ving ik binnen één dag in één van de drie beaasde korven een gevlekte Amerikaanse rivierkreeft. Toen ben ik zonder aas nog vier ochtenden doorgegaan om te kijken of de vangkans groot genoeg is. De laatste van de vier dagen zonder aas ving ik in een korf één vrouwtje. Bijgesloten een foto." [Maarten Loonen]

"Even the police stopped me. They asked me what I was doing. After explaining, they looked rather flabbergasted. However, after showing my papers, they seemed reassured that it was ok."
[Richard van Sluis]

"Ik ben zelfs aangehouden door de politie met de vraag wat ik wel niet aan het doen was. Nadat ik het verhaal had uitgelegd stonden ze heel vreemd te kijken, maar door de papieren te laten zien waren ze snel van mening dat het wel goed zou zijn."
[Richard van Sluis]

Box 4. Comments from the field (continuation)

“Monday: placing the traps. The designated location is unsuitable due to boats waiting in front of the sluice. Therefore, I placed the traps a bit further away. The trap lines provided are too short, but I’ve taken that into account. Tuesday: the rope of trap 1 was cut. I tried to relocate it with a hook of a helpfull boat, but nothing’s there. The trap was probably taken away by someone. Trap 2 was empty, in trap 3 one crayfish. Wednesday: two crayfish in trap 2. Thursday: the bank was mowed and the rope of trap 2 was cut because of that. I fetched a rake from home, but found nothing again - the canal is flowing fast. Friday: in the last remaining trap three crayfish, one with juveniles.” [Corry van Leeuwen]

Red swamp crayfish at day four at site HHD-17. Photo: C. van Leeuwen

“Maandag: Korven uitzetten. De aangewezen plek lijkt niet handig i.v.m. boten die wachten tot ze de sluis in kunnen. Daarom ga ik een stukje verderop. Meegeleverde touwen zijn te kort, daar had ik gelukkig rekening mee gehouden. Dinsdag: het touw van korf 1 is doorgesneden. Met een boothaak van een behulpzame schipper de bodem afgezocht, niets gevonden. Waarschijnlijk door iemand meegenomen. Korf 2 is leeg, korf 3, één kreeft. Woensdag: in korf 2, twee kreeften (...). Donderdag: de slootkant is gemaaid, en daarmee is het touw van korf 2 doorgesneden. Thuis een hark gehaald, maar ook deze korf was weg -er staat een sterke stroming. Vrijdag: in de overgebleven korf drie kreeften, waarvan één met jongen.” [Corry van Leeuwen]

I caught: tomatoes, pieces of bread for feeding ducks, almost entire grass lawns, fruit from the trees, chicory, mushrooms, onion skins, macaroni and leaves. Despite all the bait, I have the impression that the traps do not work very well in fast flowing streams. You need to fill the traps with many stones in order to prevent them from washing away. Even then, it’s hardly possible to place the traps crossways to avoid the income of debris. And that with so many crayfish in the Meuse nearby.” [Ivo Raemakers]

“Ik heb gevangen: tomaten, stukjes brood voor de eenden, halve gazons (het maaisel), valfruit in de vorm van peren en appels, witlof, champignons, uienschillen, macaroni en herfstbladeren. Ondanks al het aas heb ik de indruk dat de fuiken niet erg goed werken in snel stromend water. Om te beginnen moet je ze vullen met kilo’s stenen en dan blijk je ze *nog* niet dwars te kunnen leggen in de hoop instroom van materiaal te beperken. En dat terwijl het in de Maas vlakbij wemelt van de kreeften.” [Ivo Raemakers]

“I was lucky with a crayfish in my first test trap near Oude Tonge. Unfortunately, I couldn’t enjoy my discovery for long, because I slipped into the water. I landed full weight on my shin-bone at the innerside of the shoring and went straight to the hospital. I’m still recovering” [Hendrik Baas]

“Direct de eerste testfuik bij Oude Tonge was het raak. Helaas heb ik niet echt genoten, doordat ik zelf in het water terecht kwam -met mijn scheenbeen met volle gewicht tegen de binnenkant van de beschoeiing- en vervolgens in het ziekenhuis. Ik ben nog steeds aan het herstellen.” [Hendrik Baas]

“Because we went diving, we placed the traps at three different depths. The first at 4.2 m, the second at 6.2 m and the third at 9.8 m. Unfortunatally, we haven’t caught any crayfish. We made it to the local media, though.” [Margreet Dekker/Dive Post Zoetermeer]

“Omdat we duikend de korven hebben gecontroleerd hebben we ze op drie verschillende diepten geplaatst: de eerste op 4,2 m, de tweede op 6,2 m en de derde op 9,8 m. Helaas hebben we geen rivierkreeften aangetroffen. We hebben wel de media gehaald met het onderzoek.” [Margreet Dekker/Dive Post Zoetermeer]

Discussion

PROTOCOL

The protocol used for this survey was based on a reference dataset of daily, unbaited trap controls of one species at one location (see box 3). We took a risk by assuming that the capture probabilities in winter at this particular location (with high densities of crayfish), could reflect a summer situation at other sites (with probably lower densities of crayfish).

This assumption was indeed critically received by some of the volunteers. Therefore, all volunteers with few or no results or sceptical feelings about the protocol were invited to verify their doubts by using bait in the traps *after* the prescribed four day sampling period without bait. The use of bait was generally discouraged to avoid bycatches, to avoid damage to the traps by other species, and to standardize the protocol as much as possible. However, bait could greatly enhance the catches (up to a three-folded capture probability, pers. obs. P. Heemskerk). In total, 25 volunteers used baited traps for one or many more days after the regular sampling.

Crayfish actually did appear at two sites after the use of bait (with the use of algae tablets and cat-food respectively), which is 8% of the sites where previous sampling revealed no crayfish. This error is very close to the standard error of 5% already taken into account, which confirms the reliability of the protocol, althought it remains hard to tell whether the effort is enough to detect the leading edge of an invading population.

Although we recommended the use of Euroshopper© catfood as bait, volunteers used at least four different kinds. This emphazises that standardization of the protocol is indeed very hard to achieve once bait is allowed.

PERIOD

The sampling was performed in late summer, partly because of the high activity of crayfish in this period and partly to avoid by-catches of amphibians as much as possible.

Although we have no spring data to compare with, the number of by-catches among amphibians was very low (n=4) compared to the number of crayfish (390). As far as reported, two green water frogs (*Rana esculenta synklepton*) drowned during the inventory. Altogether, the recommended sampling period seems to coincide well with a low activity of adult amphians (active tadpoles could swim through the mesh of the traps) and a high activity of crayfish.

DISTRIBUTION

Although many new sites with crayfish were found, the overall distribution had not changed for any of the four species detected during the survey (fig. 10). Even in sparsely populated areas of the Netherlands, the amount of crayfish data actively acquired with the survey in 2010 is quickly exceeded by random, mostly non-intentional observations by people who stumbled upon a crayfish. However, striking differences between the species occur. The spiny cheek crayfish seems to be one of the most cryptic species. The number of 5x5 km squares where the species was detected during the inventory in late summer 2010 (n=65) was similar with the year total of passively acquired data in 2010 (n=73, fig. 10). In contrast, passively acquired data for the red swamp crayfish (n=57 5x5 km squares in 2010) are much more numerous than actively collected records (n=16) (fig. 10b). This clearly demonstrates the impact of behavioural differences on the likelihood of being found by someone. The red swamp crayfish frequently wanders over land, especially on humid days in late summer (fig. 30), while the spiny cheek

Fig. 30. A red swamp crayfish wandering over land. Crayfish species that regularly break through the water surface have a much larger probability of being recorded compared to species that almost never leave the water. Photo: Bart Noort.

crayfish is almost never observed on the land.
Although the overall distribution of crayfish in the Netherlands seemed to remain unchanged after the survey compared to previous records, the survey gave the first overview of sites of where we can assume that crayfish do *not* occur. This gave us the opportunity to compare water quality parameters at sites with and without crayfish.

HABITAT PREFERENCES OF THE SPINY CHEEK CRAYFISH

The spiny cheek crayfish is, like most of the other invasive species, known as a very tolerant species. As shown in fig. 11, its requirements seem to fit the quality of the Rhine and the Meuse almost perfectly, which might explain its current widespread distribution in the Netherlands. However, the spiny cheek crayfish has its ecological constraints.
Conditions that appear to be limiting for the presence of the spiny cheek crayfish, as determined with this study, mostly confirm prior expectations. The observed minimum pH of 6.4 (fig. 12) fits well with other studies and the preliminary risk analysis (Soes & Koese 2010). If the acidity falls below pH 6, the calcium metabolism of crayfish will be disturbed, which results in weak specimens and infertile eggs (Nyström 2002).
For salinity, the boundaries are not as sharp as for acidity. Over 95% of the sites have chloride levels well below 300 mg/l, but there are two exceptions: WAN-16 and WHD-01 with levels up to 1.5 g/l From literature, it is known that, depending on the species, growth and reproduction start to be problematic at chloride levels of 3-5 g/l (Nyström 2002), which is brackish water. This study suggests that in practice, the prefered levels might be lower. The lower limit for chloride (<20mg/l) might reflect other, more stringent relations. For example, low chloride sites might also be sites with a low pH.
The spiny cheek crayfish was only found in waters where the temperature exceeded 20° C in summer (fig. 16). Intuitively, one is eager to think that crayfish are limited by minimum temperatures. Most species however can withstand very cold water for a long time (provided they don't freeze) as long as their habitat is sufficiently heated in summer. For example, the native noble crayfish (*Astacus astacus*) needs a temperature of 15° C for at least three subsequent months to fulfill its life cycle (Abrahamsson 1972). This study indicates that a temperature of at least 20° C in summer is required for the spiny cheek crayfish for long-term survival of the species. Temperature limits may also explain the sparse number of records from small waterbodies. Most narrow water bodies (<5m) in this dataset refer to upstream locations that remain relatively cool throughout the year due to shadowing effects by vegetation or groundwater influences. Water bodies wider than 5 metres nearly all warm up sufficiently (fig. 16). Strikingly, the spiny cheek crayfish is also rare in warmer, very narrow water bodies (<2 metres wide). These are almost all flowing waters (and a ditch) which is especially remarkable, since the percentage of flowing waters where the species does occur is very high: in around a third of all sampled running waters (fig. 17) the spiny cheek crayfish was found. The fact that they were rarely found in narrow water courses might be because they have not been able to reach these locations.
A remarkable finding is the (almost) complete absence of the species in waters with a low nutrient loading (fig. 20). This could be the result of its own presence (in other words the spiny cheek crayfish turned its own habitat into unfavourable conditions). The fact that the species entered the country through the Meuse and Rhine (and related waterbodies) at a time when both rivers contained extremely high levels of nutrients suggests however a preference for high level nutrients (Ten Berge et al. 1973). It is also possible that its capacity to increase the nutrient state of the water, was masked by the fact that watercourses being invaded were already eutrophic.

HABITAT PREFERENCES OF OTHER SPECIES

Investigating the habitat preferences of other invasive species was not a part of this study. Because all other species are still expanding their range, it would have been too difficult to determine whether the absence of a species is caused by ecological constraints or an unfinished expansion. However, some observations are worth pointing out because of differences with the ecological boundaries of the spiny cheek crayfish. Based on this study, the red swamp crayfish seems to be slightly more tolerant towards low pH-levels. Four out of sixteen sites where the species was found, had an average acidity value (pH) between 5.9 and 6, whereas none of the 70 sites with spiny cheek crayfish had a pH value below 6.4. Additionally, both the red swamp crayfish and the virile crayfish were found in habitats with (on average) lower levels of oxygen compared to the spiny cheek crayfish. The spiny cheek crayfish was hardly ever found at locations with more than 20% of the oxygen measurements <5 mg/l (fig. 14). Both the other species however, were found at sites with more frequent measurements of dissolved oxygen levels below 5 mg/l. A tolerance for low levels of oxygen seems logically related to a preference for more stagnant waters compared to the spiny cheek crayfish, as indicated by the DCA-analysis (fig. 22).

ECOLOGICAL EFFECTS

It is hard to determine the impact of the present species, because it is not well known when the species first appeared at the location where they were recorded. There seems to be a decline in ecological quality of macrophytes in the past 10 years, when comparing the periods 2000-2005 and 2006-2009. However, this effect is not significant, due to a large variance in the measurements, and due to the low availability of macrophyte data at sites with invasive crayfish. Also, other factors could have influenced the EQR's. For example, improvements of water quality and structure might have compensated the negative effect of crayfish on some locations. For macroinvertebrates, the trend is even less obvious. The EQR is just *one* score. Damaging effects on some (groups of) taxa such as molluscs can be obscured by groups that are hardly affected or may even benefit. In other words: the EQR score may mask some significant impacts.

MEASURES

Any impact of invasive crayfish on water quality could not be proven with this study. Therefore, specific measures to reduce crayfish populations are not justified for quality reasons alone at this point. In practice however, there still can be a need for controlling crayfish for other reasons (e.g. burrowing behaviour, precautionary motives). Here, we give an overview of some possible measures for controlling crayfish based on the results of this study.

An interesting finding of this study is that hardly any crayfish are found in waters where macrophyte or macroinvertebrate communities are in 'good condition' (fig. 23). Additionally, most specimens of the spiny cheek crayfish are found in waters that are classified as bad, poor or moderate on the nutrient standards for phosphorus and nitrogen (fig. 20). In other words, the majority of crayfish are found in waterbodies that do *not* fulfill the miminum objectives for the European Water Framework Directive. Therefore, diminishing the nutrient loading might become a tool for surpressing crayfish in the long run. It is not only the results for nutrient loading that indicate disturbance of the habitat enhances crayfish distribution. Other measures that help to improve the quality seem promising for dimishing or reducing populations of crayfish as well, for example:

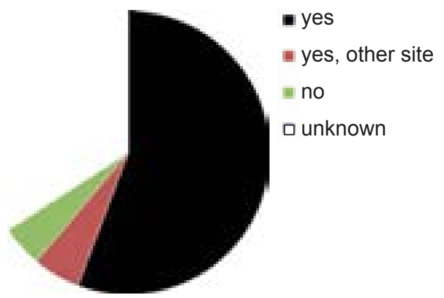


Fig. 31. Pie plot of willingniss among volunteers to participate with an inventory again. The question about willingness was introduced one month after the start of the project. Therefore, the opinion of 'early data submitters' (about 30% of the volunteers) is unknown.

- reducing the inlet of foreign water;
- counteracting the freshening of brackish waters;
- restore the natural seepage (temperature remains low);
- creating shade along streams. Especially small streams profit from shading due to less warming and more natural particulate matter in the water (branches and leaves).

FUTURE

Atlhough the impact of crayfish on water quality could not be proven with this study, it does not mean that we can reject the hypothesis of crayfish affecting the quality. However, insignificant results for changes in macrophytes and macrofauna, caused by a small dataset and a large variance of the data, do not allow a firm conclusion at the moment. Unfortunately, up-to-date quality measurements are missing from many sites, particularly from some of the largest hotspots of invasive crayfish in the Netherlands (e.g. the lake-region between Tienhoven and Loosdrecht, managed by Waternet and the Alblasserwaard managed by the waterboard Rivierenland). In order to visualize the consequences of invasive crayfish, it is necessary that measurements of macrophytes, macrofauna and physico-chemical parameters are continued or initiated, at least at the 20 sites where this study revealed one of the potentially harmful invaders (i.e. the red swamp crayfish and the virile crayfish).

Also, to detect colonization events of crayfish earlier, waterboards need to become aware of the weakness of the current sampling protocols for detecting crayfish. Monitoring for crayfish needs to become an essential part of the monitoring schemes of the waterboards. Volunteers could play and important role within such a monitoring. About 85% of the volunteers who participated this study, would like to repeat their sampling at the same location (fig. 31).

BOX 5. OVERVIEW OF SPECIES SPECIFIC DEVELOPMENTS IN 2010/2011

Recently, a risk analysis about the invasive crayfish in the Netherlands was conducted (Soes & Koese 2010). The main conclusions of the risk analysis remain unchanged after this study. Most results, such as pH levels, velocity preferences and distribution patterns confirm the assumptions made in the risk analysis. New developments, particular ones that have led to new insights compared to the preliminary risk analysis are pointed out here.

Narrow clawed crayfish - *Astacus leptodactylus*
In 2010/2011, the presence of the species was confirmed at all three familiar sites (Tynaarlo, Kerkrade and eastern Zeeuws Vlaanderen). Ovigerous females were caught in Kerkrade in March 2011.

Spiny cheek crayfish - *Orconectes limosus*
The overall distribution of this species has not changed in 2010/2011. Based on this study, the tolerance of this species for higher salinity levels might be slightly overestimated in the risk analysis. In the risk analysis, the provinces of Zeeland and Noord-Holland are indicated as very suitable, potential areas for the species. It is unlikely however, that the species can have sustainable populations in most areas in these provinces, because of high salinity levels (see discussion, p. 33).

Virile crayfish - *Orconectes virilis*
A major collapse of the population of this species west from Utrecht occured in (early) summer 2010 for unknown reasons. Numerous dead specimens were found by volunteers in and outside traps in this period (Koese 2011). The catchments at well known hotspots remained extremely low since then (pers. obs. P. Heemskerk & B. Koese). For example, daily trap controls of a single LiNi® trap near Wilnis, close to a well known site of this species (Emmerik & De Laak 2008), haven't revealed a single crayfish(!) since November 2010 (pers. com. A. de Kruijf). Catchments east from Utrecht still seem 'normal'. Although this species wasn't stigmatized as a serious burrower in the risk analysis, long (ca. 50 cm). subvertical burrows created by this species were discovered near Houten (province of Utrecht) in February 2011. Lowered temperatures most likely caused the behaviour in order to escape freezing (Koese et al. 2011). The species has a high potential to become a threatening burrower, although no serious damage has been reported yet.

Signal crayfish - *Pacifastacus leniusculus*
No changes compared to the risk analysis have been reported.

Red swamp crayfish - *Procambarus clarkii*
As expected, this species is steadily expanding its range. Two hard winters don't seem to have affected the population. Most new records add to the well known hotspots. Two sites are further away: a specimen was found on the road in Wieringen (province Noord-Holland), september 2010. It's unknown whether this was a single individual or an indication of a larger population. The existence of a population on the isle of Flakkee near Ooltgensplaat (province Zuid-Holland) was confirmed in May 2011. Its presence was presumed here, after the catchment of very early staged *Procambarus* sp. juveniles in March 2009. Substantial damage due to extensive burrowing behaviour of this species was recorded for the first time in the Netherlands near The Hague in september 2010. Lowered water levels in combination with the reproductive cycle (ovigerous females) are likely to have caused the behaviour (Koese et al. 2011). At the moment, this species is ecologically and economically by far the most threatening species. This study demonstrates that the red swamp crayfish has a higher tolerance for weakly buffered, oxygen poor and possible more brackish conditions compared to most other species.

White river crayfish - *Procambarus acutus*
In 2010/2011, two specimens were found far from the existing population in the Alblasserwaard (province of Zuid Holland). A specimen was found on the street in Den Helder (province of Noord-Holland). Another individual was found in the Leidsche Rijn (province of Utrecht), also far from water. Similar curious findings were done in 2009, which indicate that the species is transported by human intervention a lot. It seems only a matter of time before new populations will become established. The identity of this species remains problematic. A recent, preliminary DNA study indicates that two species might have become established in the Netherlands.

Marbled crayfish - *Procambarus fallax*
Finally, the species, well known from the aquarium trade, but not known from the wild, received a scientific identity: *Procambarus fallax* (Martin et al. 2010). Other than that, its status remained unchanged, i.e. the species is still considered as extinct.

Conclusions & recommendations

- The sampling protocol, developed for this project, which consisted of the use of three unbaited LiNi® traps that had to be examined at four consecutive mornings, turned out to be a reliable method to determine presence of crayfish at a particular site with a 95% capture probability, if present;

- The overall distribution hasn't changed for any of the four species detected after this survey;

- Both the amount of data collected and the geographical distribuition of the data collected with this study are easily exceeded by unintentional collected data (submitted to the EIS office or web-portals). The discrepancy is most profound for the red swamp crayfish. This species can often be found on the land compared to the other species detected with this survey (narrow clawed crayfish, spiny cheek crayfish, virile crayfish). This behaviour results in many more sightings by people;

- This study resulted in the first overview of sites of where we can assume that crayfish do *not* occur with a high level of certainty. This gives us the opportunity to compare waters with and without crayfish;

- The distribution of the spiny cheek crayfish seems to be limited by the following factors:
  - Acid waters: the minimal pH value where the species was found was pH 6.4;
  - Brackish waters: the maximum chloride content where the species was found was at 1000 mg/l, but 98% of the populations recorded were found in waters with less than 300 mg/l of chloride.
  - Oxygen: at least 6.6 mg/l on average. Additionally the species is also absent at sites where over 20% of the measurements had values below 5 mg/l of dissolved oxygen (fig. 14);
  - Temperature: the species seems to require a minimum temperature of at least 20° C in summer (fig. 16);
  - Nutrient level: the species is absent in waters with a low nutrient content (fig. 20) i.e. waters with less than 0.8 mg P¯/l and 1.8 mg N¯/l

- Compared to the spiny cheek crayfish, the red swamp crayfish (and probably the virile crayfish) seems to have a higher tolerance for weakly buffered and oxygen poor conditions:
  - The minimal pH value where the red swamp crayfish was found was 5.9 (fig. 13);
  - The red swamp crayfish was found at sites where on average 20% of the measurements resulted in values below 5 mg/l of oxygen (fig. 15);

- Insignificant results for changes in macrophytes and macrofauna, probably caused by a small dataset and a large variance of the data, do not allow any firm conclusions about impact of recent invaders at the moment.

- Up-to-date quality measurements are missing from many sites, particularly from some of the largest hotspots of invasive crayfish in the Netherlands;

- Measurements of macrophytes, macrofauna and physico-chemical parameters need to be continued or initiated, especially at targeted sites where crayfish species are expected to be invasive in the not-too-distant future and at least at the 20 sites where this study revealed one of the potentially harmful invaders (i.e. the red swamp crayfish and the virile crayfish).

- Monitoring for crayfish needs to become a essential part of the monitoring schemes of the water-boards in order to detect colonization events of crayfish in time.

Conclusies en aanbevelingen

- De voor dit project ontwikkelde meetstrategie, het controleren van drie onbeaasde LiNi gedurende vier achtereenvolgende ochtenden blijkt een goede methode om de aan- of afwezigheid van rivierkreeften vast te stellen met een hoge mate van betrouwbaarheid (circa 95%);

- De reeds bekende verspreiding van de verschillende soorten rivierkreeften is door dit onderzoek op hoofdlijnen niet veranderd;

- Losse, ongerichte waarnemingen ('toevalstreffers') leveren op jaarbasis meer data en een hogere spreiding van de gegevens op in vergelijking met de data die dit onderzoek heeft opgeleverd. Dit verschil is veruit het grootst bij de rode Amerikaanse rivierkreeft, die vaak het land op klimt en daardoor naar verhouding veel meer gezien wordt dan de andere soorten die bij dit onderzoek zijn aangetroffen (Turkse, gevlekte Amerikaanse en geknobbelde Amerikaanse rivierkreeft);

- Dankzij dit onderzoek is voor het eerst een grote hoeveelheid nulwaarnemingen beschikbaar gekomen, waardoor het mogelijk is geworden wateren met en zonder kreeften met elkaar vergelijken;

- De gevlekte Amerikaanse rivierkreeft lijkt in zijn verspreiding begrensd te worden door de volgende factoren:
  - Zuurgraad: de minimale pH waarde waarbij de soort is aangetroffen bedraagd 6.4 (fig. 12);
  - Brakke wateren: het maximale chloridegehalte bedraagd 1000 mg/l. Mogelijk is 300 mg/l al beperkend (fig. 12);
  - Zuurstof: minimaal 6,6 mg/l. De soort is evenmin aangetroffen in wateren waarbij meer dan 20% van de metingen lager uitvallen dan 5 mg/l zuurstof (fig. 14);
  - Temperatuur: de soort lijkt een opwarming van tenminste 20° C in de zomer te verlangen (fig. 16);
  - Nutriëntengehalte: de gevlekte Amerikaanse rivierkreeft is afwezig in wateren met een lage nutrientenbelasting (fig. 20), dat wil zeggen wateren die minder dan 0,8 mg P¯/l en 1,8 mg N¯/l bevatten.

- Vergeleken met de gevlekte Amerikaanse rivierkreeft lijkt de rode Amerikaanse rivierkreeft toleranter ten aanzien van zwak gebufferde en zuurstofarme omstandigheden:
  - De minimale pH waarde waarbij de soort is aangetroffen bedraagd 5,9 (fig. 13).
  - Gemiddeld genomen komt de soort voor in wateren waarbij meer dan 20% van de metingen lager uitvallen dan 5 mg/l zuurstof (fig. 14);

- Bij het onderzoek naar de mogelijke beïnvloeding van de waterkwaliteit op basis van macrofauna en waterplanten zijn geen significant negatieve effecten aan het licht gekomen, wat echter mogelijk het gevolg is van een (te) kleine dataset en een grote spreiding van de meetwaarden. Daarmee is het op basis van deze studie niet mogelijk om harde conclusies te trekken ten aanzien recent gevestigde rivierkreeften.

- Momenteel worden er op veel plaatsen (onder andere op locaties met hoge dichtheden aan rivierkreeften) nog geen waterkwaliteitsmetingen verricht;

- Om een vinger aan de pols te kunnen houden is het noodzakelijk om physisch-chemische metingen en metingen op het gebied van waterplanten, macrofauna te blijven verrichten of te initiëren, met name op locaties waar kreeften binnen afzienbare tijd verwacht worden en tenminste op de 20 locaties waar tijdens dit onderzoek potentiëel schadelijk soorten zijn aangetroffen (de rode Amerikaanse rivierkreeften en de geknobbelde Amerikaanse rivierkreeft).

- Monitoring van rivierkreeften behoort een essentieel onderdeel te worden van de meetprogramma's van de waterschappen.

References

Abrahamsson, S.A.A. 1972. Fecundity and growth of some populations of *Astacus astacus* Linné in Sweden with special regard to introductions in northern Sweden. Report of the Institute of Freshwater Research, Drottningholm 52: 23-37.

Adema, J.P.H.M. 1982. *Astacus leptodactylus* - Eschscholz introduced in the Netherlands. Bijdragen tot de Faunistiek van Nederland. Zoologische Bijdragen 28: 5-8.

Adema, J.P.H.M. 1989. De verspreiding van rivierkreeften in Nederland. Nieuwsbrief EIS-Nederland 19: 3-10.

Berge, W. ten, M. Bik, E. de Bloeme, J.G.W. Bolomy, D. Eisma, P.J. Konijn, D.M.J. Lasonder, M. van der Loeff, J. Moller, A.J.C. Vierling, J. Woets & J.S. van der Zwaag 1973. Rijn Nota. Internationale Rijngroep, Vereniging Milieudefensie, Amsterdam.

Dawaco Ecologie, 2010. Software for data storage and analysis. Developed by Royal Haskoning.

Emmerik, W.A.M. & G.A.J. de Laak 2008. Oriënterend onderzoek exotische rivierkreeften Wilnisse Bovenlanden, Polder Groot Wilnis-Vinkeveen. Sportvisserij Nederland, Bilthoven.

Evers, C.H.M. & R.A.E. Knoben (ed.), 2007. Description MEP and metrics for ditches and canals for the Water Framework Directive. STOWA 2007-32b and RWS-WD 2007-019.

EU, 2000. Water Framework Directive; Directive 2000/60/EG from the European Parliament and the Council. October 23rd 2000.

Geelen, J.F.M. 1978. The distribution of the crayfishes *Orconectes limosus* (Rafinesque) and *Astacus astacus* (L.) (Crustacea, Decapoda) in The Netherlands. Bijdragen tot de Faunistiek van Nederland 5. Zoologische Bijdragen 23: 4-19.

Heinis F. & C.H.M. Evers [red.], 2007. Nutrient values for the GET for natural water bodies. Heinis Waterbeheer, Royal Haskoning, Alterra, LNV and RIKZ. RIZA-001 and STOWA-02.

Koese, B., 2011. De geknobbelde Amerikaanse rivierkreeft rond Kamerik en Kockengen in 2010. Stichting EIS-Nederland, Leiden.

Koese, B., E.P. Raaphorst, P.G.M. Heuts & A.E. Kolff 2011. Gravende rivierkreeften: waar gaat het heen? De Levende Natuur 112(3): 120 - 123.

Knol, B. 2005. Californische rivierkreeft in Twenthe. Macrofaunanieuwsbrief 56.

Limnodata Neerlandica, 2011. Database with water board data from the chemical and biological water quality samples. Managed by Royal Haskoning on behalf of STOWA.

Lodge, D.M, C.A. Taylor, D.M. Holdich & J. Skurdal 2000. Nonindigenous crayfishes threaten North American Freshwater Biodiversity: lessons from Europe. Fisheries 25(8): 7-20.

Lipmann, R. 2007. Maagdelijke bevalling. Duiken 7: 5.

Martin, P., N.J. Dorn, T. Kawai, C. van der Heiden & G.Scholtzl, 2010. The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870). Contributions to Zoology 79(3): 107-118.

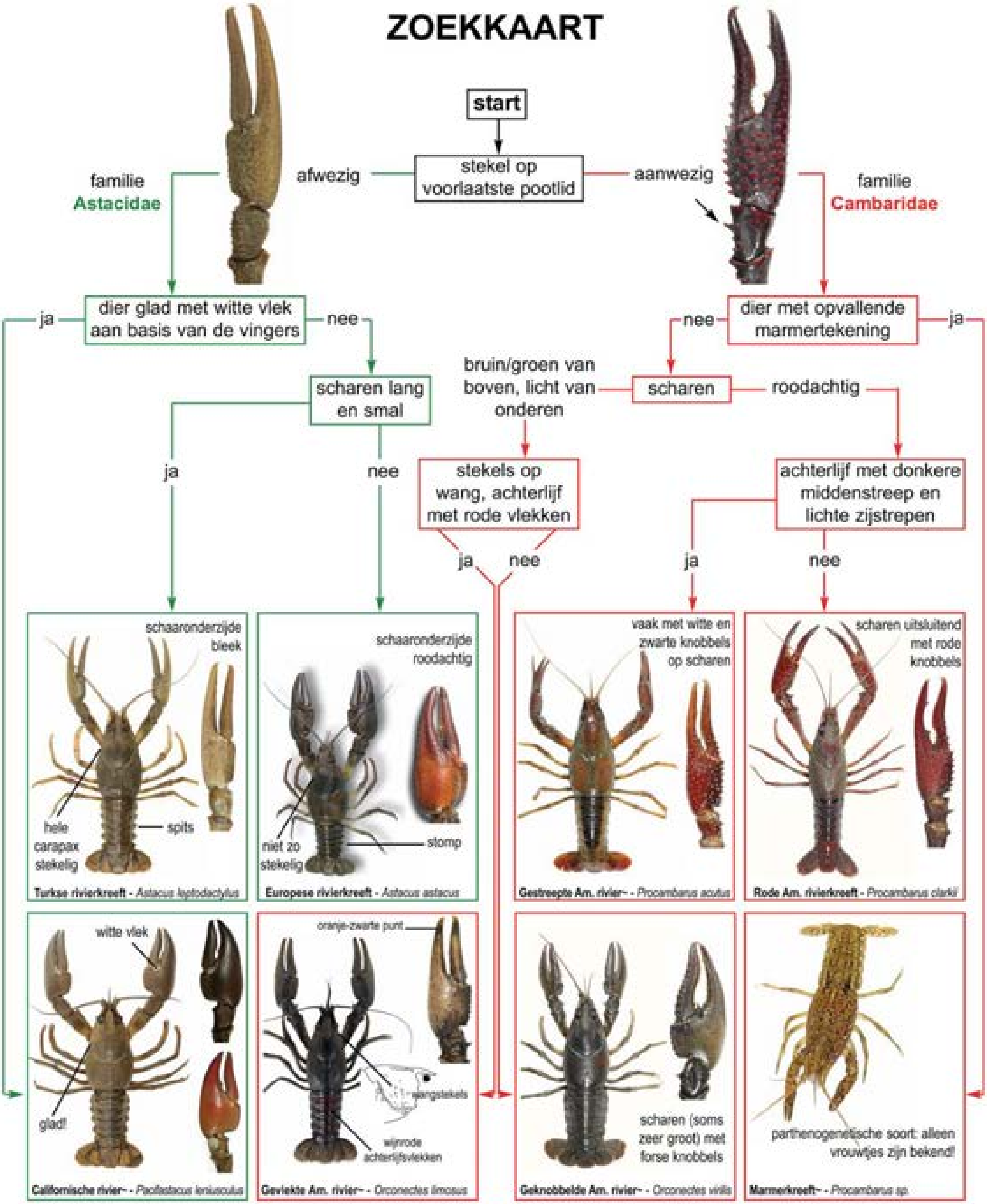
Nyström, P. 2002. Ecology. In: Holdich, D. (red) 2002. Biology of Freshwater Crayfish. Blackwell Science, Oxford.

Soes, M. & R. van Eekelen, 2006. Rivierkreeften? Een oprukkend probleem? De Levende Natuur 107(2): 56-59.

Soes, M. & B. Koese 2010. Invasive freshwater crayfish in the Netherlands: a preliminary risk analysis. Stichting EIS-Nederland, Leiden & Bureau Waardenburg, Culemborg. [www.repository.naturalis.nl/document/200127].

Van der Molen & Pot (ed.), 2007. References and metrics for natural water types for the Water Framework Directive. STOWA 2007-32 RWS-WD 2007-018.

APPENDIX 1. IDENTIFICATION KEY



APPENDIX 2. VOLUNTEERS

All volunteers who supplied data are listed below.

People

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Bekker, Eduard
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Blankena, Gert Jan
Blokland, Andre
Bodegom, Tom van
Boersma, Tekla
Boomstra, Bart
Boonstra, Frans
Bos, Walter
Bosma, Harrie
Bouwmeester, Paul
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Broek, Jan van der
Broekert, Auke
Broekman, Martijn
Burkhardt, Radboud
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Dongen, Peter van
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Joosse, Cees
Kalis, Fer
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Koster, Hans
Kuijs, Emil
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Timmermans, Geert
Toebes, Ruben
Traas, Henk
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Tuinstra, Gerrit
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Verburg, Peter
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Vlemmix, Cees
Vorsselman, Bert
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Vries, Paul de
Vries, Robin de
Vroome, Arjen de
Waal, Anthony de
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Westerink, Astrid
Wetzels, Peter
Wieland, Alex
Wielen, Paul van der
Wiersma, Tjitske
Wijnbergen, Rob
Wijnen, Bram
Wind, Julia
Zanten, Martijn van
Zwanepol, Henk

Organisations
Dive Post Zoetermeer
Hoogheemraadschap Hollands Noorderkwartier
IVN Boxmeer
IVN Laarbeek
IVN Someren/Asten
IVN Veghel
KNNV afdeling Delfland
KNNV afdeling Rosendaal
NJN summercamp 'Haaksbergen 3'
NJN summercamp 'Ouddorp 4'
NMV Gemeente Heusden

APPENDIX 3. RESULTS PER SAMPLING SITE

SCC = spiny cheek crayfish; VC = virile crayfish; RSC = red swamp crayfish; NCC = narrow clawed crayfish

Project code	Waterboard code	Coordinate X (Dutch grid)	Coordinate Y (Dutch grid)	Crayfish presence(+) or absence(-)	SCC	VC	RSC	NCC
HHD-01	HHD-202-000	089,257	443,578	-	0	0	0	0
HHD-02	HHD-026-000	081,827	439,980	-	0	0	0	0
HHD-03	HHD-004-001	080,270	447,486	+	0	0	9	0
HHD-04	HHD-056-000	073,264	447,702	+	0	0	1	0
HHD-05	HHD-043-002	078,839	455,480	+	0	0	1	0
HHD-06	HHD-062-002	085,195	445,310	-	0	0	0	0
HHD-07	HHD-062-008	088,461	439,443	+	1	0	0	0
HHD-08	HHD-044-000	082,925	453,538	+	0	0	8	0
HHD-09	HHD-221A013	086,743	444,776	-	0	0	0	0
HHD-12	HHD-402A021	077,555	454,974	+	0	0	23	0
HHD-16	HHD-215-030	090,005	451,234	-	0	0	0	0
HHD-17	HHD-047-001	086,869	454,972	+	0	0	6	0
HHD-18	HHD-213B024	090,323	447,581	-	0	0	0	0
HHD-19	HHD-401C012	078,755	452,178	-	0	0	0	0
HHD-21	HHD-042-003	080,967	457,002	-	0	0	0	0
HHD-22	HHD-215-026	085,630	449,527	+	0	0	3	0
HHD-25	HHD-048-001	083,528	450,046	-	0	0	0	0
HHN-01	HHN-002002	129,544	512,754	-	0	0	0	0
HHN-02	HHN-116102	110,520	532,780	-	0	0	0	0
HHN-03	HHN-119201	106,323	515,380	-	0	0	0	0
HHN-04	HHN-146402	128,473	503,902	+	2	0	0	0
HHN-05	HHN-770305	131,426	545,936	-	0	0	0	0
HHN-06	HHN-184501	121,580	531,510	-	0	0	0	0
HHN-08	HHN-206003	112,378	547,627	-	0	0	0	0
HHN-09	HHN-084001	124,054	528,072	-	0	0	0	0
HHN-10	HHN-802014	114,480	560,770	-	0	0	0	0
HHN-11	HHN-802003	118,810	563,300	-	0	0	0	0
HHN-12	HHN-803007	121,180	567,880	-	0	0	0	0
HHN-13	HHN-007001	120,378	516,406	-	0	0	0	0
HHN-14	HHN-013001	112,716	497,593	-	0	0	0	0
HHN-15	HHN-072001	129,030	546,080	-	0	0	0	0
HHN-16	HHN-088001	115,046	527,147	-	0	0	0	0
HHN-17	HHN-135701	115,165	547,466	-	0	0	0	0
HHN-18	HHN-276401	110,162	534,722	-	0	0	0	0
HHR-01	HHR-RO092A	099,367	460,299	-	0	0	0	0
HHR-03	HHR-RO017	099,145	480,940	+	0	0	2	0
HHR-04	HHR-RO169	087,696	459,730	+	1	0	1	0
HHR-05	HHR-RO275	116,200	482,810	-	0	0	0	0
HHR-06	HHR-RO526	097,178	464,402	-	0	0	0	0
HHR-07	HHR-ROP13420	112,542	451,225	-	0	0	0	0
HHR-09	HHR-RO084	096,094	459,557	-	0	0	0	0
HHR-10	HHR-ROP16702	092,520	455,173	-	0	0	0	0
HHR-12	HHR-RO549	086,321	463,413	-	0	0	0	0
HHR-16	HHR-RO185	100,244	466,928	-	0	0	0	0
HHR-18	HHR-RO236	103,919	486,560	+	1	0	0	0
HHS-01	HHS-00907	102,664	440,373	-	0	0	0	0
HHS-02	HHS-00804	100,077	441,808	-	0	0	0	0

Project code	Waterboard code	Coordinate X (Dutch grid)	Coordinate Y (Dutch grid)	Crayfish presence(+) or absence(-)	SCC	VC	RSC	NCC
HHS-03	HHS-00040	098,332	443,703	+	2	0	0	0
HHS-04	HHS-00303	098,938	452,044	-	0	0	0	0
HHS-05	HHS-00504	099,644	445,673	-	0	0	0	0
HHS-07	HHS-00402	099,049	448,849	-	0	0	0	0
HHS-08	HHS-00607	103,222	444,796	-	0	0	0	0
HHS-14	HHS-01203	097,212	447,897	-	0	0	0	0
HHS-15	HHS-01205	097,006	446,061	+	1	0	0	0
HHS-16	HHS-01212	095,484	448,599	-	0	0	0	0
HHS-18	HHS-01214	093,324	444,368	-	0	0	0	0
HSR-02	HSR-A59	154,240	445,810	-	0	0	0	0
HSR-03	HSR-D12	123,980	450,180	+	1	1	0	0
HSR-04	HSR-AB14	148,000	449,000	+	0	5	0	0
HSR-06	HSR-AB-47	139,624	445,978	+	4	0	0	0
HSR-07	HSR-WB15	117,489	450,371	-	0	0	0	0
HSR-09	HSR-SB01	136,133	453,402	+	0	1	0	0
HSR-10	HSR-SB03	132,996	449,530	+	0	0	19	0
HSR-11	HSR-WB16	123,683	453,456	-	0	0	0	0
HSR-12	HSR-WB06	117,538	462,562	-	0	0	0	0
HSR-14	HSR-WB21	122,855	458,997	+	0	1	0	0
HSR-16	HSR-A01B	138,700	454,460	+	2	0	0	0
WAM-01	WAM-342410	134,459	413,199	-	0	0	0	0
WAM-02	WAM-342407	134,400	412,750	+	3	0	0	0
WAM-03	WAM-340442	153,115	417,438	-	0	0	0	0
WAM-04	WAM-340452	163,650	423,170	+	3	0	0	0
WAM-05	WAM-341427	179,511	416,981	-	0	0	0	0
WAM-07	WAM-343506	142,000	410,700	-	0	0	0	0
WAM-09	WAM-140233	178,016	383,343	-	0	0	0	0
WAM-10	WAM-140289	161,139	405,128	-	0	0	0	0
WAM-12	WAM-140216	160,182	406,573	-	0	0	0	0
WAM-14	WAM-140244	177,630	389,080	+	2	0	0	0
WAM-15	WAM-340415	193,434	412,995	-	0	0	0	0
WAM-16	WAM-342408	144,490	409,070	+	1	0	0	0
WAM-17	WAM-343507	136,500	414,350	-	0	0	0	0
WAM-18	WAM-340410	195,644	405,805	+	0	0	1	0
WAM-20	WAM-343515	155,800	420,500	-	0	0	0	0
WAM-21	WAM-341421	181,375	402,713	-	0	0	0	0
WAM-22	WAM-343521	143,671	409,587	-	0	0	0	0
WAM-24	WAM-140218	173,550	391,606	-	0	0	0	0
WAM-26	WAM-140221	181,979	375,464	-	0	0	0	0
WAN-01	WAN-SBI003	116,832	485,037	-	0	0	0	0
WAN-02	WAN-SBI007	116,294	485,313	-	0	0	0	0
WAN-04	WAN-OBL019	114,585	488,286	-	0	0	0	0
WAN-06	WAN-SBI006	114,637	488,226	-	0	0	0	0
WAN-07	WAN-OBL022	112,302	488,355	-	0	0	0	0
WAN-11	WAN-SBI008	114,241	485,903	-	0	0	0	0
WAN-12	WAN-SBI014	116,793	488,649	-	0	0	0	0
WAN-14	WAN-SBI016	116,757	485,758	-	0	0	0	0
WAN-15	WAN-VLP001	124,460	489,201	-	0	0	0	0
WAN-16	WAN-BLM001	127,958	481,664	+	2	0	20	0
WAN-17	WAN-OBL012	114,562	488,548	-	0	0	0	0

Project code	Waterboard code	Coordinate X (Dutch grid)	Coordinate Y (Dutch grid)	Crayfish presence(+) or absence(-)	SCC	VC	RSC	NCC
WAN-18	WAN-RWM002	124,657	485,695	+	0	0	4	0
WBD-02	WBD-210016	112,050	381,130	+	4	0	0	0
WBD-05	WBD-210803	115,740	394,180	-	0	0	0	0
WBD-06	WBD-200029	102,360	404,170	+	0	0	2	0
WBD-07	WBD-240103	091,550	388,550	-	0	0	0	0
WBD-08	WBD-210703	111,880	392,580	-	0	0	0	0
WBD-13	WBD-230001	114,800	406,400	-	0	0	0	0
WBD-16	WBD-221302	104,480	393,180	-	0	0	0	0
WD-01	WD-254154	136,369	395,254	-	0	0	0	0
WD-02	WD-250015	161,257	381,703	+	3	0	0	0
WD-03	WD-250013	157,778	379,239	+	1	0	0	0
WD-04	WD-251018	162,718	391,341	-	0	0	0	0
WD-06	WD-251016	163,383	386,006	+	19	0	0	0
WD-07	WD-253010	162,721	385,385	+	4	0	0	0
WD-10	WD-250092	145,026	400,200	+	1	0	0	0
WD-11	WD-250117	138,859	397,236	-	0	0	0	0
WD-12	WD-253020	152,312	406,439	+	1	0	0	0
WD-15	WD-250035	156,045	378,161	-	0	0	0	0
WD-17	WD-250087	149,201	397,760	-	0	0	0	0
WF-02	WF-0218	189,722	559,793	+	3	0	0	0
WF-03	WF-0281	205,600	583,430	-	0	0	0	0
WF-04	WF-0882	198,370	568,638	+	2	0	0	0
WF-06	WF-0261	192,670	570,460	-	0	0	0	0
WF-07	WF-9032	183,658	554,285	-	0	0	0	0
WF-09	WF-0079	192,390	559,130	-	0	0	0	0
WF-10	WF-0106	176,010	547,050	+	3	0	0	0
WF-11	WF-0896	189,754	552,688	-	0	0	0	0
WF-12	WF-0254	191,335	591,158	-	0	0	0	0
WF-13	WF-0045	200,100	574,860	-	0	0	0	0
WF-14	WF-0075	179,470	560,860	-	0	0	0	0
WF-16	WF-0895	196,416	592,006	-	0	0	0	0
WF-18	WF-0312	212,800	562,700	-	0	0	0	0
WGS-01	WGS-PRI99	194,070	502,930	-	0	0	0	0
WGS-02	WGS-QMV60	198,380	511,610	+	14	0	0	0
WGS-03	WGS-USW89	206,940	497,320	-	0	0	0	0
WGS-04	WGS-RMW05	220,820	501,860	-	0	0	0	0
WGS-05	WGS-VDR12	211,380	475,780	-	0	0	0	0
WGS-06	WGS-RMW40	216,280	501,020	-	0	0	0	0
WGS-07	WGS-LVE92	206,400	504,850	-	0	0	0	0
WGS-09	WGS-VSW27	218,480	477,680	-	0	0	0	0
WGS-10	WGS-RNW74	206,570	500,340	-	0	0	0	0
WGS-11	WGS-RMW30	217,870	501,610	+	2	0	0	0
WGS-12	WGS-RMW55	214,120	500,040	-	0	0	0	0
WGS-14	WGS-OSL17	193,600	500,780	+	1	0	0	0
WGS-15	WGS-QMO70	201,680	510,580	-	0	0	0	0
WGS-16	WGS-SNZ40	223,620	490,140	-	0	0	0	0
WGS-18	WGS-SBS01	225,100	492,300	-	0	0	0	0
WGS-29	WGS-PND30	187,400	512,900	-	0	0	0	0
WHA-01	WHA-5601	245,150	579,525	-	0	0	0	0
WHA-03	WHA-2207	237,800	551,180	-	0	0	0	0

Project code	Waterboard code	Coordinate X (Dutch grid)	Coordinate Y (Dutch grid)	Crayfish presence(+) or absence(-)	SCC	VC	RSC	NCC
WHA-04	WHA-4608	244,150	572,650	-	0	0	0	0
WHA-05	WHA-2615	235,070	574,625	-	0	0	0	0
WHA-06	WHA-2616	235,350	573,910	-	0	0	0	0
WHA-07	WHA-4609	243,920	572,870	-	0	0	0	0
WHA-08	WHA-2213	238,490	559,465	-	0	0	0	0
WHA-09	WHA-5241	249,555	587,420	-	0	0	0	0
WHA-10	WHA-2212	238,500	560,245	-	0	0	0	0
WHA-11	WHA-2206	236,950	556,450	-	0	0	0	0
WHA-12	WHA-1218	272,400	559,065	-	0	0	0	0
WHA-14	WHA-1626	265,580	533,220	-	0	0	0	0
WHA-15	WHA-2240	242,970	555,460	-	0	0	0	0
WHA-16	WHA-2204	237,440	564,370	-	0	0	0	0
WHA-17	WHA-2211	240,420	563,560	-	0	0	0	0
WHA-18	WHA-4610	243,000	572,000	-	0	0	0	0
WHD-01	WHD-BOP1524	079,184	428,562	+	1	0	0	0
WHD-02	WHD-BOP2902	073,432	435,378	-	0	0	0	0
WHD-12	WHD-GOP0205	055,597	425,072	-	0	0	0	0
WHD-13	WHD-HOP0102	081,177	419,039	+	2	0	0	0
WHD-14	WHD-HOP0103	082,046	420,633	+	2	0	0	0
WHD-17	WHD-BOP0702	069,189	438,464	+	1	0	0	0
WHD-18	WHD-BOP2201	076,835	436,071	-	0	0	0	0
WN-01	WN-5532	232,100	573,100	+	7	0	0	0
WN-02	WN-6501	228,200	567,900	-	0	0	0	0
WN-03	WN-6527	223,100	571,200	-	0	0	0	0
WN-04	WN-5502	227,200	574,200	+	7	0	0	0
WN-05	WN-5528	228,600	574,100	+	3	0	0	0
WN-06	WN-6525	224,800	561,800	-	0	0	0	0
WN-07	WN-6526	225,800	571,300	+	4	0	0	0
WN-08	WN-4159	218,780	589,190	-	0	0	0	0
WN-09	WN-4502	225,730	591,150	-	0	0	0	0
WN-10	WN-5503	231,700	577,400	-	0	0	0	0
WN-11	WN-6301	223,600	565,800	-	0	0	0	0
WN-12	WN-5428	230,200	576,770	+	14	0	0	0
WN-13	WN-3103	221,000	596,080	-	0	0	0	0
WN-15	WN-4122	224,440	594,060	+	1	0	0	0
WN-16	WN-4160	221,900	588,350	-	0	0	0	0
WN-17	WN-4501	213,800	582,190	-	0	0	0	0
WN-18	WN-4503	226,810	587,520	-	0	0	0	0
WPM-01	WPM-ONIER200	199,260	413,710	+	1	0	0	0
WPM-02	WPM-OPEKA100	188,930	384,380	+	25	0	0	0
WPM-03	WPM-OGELD100	211,190	392,950	-	0	0	0	0
WPM-04	WPM-OHELE800	190,060	382,510	-	0	0	0	0
WPM-05	WPM-OKDEU200	190,590	374,770	-	0	0	0	0
WPM-06	WPM-OAFLE900	197,730	398,110	+	1	0	0	0
WPM-07	WPM-OITTE200	183,330	352,030	-	0	0	0	0
WPM-09	WPM-OUFFE050	181,770	353,450	+	4	0	0	0
WPM-11	WPM-ORAAM100	172,900	356,910	-	0	0	0	0
WPM-12	WPM-OTUNG100	171,630	360,170	+	1	0	0	0
WPM-16	WPM-OLING300	212,260	387,740	-	0	0	0	0
WPM-17	WPM-ONIER900	194,540	414,110	+	4	0	0	0

Project code	Waterboard code	Coordinate X (Dutch grid)	Coordinate Y (Dutch grid)	Crayfish presence(+) or absence(-)	SCC	VC	RSC	NCC
WRD-05	WRD-01.300	223,181	503,062	+	4	0	0	0
WRD-08	WRD-15.012	245,772	480,733	+	1	0	0	0
WRD-09	WRD-15.099	246,465	483,437	-	0	0	0	0
WRD-11	WRD-40.018	266,706	481,013	+	1	0	0	0
WRD-14	WRD-20.008	237,125	470,809	-	0	0	0	0
WRD-17	WRD-39.002	266,127	486,082	-	0	0	0	0
WRD-21	WRD-32.200	268,659	485,082	+	1	0	0	0
WRIJ-04	WRIJ-BUB01	254,800	462,900	+	1	0	0	0
WRIJ-07	WRIJ-WEB02	209,700	443,700	+	3	0	0	0
WRIJ-10	WRIJ-BOS02	226,100	442,350	+	1	0	0	0
WRIJ-12	WRIJ-BOS01	243,600	440,800	+	1	0	0	0
WRIJ-15	WRIJ-KEB01	233,700	436,400	+	1	0	0	0
WRIJ-18	WRIJ-RDB01	211,550	447,300	-	0	0	0	0
WRL-01	WRL-MMW0020	194,800	427,650	-	0	0	0	0
WRL-03	WRL-MNB0007	164,600	440,200	+	1	0	0	0
WRL-06	WRL-MLI0007	166,300	436,600	+	2	0	0	0
WRL-07	WRL-MMW0001	158,250	426,000	-	0	0	0	0
WRL-08	WRL-MMW0014	182,300	425,750	+	2	0	14	0
WRL-09	WRL-MMW0019	188,750	428,850	-	0	0	0	0
WRL-10	WRL-MNB0003	152,950	437,750	+	4	0	0	0
WRL-12	WRL-MTW0009	132,200	429,400	+	5	0	0	0
WRL-18	WRL-PMW0082	162,950	428,690	+	0	0	1	0
WRO-02	WRO-OVOER100	179,650	308,300	-	0	0	0	0
WRO-08	WRO-ORODE800	189,340	337,230	-	0	0	0	0
WRO-09	WRO-ORODE500	196,420	332,630	+	3	0	0	0
WRO-10	WRO-OMUHL800	202,890	347,790	+	5	0	0	0
WRO-11	WRO-ORBRO500	206,220	350,360	-	0	0	0	0
WRO-15	WRO-OVOER900	177,010	309,030	-	0	0	0	0
WRO-16	WRO-OGELE900	186,160	343,330	+	7	0	0	0
WRO-17	WRO-OJEKE900	176,750	317,330	-	0	0	0	0
WRW-01	WRW-3PVEN4	198,500	519,600	+	2	0	0	0
WRW-03	WRW-2REES7	215,650	520,350	+	2	0	0	0
WRW-07	WRW-2SLEI5	215,140	518,000	-	0	0	0	0
WRW-08	WRW-2OUDD3	232,340	533,550	-	0	0	0	0
WRW-13	WRW-2OUDD5	229,950	531,450	-	0	0	0	0
WRW-14	WRW-1OUDV9	210,300	525,880	-	0	0	0	0
WV-01	WV-230010	205,500	471,550	-	0	0	0	0
WV-02	WV-210510	196,089	477,518	-	0	0	0	0
WV-03	WV-222510	196,820	464,120	-	0	0	0	0
WV-05	WV-240010	185,836	495,350	+	1	0	0	0
WV-06	WV-243010	178,000	488,750	-	0	0	0	0
WV-08	WV-200090	201,700	495,250	+	8	0	0	0
WV-09	WV-210020	199,600	487,600	-	0	0	0	0
WV-10	WV-221570	200,030	460,150	-	0	0	0	0
WV-11	WV-233510	200,550	468,300	-	0	0	0	0
WV-12	WV-204060	192,990	466,190	-	0	0	0	0
WV-13	WV-208510	191,720	483,910	-	0	0	0	0
WV-14	WV-200060	199,900	487,900	-	0	0	0	0
WV-15	WV-202010	197,700	482,800	-	0	0	0	0
WVE-01	WVE-29729	162,140	451,180	+	3	0	0	0

Project code	Waterboard code	Coordinate X (Dutch grid)	Coordinate Y (Dutch grid)	Crayfish presence(+) or absence(-)	SCC	VC	RSC	NCC
WVE-03	WVE-29879	152,000	468,520	-	0	0	0	0
WVE-04	WVE-29862	147,910	475,560	+	1	0	0	0
WVE-05	WVE-29051	180,540	443,350	-	0	0	0	0
WVE-06	WVE-29771	154,550	464,160	-	0	0	0	0
WVE-10	WVE-29991	148,730	476,590	-	0	0	0	0
WVE-12	WVE-27201	167,450	460,500	-	0	0	0	0
WVE-13	WVE-29732	156,170	454,550	+	4	0	0	0
WVE-14	WVE-29781	148,480	467,490	-	0	0	0	0
WVE-15	WVE-29861	148,175	473,440	-	0	0	0	0
WVE-16	WVE-27202	165,900	462,100	-	0	0	0	0
WVE-17	WVE-28003	161,650	453,500	-	0	0	0	0
WVV-05	WVV-ALOO80	246,050	522,850	-	0	0	0	0
WVV-09	WVV-CSLE65	254,550	529,450	+	1	0	0	0
WVV-12	WVV-BDDI60	248,980	528,870	-	0	0	0	0
WVV-14	WVV-FVHV80	245,030	528,380	+	2	0	0	0
WZE-02	WZE-MPN1333	035,604	396,243	-	0	0	0	0
WZE-03	WZE-MPN1468	041,570	393,560	-	0	0	0	0
WZE-06	WZE-MPN1503	063,320	381,310	-	0	0	0	0
WZE-09	WZE-MPN1330	031,450	390,710	-	0	0	0	0
WZE-12	WZE-MPN1440	042,590	397,510	-	0	0	0	0
WZE-14	WZE-MPN1499	056,580	394,100	-	0	0	0	0
WZE-15	WZE-MPN3946	053,230	386,060	-	0	0	0	0
WZE-17	WZE-MPN1236	073,710	393,640	-	0	0	0	0
WZE-18	WZE-MPN1481	040,180	381,650	-	0	0	0	0
WZV-01	WZV-O70150	057,080	361,730	-	0	0	0	0
WZV-04	WZV-O70590	057,100	361,450	-	0	0	0	0
WZV-06	WZV-O60400	062,250	362,980	-	0	0	0	0
WZV-07	WZV-O70400	053,730	360,180	+	0	0	0	2
WZV-08	WZV-O60390	062,450	362,500	-	0	0	0	0
WZZ-01	WZZ-AKP09	182,640	533,480	-	0	0	0	0
WZZ-02	WZZ-ACP18	181,000	528,300	-	0	0	0	0
WZZ-03	WZZ-ALH90	181,500	537,037	-	0	0	0	0
WZZ-04	WZZ-01080	178,057	504,481	+	3	0	0	0
WZZ-05	WZZ-QKM12	195,158	519,154	-	0	0	0	0
WZZ-06	WZZ-ALV75	179,157	535,809	-	0	0	0	0
WZZ-07	WZZ-ALH35	179,410	539,166	-	0	0	0	0
WZZ-08	WZZ-00850	160,295	496,456	-	0	0	0	0
WZZ-09	WZZ-01004	159,345	501,194	-	0	0	0	0
WZZ-10	WZZ-00011	157,541	482,386	-	0	0	0	0
WZZ-11	WZZ-00543	180,692	502,403	-	0	0	0	0
WZZ-12	WZZ-01002	160,029	502,914	-	0	0	0	0
WZZ-13	WZZ-00261	154,533	486,420	-	0	0	0	0
WZZ-14	WZZ-CSS01	181,500	518,500	+	2	0	0	0
WZZ-15	WZZ-00551	170,821	489,616	-	0	0	0	0
WZZ-16	WZZ-CEV45	183,660	519,920	-	0	0	0	0
WZZ-17	WZZ-CZV00	191,686	520,234	-	0	0	0	0
WZZ-18	WZZ-00564	168,667	491,869	-	0	0	0	0

APPENDIX 4. INSTRUCTIONS

Voorbereiding

- Leg de fuiken in een emmer sloot- of regenwater, hoe langer hoe beter, bij voorkeur minimaal een week.

Het vangen

- Plaats de fuiken 's middags of 's avonds
- Plaats de fuiken op de bodem en zorg dat er geen onderdelen boven het water uitsteken (met het oog op diefstal of vandalisme). Plaats eventueel een steen in de fuik om te zorgen dat de fuik stevig op de bodem verankerd ligt.
- Bevestig de fuiken met het bijgeleverde touw of kabel onopvallend aan de oever
- Nummer de fuiken van 1-3.
- Controleer de fuiken in de ochtend. 's Ochtends is de kans het grootsts om de nachtactieve kreeften in de fuik te treffen. Overdag zullen de dieren pogen uit de fuik te ontsnappen terwijl de kans op nieuwe kreeften gering is.
- Laat de fuiken tijdens de waarnemingsperiode in het water staan.
- Noteer per fuik je vangsten en maak daarbij onderscheid tussen mannetjes en vrouwtjes en eventuele eieren of jongen onder de staart!
- Maak van elke soort die je denkt gevangen te hebben een foto (van de boven- en onderzijde).
- Plaats alle vangsten na registratie terug in het water.

Let op!

- De drie fuiken mogen op maximaal 20 meter afstand van elkaar geplaatst worden per meetpunt. Zorg er altijd voor dat de fuiken in dezelfde watergang staan!
- Probeer zoveel mogelijk verschillende 'microhabitats' te bemonsteren (bijvoorbeeld een brug, een rietkraag, en een aanlegsteiger).
- Gebruik het formulier (appendix 5) om tijdens ééen bezoek enkele vragen over het meetpunt (bodemstructuur, oeverstructuur, kroosdek en weersgesteldheid) te beantwoorden.
- Hoewel een 'aasklip' in de fuik aanwezig is, is het nadrukkelijk niet de bedoeling dat je aas gebruikt in de fuik. De voornaamste reden hiervoor is dat we bijvangsten (en daarmee een verhoogde kans op sterfte) zoveel mogelijk willen vermijden. Het feit dat fuiken op zichzelf al interessant gevonden worden door kreeften, maakt onbeaasde fuiken juist een zeer selectief vangmiddel.

Preperations

- Place the traps in a bucket with water. The longer the better. Preferably, at least a week.

The catchment

- Place the traps in the afternoon or evening.
- Place the traps on the bed of the waterbody. Make sure that the traps are not visible above the waterline (to avoid theft or vandalism). If necessary, place a stone in the trap, to reassure that the traps are anchored to the bottom.
- Use the given ropes to attach the traps inconspicuously to the bank.
- Assign a number from 1 to 3 to the traps.
- Check the traps in the morning. The night-active crayfish are most likely to be in the traps in the morning. Throughout the day, the animals will try to escape, while no new specimens will enter.
- Leave the traps in the water throughout the sampling period.
- Note the catches per trap. Make a distinction between males, females and the possible presence of eggs of juveniles under the tail of the female.
- Take a picture of every species (dorsally and ventrally).
- Place all the specimens back in the water after recording.

Beware!

- The distance between each trap may not exceed 20 metres. Make sure all the traps are placed in the same waterway!
- Try to sample several 'microhabitats' (place the traps for example below a bridge, near a reedbed and near a boardwalk).
- Use the form to answer some questions about the sample site (about the structure of the bank and bottom, presence of duckweed, weather, etc.).
- Although a 'bait-hook' is present in the traps, please don't use bait! The main reason for not using bait is to avoid (mortality among) by-catches. Traps without bait are highly selective for just crayfish (which are looking for shelter).

Voorwaarden gebruik kreeftkorven

Kreeftkorven zijn zonder ontheffing een verboden vangmiddel! Stichting EIS kan een machtiging verstrekken voor het gebruik aan kreeftkorven aan een vrijwilliger die de visserij uit wil oefenen op basis van een ontheffing van het ministerie van LNV. Het is NIET toegestaan om visserij op kreeften uit te voeren met een machtiging van Stichting-EIS met andere vangmiddelen dan de korven die via Stichting EIS verstrekt worden. Alle vangsten, OOK de kreeften, dienen zorgvuldig te worden behandeld en na registratie levend in hetzelfde water te worden teruggezet.

Voor de uitvoering van de visserij is, behalve een machtiging, ook schriftelijke toestemming nodig van de visrechthebbende. Dit kan een eigenaar zijn, maar ook een huurder van het visrecht op het betreffende water. Zonder deze toestemming mag de visserij niet worden uitgeoefend. In het kader van dit onderzoek is het waterschap in de meeste gevallen de directe eigenaar.

Indien u via EIS nog geen schriftelijke toestemming heeft ontvangen, dient u toestemming te vragen aan het betreffende waterschap. Op een aantal plaatsen zijn de visrechten echter verhuurd aan o.a. hengelsportverenigingen en/of beroepsvissers. Indien sprake is van verhuring van visrechten is van belang of het volledige visrecht is verhuurd.

Indien dit zo is, dan moet de huurder toestemming geven om de visserij op kreeft uit te mogen oefenen. Is niet het volledige visrecht verhuurd, maar enkel het aalvisrecht en/of het schubvisrecht, dan is hoogstwaarschijnlijk het schaaldierrecht niet verhuurd en is de eigenaar van het water degene die toestemming moet geven om de visserij op kreeft uit te mogen oefenen.

Binnen verschillende waterschappen en regio's zijn inmiddels goede afspraken gemaakt met de visrechthebbenden. Als u aan de slag wilt en nog geen bericht van ons heeft ontvangen doet u er goed aan om uw watergang even op te zoeken op www.visplanner.nl of www.combinatievanberoepsvissers.nl voor de contactgegevens van de plaatselijke visrechthebbenden.

Bottom line: werk zorgvuldig, veroorzaak geen overlast en houd, behalve met visrechthebbenden, zorgvuldig rekening met aanliggende terreineigenaren zoals particulieren, natuurmonumenten of staatsbosbeheer.

Terms and conditions for using traps

Crayfish traps are prohibited without a permit! EIS is authorized to give a licence to volunteers (based on a general dispensation of the Ministery of Economic Affairs, Agriculture and Innovation) for trapping crayfish for registration. It is NOT allowed to use the permit for other traps than the ones provided by EIS. Also, all crayfish should be handled with care and released back into the same water as they come from.

Besides the licence, volunteers need to have a permission by letter from the owner of the fishing rights. This could be the owner of the water, or someone who rents the fishing rights from the owner. Trapping must not be carried out without this permission. Within the framework of this study, the water boards are usually the owner of the water.

APPENDIX 5. FORM

Verspreidingsonderzoek uitheemse rivierkreeften

Mail uw ingevulde formulier naar: eis@ncbnaturalis.nl

Gegevens waarnemer	
Voornaam:	*Jeroen*
Achternaam:	*Brand*

Meetlocatie

WD-06

Eerste controle dag

11-Oct-10

Breedte van de watergang

c) >5 m

Beschoeiing langs de kant op het traject waar de fuiken staan

b) Oever gedeeltelijk beschoeid en gedeeltelijk 'natuurlijk'

Welk percentage van wateroppervlak wordt, van boven af gezien, door drijvende en emergente (=boven water uitstekende) vegetatie bedekt?

2

Welk percentage hiervan wordt ingenomen door kroos?

0

100% sterrekroos

Weersomstandigheden gedurende de meetperiode

b) Neerslag

Zou u volgend jaar in dezelfde periode (15 augustus - 15 oktober) opnieuw willen meewerken aan een eventueel vervolgonderzoek?

Ja, op dezelfde of op een andere meetlocatie

Bijvangsten (optioneel)

[Voer hier eventueel uw bijvangsten in die u gedurende de meetperiode heeft gevangen]

Kreeften gevangen?

Ik heb geen rivierkreeften gevangen ----> ☐ *[u hoeft onderstaande tabellen niet meer in te vullen]*

Indien u geen rivierkreeften heeft gevangen, heeft u bewijzen/vermoedens dat er desondanks wel kreeften voorkomen op uw meetpunt?

[Vul hier uw antwoord in]

Waarnemingen

Voer de aantallen in in onderstaande tabel(len. Gebruik voor iedere kreeftensoort een nieuwe tabel! Markeer gefotografeerde dieren rood in de tabel en geef de foto's de volgende naam: jjjjmmdd_korf# (bijvoorbeeld 20100927_korf2)

SOORT-1 --->	Gevlekte Amerikaanse rivierkreeft - Orconectes limosus					
		DAG1	DAG2	DAG3	DAG4	
KORF1	MN		2	1	4	korf geplaatst benedenstrooms va
	VR					brug aan 'natuurlijke' oever
	VR+EI					
	VR+JUV					
KORF2	MN		2	3	5	korf geplaatst onder brug op steer
	VR					
	VR+EI					
	VR+JUV					
KORF3	MN	1			1	korf geplaatst bovenstrooms van
	VR					brug aan 'natuurlijke' oever
	VR+EI					
	VR+JUV					
	TOTAAL	1	4	4	10	LET OP: Uitsluitend mannelijke exe
						gevangen

SOORT-2 --->					
		DAG1	DAG2	DAG3	DAG4
KORF1	MN				
	VR				
	VR+EI				
	VR+JUV				
KORF2	MN				
	VR				
	VR+EI				
	VR+JUV				
KORF3	MN				
	VR				
	VR+EI				
	VR+JUV				
	TOTAAL				

SOORT-3 --->					
		DAG1	DAG2	DAG3	DAG4
KORF1	MN				
	VR				
	VR+EI				
	VR+JUV				
KORF2	MN				
	VR				
	VR+EI				

APPENDIX 6. PHOTO IMPRESSION

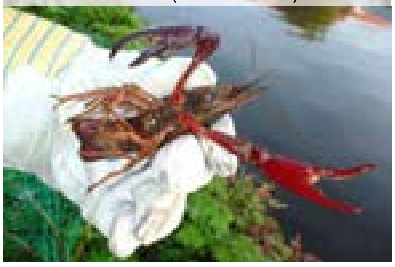
* = spiny cheek crayfish present at site; * = red swamp crayfish; * = virile crayfish.

HHD-01 (foto: K. Keijzer)

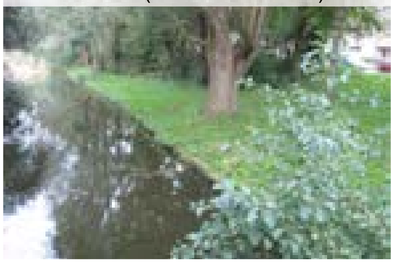
HHD-05 (E. Bekker)*

HHD-08 (foto: W. Bos)*

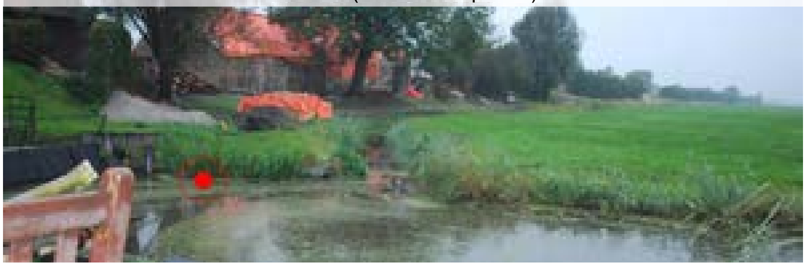
HHD-08 (foto: W. Bos)*



HHD-12 (foto: M. Scheeres)*



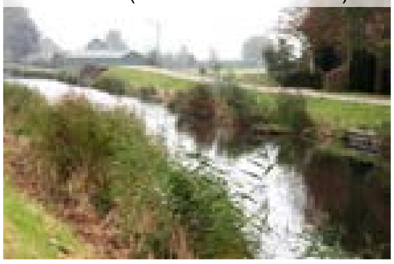
HHD-14 (foto: E. Raaphorst)



HHD-21 (foto: L. Kusse)

HHN-04 (foto: P. Koelma)*

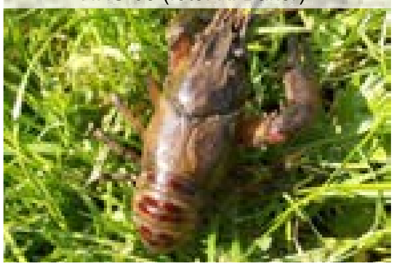
HHN-09 (foto: A. van der Kraan)



HHR-03 (foto: W. Tamis)*

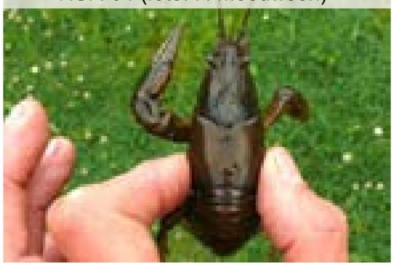
HHR-06 (foto: R. Kleukers)

HHS-03 (foto: K. Baker)*

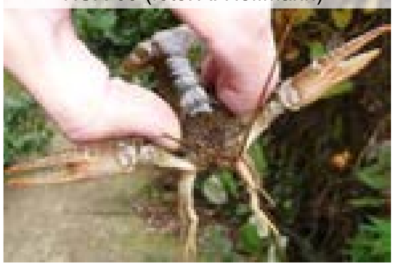


HHS-03 (foto: M. Kolpa)

HSR-04 (foto: F. Meeuwsen)*



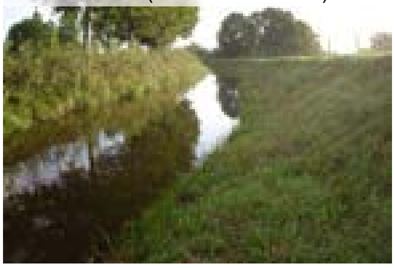
HSR-09 (foto: A. Hoffmann)*



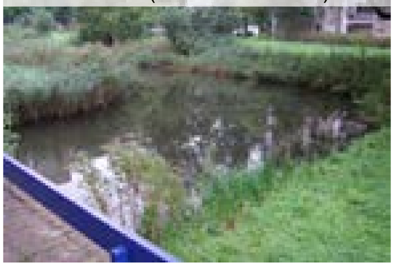
APPENDIX 6. PHOTO IMPRESSION (continuation)

* = spiny cheek crayfish present at site; * = red swamp crayfish.

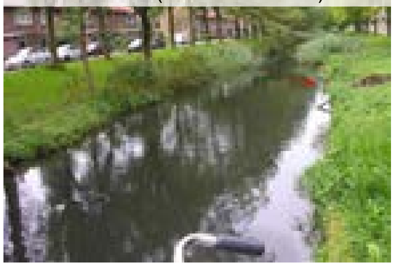
WAM-18 (foto: R. Bakkenes)*



WAN-11 (foto: R. Burkhardt)



WAN-12 (foto: T. Boersma)



(foto: P. Bouwmeester)*

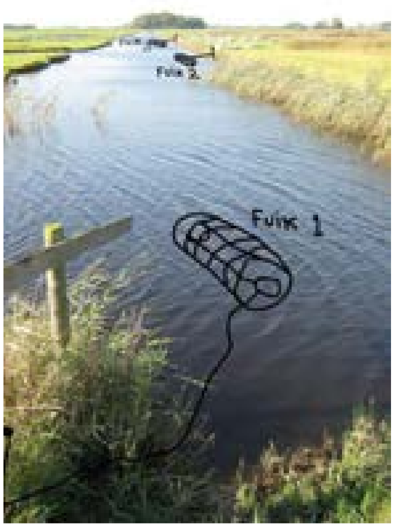
WD-02 (foto: A. de Korte)*

WD-12 (foto: J. van der Linden)*

WF-10 (foto: D.J. Venema)*

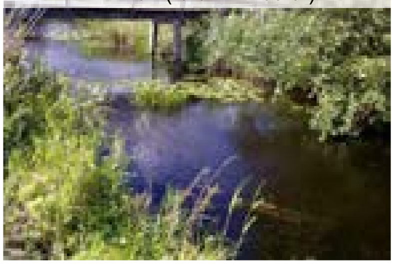
WGS-11 (foto: E. de Visser)*

WF-12 (foto: M. van der Beek)



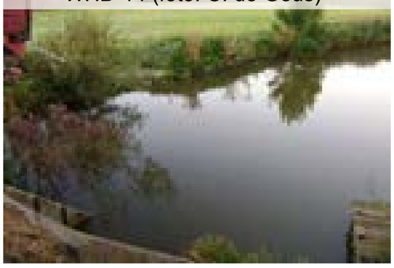
WGS-15 (foto: D. Hoekerd)

WHA-07 (foto: H. Koster)

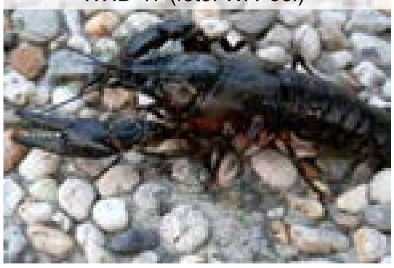


WHA-16 (foto: H. Offringa)

WHD-14 (foto: C. de Geus)*



WHD-17 (foto: W. Poel)*



WN-08 (foto: D. Alting)

WN-12 (foto: D. Bekker)*

WPM-02 (foto: J. Tienstra)*

APPENDIX 6. PHOTO IMPRESSION (continuation)

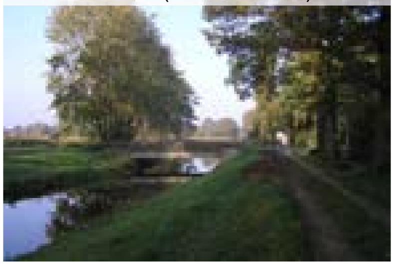
* = spiny cheek crayfish present at site; * = red swamp crayfish.


WPM-11 (foto: F. Smit)


WRD-05 (foto: M. van der Ende)*

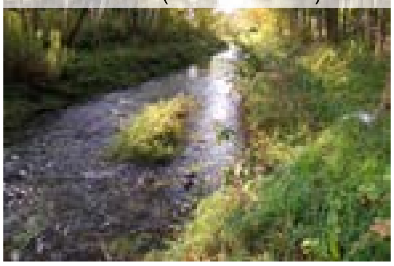

WRD-08 (foto: J. Verheijen)*


WRIJ-07 (foto: D. Eenink)*


WRIJ-10 (foto: J. Bakker)*

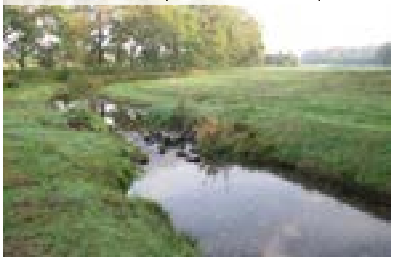

WRL-08 (foto: M. van Dongen)*

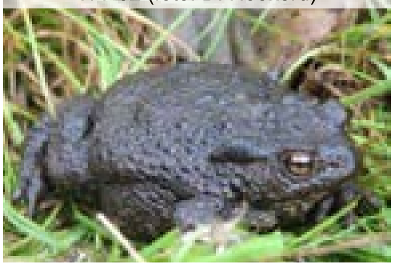

WRL-10 (foto: T. Aarts)*

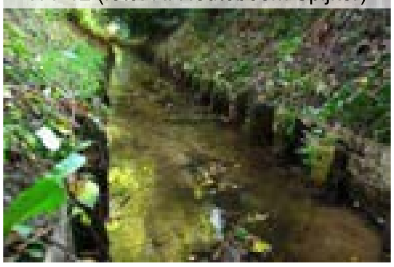

WRO-08 (foto: J. Tienstra)

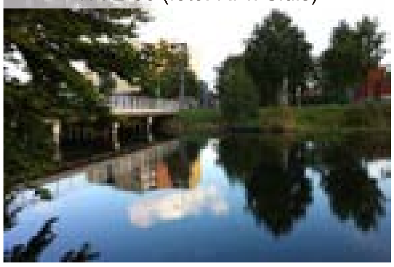

WRO-02 (foto: R. Roepers)


WRW-03 (foto: A. Broekert)*

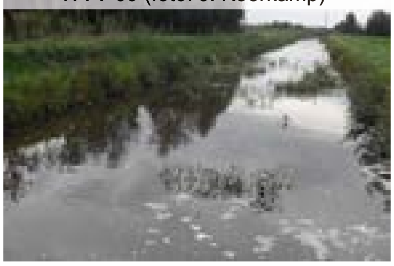

WRW-08 (foto: M. Loonen)


WV-02 (foto: D. Hoekerd)


WV-12 (foto: R. Neuteboom Spijker)


WVE-06 (foto: R. v. Sluis)


WVE-13 (foto: R. Nijland)*


WVV-09 (foto: J. Koerkamp)*

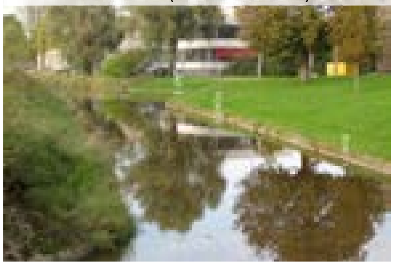

WVV-09 (foto: R. Jongepier)

APPENDIX 6. PHOTO IMPRESSION (continuation)

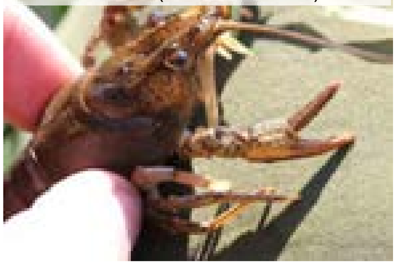
* = spiny cheek crayfish present at site; * = narrow clawed crayfish.

WZE-18 (foto: C. Vlemmix)

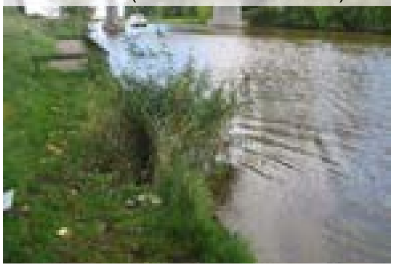
WZE-09 (foto: E. Paree)



WZV-07 (foto: A. Wieland)



WZZ-11 (foto: D. & S. Hoekerd)



WZZ-13 (foto: F. Steenhuisen)

WZZ-14 (foto: K. Kleermaker)*