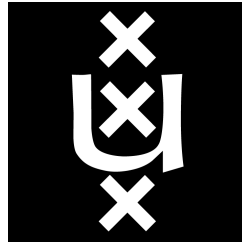


Alien crayfish distribution and species specific habitat preferences in the Netherlands

BSc. Thesis

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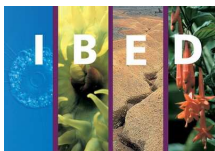
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Alien crayfish distribution and species specific habitat preferences in the Netherlands

Abstract

Several American crayfish species have invaded the Netherlands in the past decades, and have undergone a tremendous population growth since. Yet, their distribution and species specific habitat preferences is not well documented. The aim of the present study was therefore to investigate the relationship between crayfish abundance and their abiotic environment. To this purpose, crayfish were caught at several sites in the 'Amstel-, Gooi- and Vecht region' in the Netherlands. The selected sites are monitored on a regular basis by Waternet Amsterdam, involving the measurement of various environmental variables. Distribution of the crayfish *Procambarus clarkii* was found related to a combination of oxygen, pH, nitrate, sulphate and temperature and directly to pH. No relations between other crayfish species and environmental variables were found. Low crayfish numbers suggest inactivity during this study.

KEYWORDS: Alien crayfish, distribution, environmental factors, *P. Clarkii*.

Introduction

Relating the distribution of species to their abiotic environment is of fundamental importance to understand their ecology. Yet, for many species this basic information is largely lacking. Particularly little is known about non-indigenous, although they can invade successfully when they are tolerant of the local conditions or when their natural competitors or predators are absent (Sax & Brown, 2000). In some cases invasive species may become a major threat to biodiversity (Bax *et al.*, 2001; Mack *et al.*, 2000).

An example of a group of introduced species in Europe is American crayfish, such as the Red swamp crayfish (*Procambarus clarkii*), the Northern crayfish (*Orconectes virilis*) and the Spinycheek crayfish (*Orconectes limosus*). Until the 1970s the native crayfish species of the Netherlands, the Noble crayfish (*Astacus astacus*), was the only crayfish species present in the Netherlands. However, since the introduction of the American crayfish species, *A. astacus* has become nearly extinct in this part of Europe. Populations of *A. astacus* were already declining due to deteriorating water quality (Roessink *et al.*, 2009), but the situation has probably been aggravated by the introduction of American crayfish species, who are natural hosts for *Aphanomyces astaci*, the pathogen causing crayfish plague (Kozubiková *et al.*, 2009).

Besides outcompeting the native *A. astacus*, *P. clarkii* is suspected to have a dramatic impact on aquatic communities via feeding links with multiple trophic levels (Dorn & Wojdak, 2004), and to deteriorate water quality (Van der Meulen *et al.*, 2009). The influence of *P. clarkii* and other invasive American crayfish species on ecosystems has been studied extensively (Verdonschot *et al.*, 2009; Van Emmerik & De Laak, 2008), as well as the distribution of crayfish and the predictability of crayfish occurrence in relation to isolated habitat features (Naura & Robinson, 1998; Barbaresi *et al.*, 2007). Previous research in other countries considered the effects of nutrient deprivation, chemical stimulants and light preferences on individual crayfish (Kozák *et al.*, 2009; Correia *et al.*, 2007; Powell & Watts, 2010), but these studies did not relate these preferences to species distribution.

Studies on the density and distribution of American crayfish have also been performed in the Netherlands (Van Emmerik & De Laak, 2008; Van Emmerik, 2010). However, these studies were conducted on a local scale and therefore results cannot be extrapolated to a larger scale. This is partly because no standard sampling methods were used, since standard monitoring methods are not yet designed to catch crayfish. Moreover, studies on the predictability of crayfish occurrence associated with habitat features have not yet been endeavoured, as species specific habitat preferences are not well understood. This fundamental knowledge on the distribution of alien crayfish is however very important to understand the true impact of these non-indigenous invaders on the ecosystem (Van Emmerik & De Laak, 2008). The aim of the present

study was therefore to relate crayfish distribution to abiotic environmental variables, allowing to derive species specific habitat preferences. The ‘Amstel-, Gooi- and Vecht region’ was selected as a case study, since *P. clarkii* has been reported to be most abundant in this area (Koesse, 2008).

Several studies have already been performed on limiting conditions for crayfish. Although most of these studies took place in southern Europe or were performed on other crayfish species than present in the Netherlands, their results may prove useful for predictions in the present research. Naura & Robinson (1998) stated that channel vegetation is a crucial factor in crayfish presence, although the percentage of coverage was not related to crayfish abundance. Cruz & Rebelo (2007) concluded that *P. clarkii* is negatively affected by higher water flow rates. In permanent water bodies the substrate composition had a significant effect, while in temporary water bodies, this relation was absent. In these water bodies, crayfish were more likely limited by drought periods. Barbaresi *et al.* (2007) and Cruz *et al.* (2007) observed that crayfish presence is related to substrate composition, especially exposed pebbles and cobbles seem to indicate good habitats. The former study also stated that calcium concentration is a limiting factor, due to the fact that crayfish need to build a calcareous skeleton. Unlike other studies, Barbaresi *et al.* (2007) did not observe a relation between canopy cover and crayfish abundance. The different habitats investigated in these studies are mainly absent in our research area. Hence other, yet unknown, environmental variables may determine crayfish occurrence and distribution in the Netherlands.

Methods

Study Sites

Waternet regularly monitors approximately 5050 sites in the Amstel-, Gooi- and Vechtregion in the Netherlands. At these sites either physical-chemical parameters are measured or macro-fauna is sampled. Sample sites for the present research were selected from those Waternet sites where physical-chemical factors have been monitored in the past years. A total number of 48 sites was selected (Figure 1).



Fig 1. Map of sampling sites.

The blue area is the area managed by Waternet. The red dots are the selected sample sites.

Sampling

Fieldwork was performed from the start of April 2010 until the end of May 2010. Sample sites were visited for two consecutive days. On the first day, fyke nets were installed to catch crayfish. On the second day, approximately 24 hours later, the fyke nets were retrieved.

In order to catch crayfish in a standardized way, the fyke nets were placed at the bottom of the water (Figure 2). In every fyke net, a piece of bait was placed to attract the crayfish. Whiting (*Merlangius merlangus*) was used as bait.

Approximately 24 hours after placing the fyke nets they were retrieved. If the net contained crayfish, the crayfish species was identified and crayfish length was measured. After the measurement, the crayfish were released into the water.

At the study sites water depth and depth of the mudlayer at the location of the fyke net was measured. In addition the current velocity of the water was estimated (1-5: 1= no current; 5=high current).

Statistical Analysis

A total of 93 physical-chemical parameters were obtained from the Waternet database. Twenty of these parameters were monitored consistently on most locations and therefore only these were used for analysis. The selected parameters are listed in Table 1.

For each site we selected the values of these parameters measured in 2010. If data from 2010 was lacking, we used the values of December 2009. Six sites were omitted, as information from this month was also lacking and the data would be unreliable.

In order to separate the sampling sites on physical-chemical characteristics only, a Principal Component Analysis (PCA) was performed on the environmental data (without crayfish data) in SPSS. In order to reveal a correlation between the resulting PCA axes and crayfish presence, a binary logistic regression was performed, with crayfish presence as a dependent variable and the PCA axes as independent variables. To this purpose, crayfish data was converted into binary output (presence/absence). Finally a T-test was performed in Microsoft Excel, to test crayfish presence against the individual environmental parameters that had high loadings in the PCA axes.



Fig. 2: Fyke net with bait and crayfish

Table 1. Parameters from Waternet database

Physical-chemical parameter	Dimension
Absorbance at 380nm	/m
Absorbance at 440nm	/m
Acidity	dimensionless
Ammonium	mg/L
Ammonia	mg N/L
Chloride	mg/L
Chlorophyll- α	$\mu\text{g/L}$
Conductivity	m
Kjehldahl Nitrogen	mg N/L
Transparency	$\mu\text{g/L}$
Nitrate	mg N/L
Nitrite	mg N/L
Nitrogen	mg N/L
Ortho-phosphate	mg P/L
Oxygen	mg/L
Oxygen Saturation Percentage	%
Phaeophytins	$\mu\text{g/L}$
Phosphorus	mg P/L
Sulphate	mg/L

Results

A total of 16 *P. clarkii* and 7 *O. limosus* were caught at 12 sites, with a maximum of 6 crayfish per site. No other crayfish species were found. All crayfish ranged in size between 8 cm and 13 cm. The number of *O. limosus* was too low for statistical analysis, so only *P. clarkii* was used for analysis. By-catch was minimal. Only one bream (*Abramis brama*) and two European perch (*Perca fluviatilis*) were caught in the fykes during the sampling period.

The PCA resulted in three axes with an explanation of variation of respectively 28%, 16% and 12%. Axis loadings are shown in Table 2. The first axis was mainly loaded by nitrogen, phosphorus, conductivity and transparency. This suggested that this axis represented an underlying factor of eutrophication. The second axis was mainly loaded by variables chlorophyll, pheophytin and humus, and was therefore interpreted as describing levels of plant material.

There was however, no clear difference between sites with crayfish present and those with crayfish absent (Fig. 3). And consequently, the presence/absence of *P. clarkii* was not correlated to the two main PCA components of variation in sampling locations. The third PCA component however, was significant as an explaining factor for crayfish presence ($p=0.048^*$). This was a negative effect (Fig. 4), implying that probability of crayfish presence increased with decreasing values of the third PCA component. This third axis showed high loadings of oxygen, pH, nitrate, sulphate and temperature. These variables do not clearly represent an underlying mechanism.

The T-tests performed on the individual variables showed no significance, except for pH ($p=0.018^*$).

Discussion

Previous studies on crayfish distribution revealed vast numbers of alien crayfish (Kroese, 2008; Van Emmerik, 2010; Van Emmerik & Laak, 2008; Verdonschot *et al.*, 2009), while in the present study only 23 crayfish were caught in the fyke nets, with *P. Clarkii* being the most abundant species. There are two possible explanations for this change in crayfish abundance. Richards *et al.* (1996) reported that peak catches of crayfish occur later in spring, when the water is warmest. Since the Netherlands experienced the coldest winter in fourteen years and the coldest May in nearly twenty years (KNMI, 2010a; KNMI, 2010b), many of the crayfish may have perished due to cold or anoxic conditions under the ice, while surviving animals may have remained in their burrows during the present field campaign (May 2010). This inactivity is supported by Barbaresi & Gheraldi (2000), who reported that crayfish remain dormant in the colder months of the year.

Another possible explanation for crayfish absence is predation by birds and large predatory fish. Fortino & Creed (2007) suggested that especially juveniles are susceptible to fish predation. Tablado *et al.* (2010) and Roessink *et al.* (2009) reported diet shifts of large birds like storks, cormorants, herons and egrets to crayfish. More than 10% of the diet of half of the number of predators investigated by Tablado *et al.* (2010) consisted of red swamp crayfish. Moreover, these predators have increased in numbers, caused by the increase in crayfish numbers.

The principal component analysis of physical/chemical parameters showed a clear structural variation based on nutrient levels and plant material. These factors did not, however, explain the occurrence of crayfish in the study area. A third combination of parameters explained both the variation between sampling locations and occurrence of *P. clarkii*. Unlike the first two PCA axes, the third axis (mainly composed of oxygen, pH, nitrate, sulphate and temperature) did not represent a clear underlying factor, like eutrophication for the first axis. Some of the parameters associated with the third axis were also identified by Rallo & García-Arberas (2002), like nitrate and sulphate. On the contrary, Rallo & García-Arberas (2002) did not observe a relationship between pH and crayfish occurrence, while in the present study pH was the only individual parameter correlated with crayfish occurrence. Rallo & García-Arberas (2002) also observed a correlation with several minerals (Mg, K, Na and Ca), but limited data on those elements prevented us from incorporating them in the statistical analysis. The partial correlation with temperature observed in the present study, is in line with Barbaresi & Gheraldi (2000), who reported that crayfish remain dormant in the colder months of the year.

Suggestions for further research

Further research would require a shift in sampling period from spring to summer. At higher water temperature, crayfish will be very active and not only crayfish occurrence, but also abundance can then be correlated to environmental parameters. It might then also become possible to define species specific habitat preferences of other alien crayfish species as well.

Conclusions

Distribution of *P. clarkii* presence was related to pH and to a combination of oxygen, pH, nitrate, sulphate and temperature. However, the results of the present study should be interpreted with care, due to the low number of crayfish caught, combined with a high number of missing values in the Waternet database.

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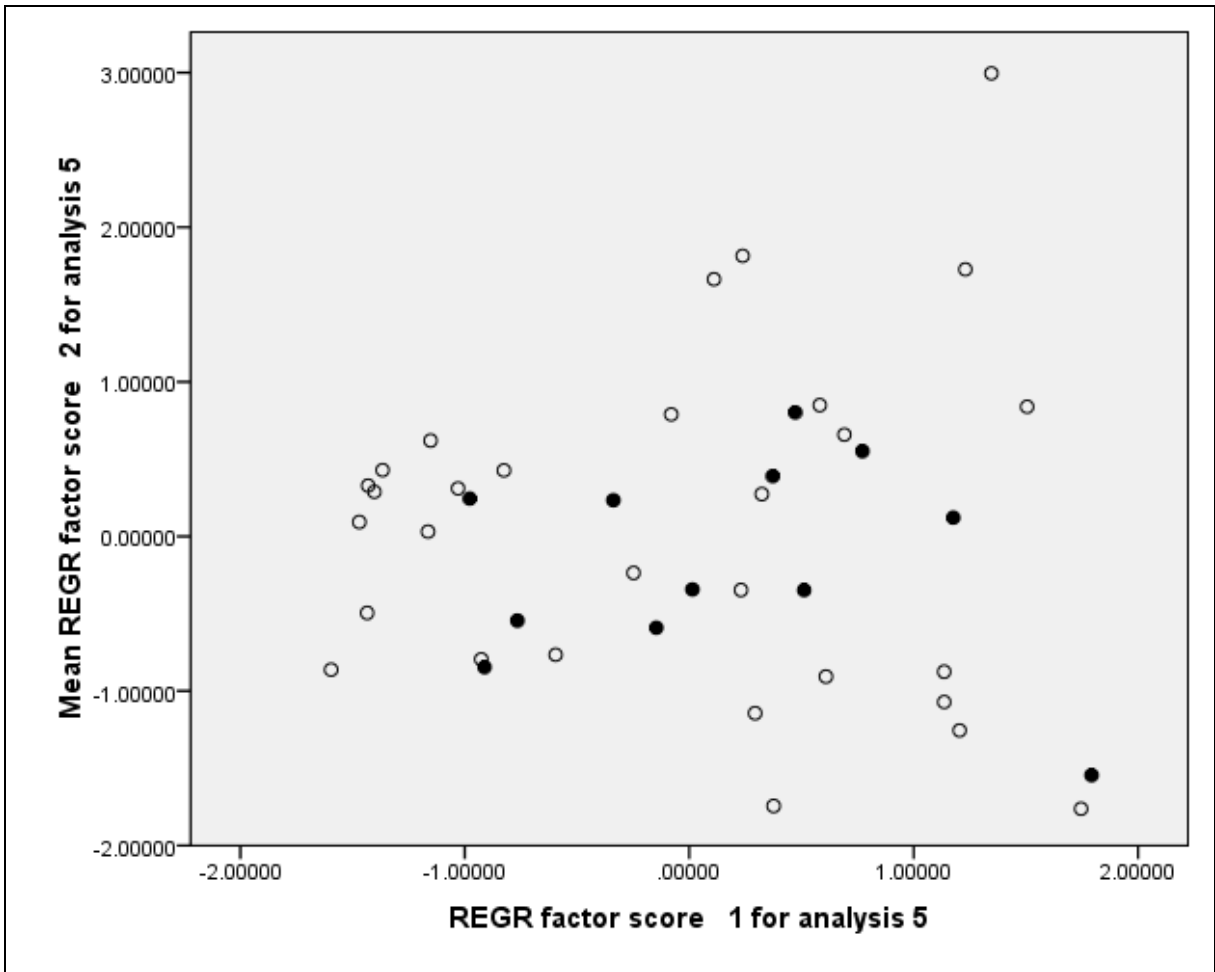


Figure 3. PCA plot of the two main components of variation. The first PCA axis is shown on the X-axis, the second on the Y-axis. Open circles represent sampling locations with crayfish absence, black circles are sites with crayfish presence.

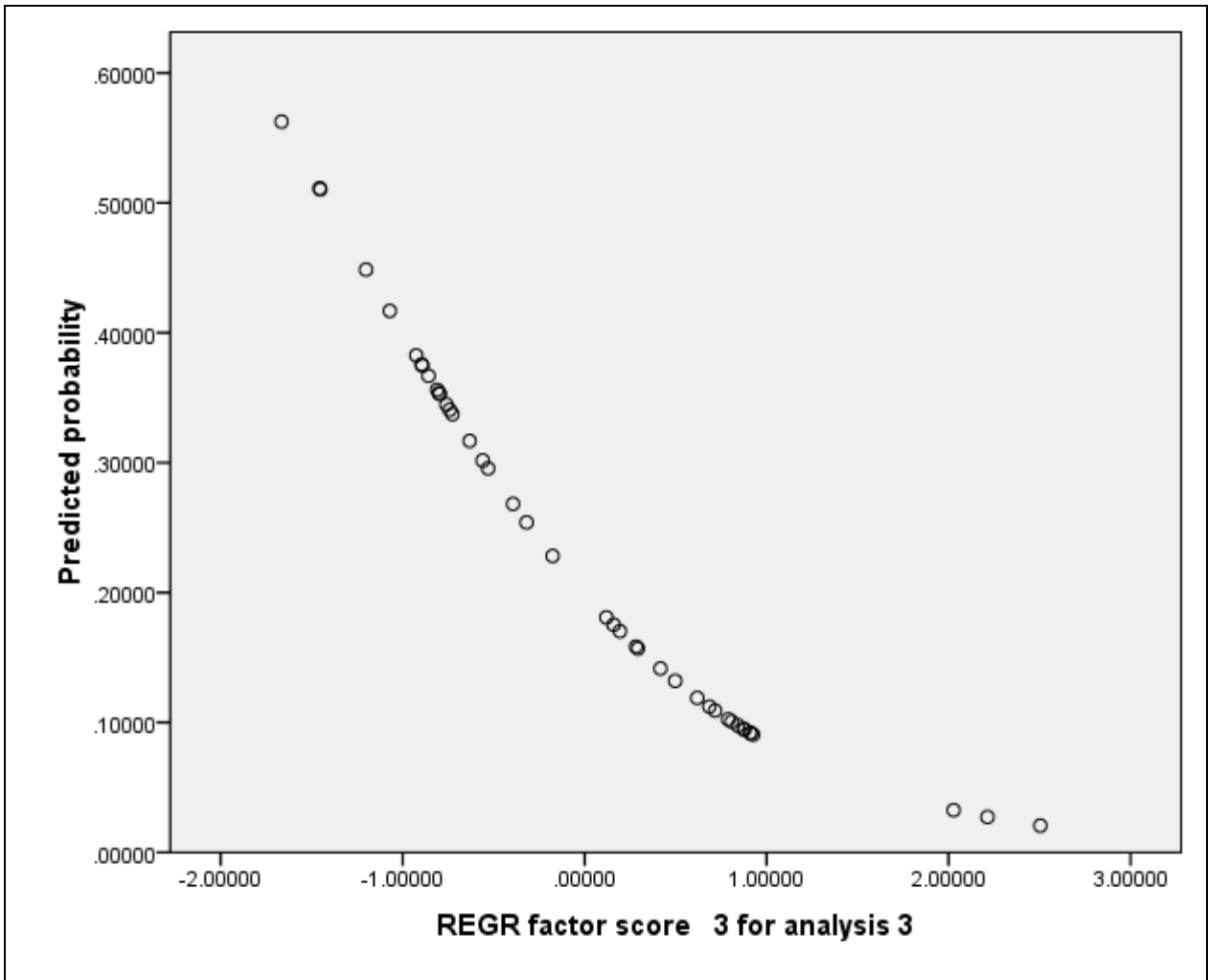


Figure 4. Regression scores for values of axis 3. The X-axis represents the third PCA axis. The Y-axis represents the probability of finding crayfish at a given location. Note that for low values of axis 3, the chance for crayfish presence is high, showing a reverse correlation of the third axis with crayfish presence.

Table 2. Loading scores of the three main PCA axes. High absolute values indicate a high correlation of the variable with the given axis. Negative values indicate a reverse correlation.

	Axis 1	Axis 2	Axis 3
Stikstof	0.913375	-0.08768	0.309652
Kjehldahl	0.88571	0.197435	-0.1238
Fosfor	0.75511	0.422873	0.051279
Ammonium	0.715033	-0.23066	-0.35831
Geleidendheid	0.617326	-0.59139	-0.06142
Nitriet	0.599407	-0.42645	0.316282
Doorzicht	-0.59328	-0.12488	0.043744
Sulfaat	0.583859	-0.38116	0.523734
Extinctie380	0.574234	0.567656	-0.25401
OrthoFosfaat	0.551034	0.332195	-0.05268
Modderdiepte	0.527049	0.264715	-0.14462
Stroming	0.468349	-0.33691	0.033237
Chlorophyl	0.143282	0.656217	0.473174
Feofytine	0.298619	0.611555	0.206808
Chloride	0.499417	-0.58656	-0.19513
Extinctie440	0.50606	0.585026	-0.26583
Zuurstof	-0.24678	0.300561	0.642813
Nitraat	0.497488	-0.35256	0.625314
Zuurgraad	-0.38657	-0.2475	0.618384
ZVP	-0.12289	0.295938	0.616822
Waterdiepte	0.016712	-0.12183	0.038109
Ammoniak	0.340659	-0.26685	0.142929
Temperatuur	-0.13979	-0.23364	-0.4567