ELSEVIER

Aquat. Living Resour. 15 (2002) 119–128

Aquatic Living Resources

www.elsevier.com/locate/aquliv

Differences in abiotic water conditions between fluvial reaches and crayfish fauna in some northern rivers of the Iberian Peninsula

Ana Rallo *, Loreto García-Arberas

Dpto. de Zoología y Dinámica Celular Animal, Universidad del País Vasco/Euskal Herriko Unibertsitatea. Spain, Apdo 644. 48080 Bilbao, Spain

Received 7 May 2001; accepted 8 January 2002

Abstract

We studied the distribution patterns of the native European white-clawed crayfish (*Austropotamobius pallipes)* and the introduced signal crayfish (*Pacifastacus leniusculus*), and looked at the water chemistry in several streams in the north of the Iberian Peninsula (Cantabrian watershed). Fifty fluvial reaches which were currently or previously inhabited by crayfish and had physical attributes similar to the known habitat requirements of crayfish were sampled. *P. leniusculus* was the most common species encountered (54% of the samples), *A. pallipes* was found in 19% and neither species was recorded in the remaining reaches. In this paper we determine the relationship between crayfish presence and water chemistry. As neither species inhabits waters with the highest NO_2^- concentrations found, it seems both species are sensitive to NO_2^-. Mg^{+2} and SO_4^{-2} concentrations are the factors separating sites with and without crayfish (crayfish inhabit water with higher concentrations of both cations). The main differences between reaches inhabited by native and signal crayfish lies in SO_4^{-2} concentration: higher concentrations favour signal crayfish. Applications to management based on the river water chemistry are proposed.

Resumen

Diferencias en las condiciones abióticas del agua en relación con la fauna de cangrejos en varios tramos fluviales del norte de la Península Ibérica. En este trabajo se estudia la distribución de las especies de cangrejos autóctona (*Austropotamobius pallipes*) e introducida (*Pacifastacus leniusculus*) en relación con las condiciones físico-químicas del agua de diversos tramos de ríos de Bizkaia (norte de la Península Ibérica; vertiente cantábrica). Se han tomado datos y muestras de cincuenta tramos fluviales, de los que algunos están habitados actualmente por cangrejos y los demás lo han estado en el pasado reciente y siguen presentando condiciones físicas que parecen adecuadas para la biología de estos crustáceos. La especie más frecuente fue *P. leniusculus*, que se encontró en un 54% de los casos, *A. pallipes* en un 19%, y no había cangrejos en el resto de los tramos fluviales. Se ha determinado la relación entre la presencia de cangrejos y las condiciones químicas del agua. Ambas especies parecen ser relativamente intolerantes al NO_2^- y, dado que la concentración de Mg^{+2} de estos tramos es relativamente baja, este elemento ha resultado ser el factor más importante, seguido por el SO_4^{-2}, que separa tramos habitados por cangrejos (concentraciones significativamente más altas) de los deshabitados. Es también el SO_4^{-2} la variable que discrimina entre tramos con cangrejo nativo (concentraciones más bajas) o con cangrejo señal. Se proponen aplicaciones para la gestión del recurso.

Keywords: *Austropotamobius pallipes*; *Pacifastacus leniusculus*; Chemical water conditions; Spain; Fluvial systems

1. Introduction

The presence, population size and productivity of aquatic invertebrates in rivers is affected by several factors. These factors include habitat (i.e., substrate, physical and chemical factors in water, vegetation), food availability, interaction with other species (competitors, predators, etc.), and disease (Lodge and Hill, 1994). The literature on the matter suggests that the abiotic factors of temperature, pH, dissolved oxygen, conductivity, calcium and substrate may all be important in governing species composition of cool-water crayfishes (Lodge and Hill, 1994). Although the plague produced by the fungus *Aphanomyces astaci* is the main reason generally given to explain the disappearance of most

---

* Corresponding author.
E-mail address: zopragra@lg.ehu.es (A. Rallo).

PII: S0990-7440(02)01156-7

native crayfish from European waters, the decline of these species has in fact probably resulted mainly from habitat degradation (Westman, 1985; Lowery and Holdich, 1988; Light et al., 1995, Smith et al., 1996). Native crayfish species have been protected since 1992 by the European Union Habitats and Species Directive.

Austropotamobius pallipes (Lereboullet, 1858) is the only species of crayfish indigenous to the Iberian Peninsula, excepting, perhaps, *A. italicum*, if it can be considered as a different species (Granjean et al., 2000). This crayfish once inhabited all the freshwater systems on the limestone areas of the Iberian Peninsula, where it was formerly very abundant and much appreciated as food (Alonso et al., 2000). But, as has happened throughout its original distribution area for the reasons indicated above, *A. pallipes* has virtually disappeared from the Iberian Peninsula over the past 30 years (Diéguez-Uribeondo et al., 1997). At present, sparse populations of *A. pallipes* are found in the Basque Country only in brooks or small springs and in the headwaters of certain streams and rivers (García-Arberas and Rallo, 2000). No outbreak of plague has been detected for at least 10 years.

In the 1980s the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), was introduced into many Iberian rivers and streams where native species had become extinct. At the time of these introductions, these systems were either without decapod fauna or inhabited by the exotic crayfish *Procambarus clarkii* (Girard, 1852), the 'red swamp crayfish', introduced in an uncontrolled manner from astaciculture farms. *P. leniusculus* was introduced into these waters devastated by aphanomycosis or depleted by pollution in an effort to restore a traditional fishing resource, to reduce the increase of red swamp crayfish populations and to control river vegetation, a role played by crayfish (Matthews et al., 1993; Nyström, 1999). As a result, breeding populations of signal crayfish are now present in many fluvial reaches.

Most studies of the habitat requirements for crayfish investigate lentic systems (lakes and ponds) and physical and structural variables (depth and substrate type, cover, riparian and aquatic vegetation, temperature, etc.) rather than the physico-chemical properties of water, with the exception of temperature, pH, conductivity, alkalinity, hardness, dissolved oxygen, CO_3^{-2} and Ca^{+2} thresholds (Laurent, 1988; Eversole and Foltz, 1993; Foster, 1995; Light et al., 1995). Data on concentrations of major cations and anions in European streams inhabited by native and introduced species have also been published (Lilley et al., 1979; Arrignon and Roché, 1983; Lachat and Laurent, 1987; Arrignon et al., 1993; Guan and Wiles, 1996; Smith et al., 1996; Troschel, 1997), though in some cases they are contradictory. For example, *A. pallipes* seems to be sensitive to both organic and chemical pollution (Hobbs and Hall, 1974), but it is also found in highly organic-enriched waters (Laurent, 1988).

In this paper, we document the presence or absence of sympatric and allopatric populations of *A. pallipes* and *P. leniusculus* in all river reaches of the province of Biscay where presence might be deemed likely on the basis of previous knowledge and adequate habitat. We then characterize and compare the water chemistry of these reaches in order to assess the relationships between water chemistry and crayfish distribution. The main aim of this work is to test whether, at present and in the fluvial reaches selected, chemical factors affect and mainly explain the distribution of native and signal crayfishes. If this expectation is confirmed, potential applications for management can be proposed.

2. Material and methods

2.1. Study area

The general conditions of the hydrographic basins of the Basque Country have already been described (Docampo et al., 1991; Rallo, 1992). The streams are short, (distance to source is 26.5 ± 8.0 km, range 3.7 to 35.6), with relatively steep slopes (0.9 ± 0.3%, range 0.4 to 1.7) and fluctuating flow rates, and with maximum levels in spring and autumn (mean annual flow = 2.7 ± 3.8 $m^3.s^{-1}$, range 0.6 to 15.4).

The geology of the drainage basin is primarily alternating layers of limestone, marl and sandstone bedrock, mainly from the Cretaceous period (Weald and Urgonian series). There are also salt and gypsum diapiric sites in some upper catchment areas; the rivers draining these zones have higher conductivity values in their upper reaches than downstream.

We selected 50 fluvial reaches in Biscay, Basque Country, Spain (Fig. 1). These were visited for at least three consecutive years. Sites were selected on the basis that they were presently or had recently been inhabited by *A. pallipes*, the native crayfish, and they appeared to be an adequate habitat for crayfish. 'Adequate habitat' was taken to mean not very fast running waters or water with sheltered zones (but in lotic systems), stones and sandy/muddy substrate, stepped channel banks and presence of riparian shrubs or trees (*Alnus glutinosa*, *Salix* sp.) with roots extending into the water (as defined by Hogger, 1988; Smith et al., 1996). No evidence of significant pollution was detected, either visually or in previous studies (Gobierno Vasco, 1995; Orive and Rallo, 1997). All selected reaches met trout or barbel habitat requirements; no special abundance of predators (trout, otter) was detected.

This study was carried out during the summer and early autumn months (July to October) over 6 years (1993-1998), and a total of 155 observations were made.

2.2. Habitat characterization

The fluvial reaches studied all met the general conditions required as adequate habitat for crayfish described above, and had both riffles and pools. In the riffles, the bottom substrate was covered by coarse sand, gravel, cobbles,

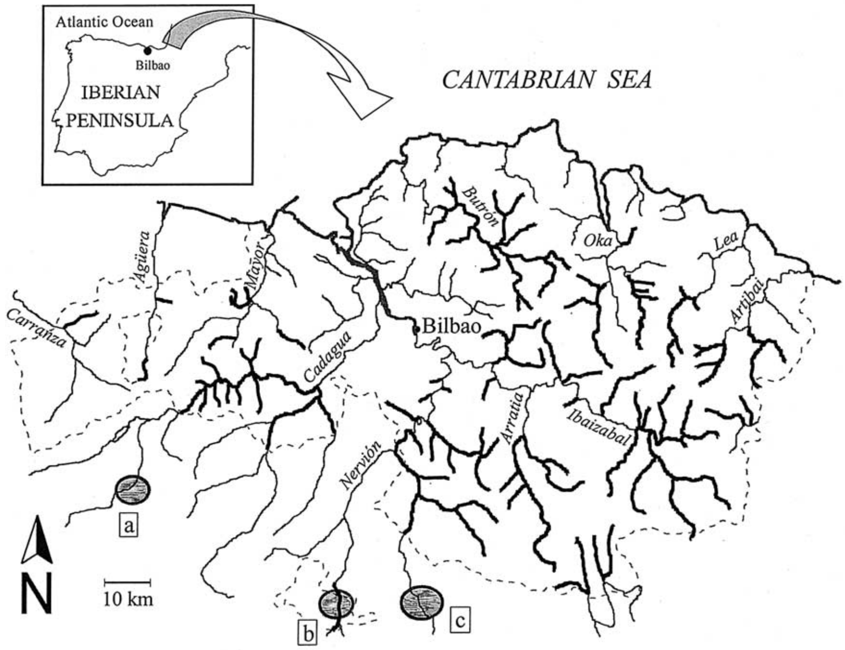

Fig. 1. The study area. Bold lines show the part of the river studied, the dashed line is the border of Biscay. Main diapiric locations: **(a)** Villasana de Mena (gypsum and limestone); **(b)** Orduña (clay, gypsum and rock salt); and **(c)** Altube (gypsum, marl and clay).

pebbles and some prominent ends of bedrock in similar proportions. There was sand and mud in pools, too. At each sampling, substrate and vegetation, mean water depth and flow were recorded to verify that they continued to fulfill the required conditions.

The most important factors for habitat characterization are the following: wetted river-bed width: median 11 m (range 1–34 m), river depth: median 21 cm (range 5–60 cm) and instant flow: median 1.1 m^3.s^{-1} (range 0.02 to 7.1 m^3.s^{-1}). Biological quality of rivers was measured by the Iberian Biological Monitoring Working Party (IBMWP) biotic index, which was adequate for our streams (Rico et al., 1992). The IBMWP classed the water quality as very good, good or slightly organic polluted (average value = 82.6 ± 29.3, range 40 to 157).

Water temperature, pH, conductivity and dissolved oxygen concentration were measured at each sampling site. We measured pH with a WTW pHmeter (model 320) following a two-part standardisation with pH 7 and pH 10 buffers. Conductivity was determined with a WTW conductivimeter (model LF320) with temperature compensated for to provide values as specific conductances at 25 °C. Dissolved oxygen was also measured by a WTW model 320 oxymeter. Temperatures were measured at a depth of approximately 0.2 m using the conductivimeter electrode. Samples of water were taken in plastic bottles (1 l capacity; two samples per site) and frozen to –30 °C before analysis by a certified laboratory using standard methods (APHA-AWWA-WPCF, 1998). Hardness, Cl$^-$, SO4^{-2}, Na$^+$, K$^+$, Mg^{+2}, SiO$_2$, PO$_4^{-3}$ Ca^{+2}, NO$_3^-$, NO$_2^-$, NH$_4^+$ and suspended solids were measured.

2.3. *Presence of crayfish*

Depending on the river conditions, crayfish presence was tested for using two methods. In small rivers and in marginal regions with shallow, clear waters, crayfish were observed and captured by hand at night while the riverbed was lit with a head lamp. Searches were limited to 30 minutes and the length of the fluvial reach covered was recorded. At larger, deeper sites, a set of 25 traps (cylinders 40 cm long × 25 cm diameter; with an 8 × 8 cm door) were used. They were baited with ox heart and deployed at dusk. The traps were collected the following morning. Crayfish cannot escape from these traps.

In all cases, a minimum stream length of 400 m was observed. If no animals were collected, the length of stream observed was increased. We made sure there were no crayfish in the reach by further inspections and by questioning local people and river guards. In those places where they were collected, crayfish were present as measurable and self-sustaining populations (Rallo et al., 1998; García-Arberas and Rallo, 2000).

2.4. *Statistical analyses*

Results were tabulated in a two-dimensional matrix where rows represent the 155 observations defined by reach and year (‘cases’), and columns (‘variables’) are the descriptors: presence/absence for each of the two species of crayfish and physical and chemical data. General statistical methods were used (Sokal and Rohlf, 1981). The skewness and kurtosis of the descriptive statistics were used to test for normal distribution of the quantitative variables. Before analysis, variables were normalized as required. No transformation was required for pH, temperature, conductivity and HCO_3^-; logarithmic transformation was used for hardness, Cl^-, $SO4^{-2}$, Na^+, Mg^{+2}, SiO_2, NH_4 and PO_4^{+3}; the square root transformation was required for Ca^{+2}, NO_3^- and K^+. No normalization was possible for NO_2^-.

Analysis of variance or the Kruskal-Wallis test and the Box-and-Whisker procedure were used to test for differences among quantitative variable values classified by the absence/presence of crayfish species. Exploratory multivariate analyses (principal components and cluster) were used as alternative and supplementary methods to define relationships between variables, in order to obtain, if possible, new complex, integrating and independent variables to be tested for differences between the classes of cases. Principal components analysis was performed on the correlation matrix and loadings were not rotated; the factors extracted were kept if they explained more variance than would be expected by chance alone under the broken-stick model (Jolliffe, 1986; Rohlf, 1997). Linear combinations of variables to predict the presence/absence of crayfish were derived by discriminant analysis. A method of stepwise, forward selection of variables was used to select and combine the most significant variables, so that correlation effects were minimized (Sokal and Rohlf, 1981; Manly, 1994). All data analyses were performed using NTSYS-PC vers. 2.0 (Rohlf, 1997) and Statgraphics Plus for Windows, vers. 4.1 (Manugistics Inc., 1997).

3. Results

3.1. Physical and chemical conditions of water

The water from the reaches of rivers studied in Biscay showed a natural hardness of 166 ± 79 $mgCaCO_3\ l^{-1}$ (*n* = 155; range = 26–390) and a relatively alkaline pH of 8.2 ± 0.3 (*n* = 155; range = 7.3–8.9), as expected given the nature of the sedimentary terrain. The dominant ions were HCO_3^-, SO_4^{-2}, Ca^{+2} and Cl^- (Fig. 2).

An exploratory classification of waters based on all water chemistry variables, temperature and suspended solids shows that they are grouped in two major clusters (Fig. 3): The first comprises dissolved minerals or 'mineralization', with the subgroups: SO_4^{-2} and Mg^{+2} (related to diapiric sites with gypsum), total suspended solids and HCO_3^- associated with conductivity, and the rest of the dissolved major ions (Ca^{+2} – with the dependent variable hardness –, Cl^- and Na^+). The second comprises dissolved nutrients, also with two subgroups: NO_3^-, PO_4^{+3}, SiO_2 and K^+, and the reduced compounds of nitrogen (NO_2^-, NH_4^+) with temperature. These groups are used to consider mineralization and nutrients as derived habitat dimensions for crayfish, as defined below.

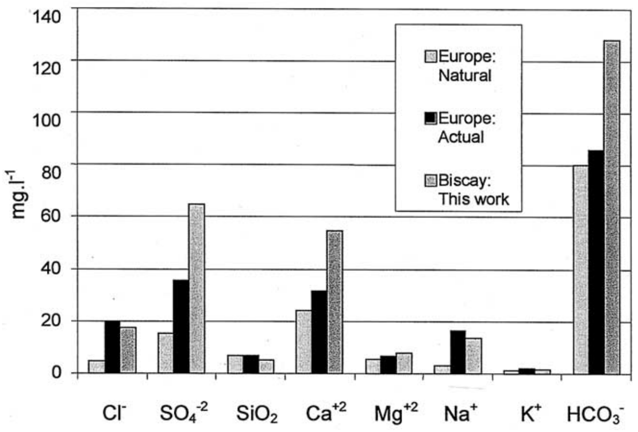

Fig. 2. Mean values of major ion concentrations in European river waters: natural and actual figures are from Berner and Berner (1987); those from Biscay are average concentrations measured in the present work.

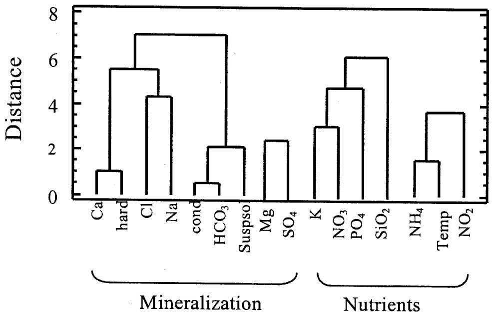

Fig. 3. Dendrogram representing similarity between variables. Procedure: standardized values, Euclidean distance, agglomeration by Ward's method.

The environmental data were reduced to subsets of variables using principal component analysis. Only the first four components are considered here, as eigenvalues after component 4 are less than 1. The first component or first axis (PCA1) explains 45% of the total sample variance (Table 1). Mg^{+2}, hardness, Ca^{+2}, Na^+, Cl^-, conductivity, SO_4^{-2} and HCO_3^- were the biggest contributors to this axis. This first PC axis represents water mineralization. The weights of nutrients and pH are shared between the two following axes, whose partial contributions to the total variance are similar. The second axis (11% of variance explained) is related to PO_4^{-3}, NO_3^- and K^+; the third (10%) represents NH_4^+ versus SiO_2 and NO_3^- (in part). The fourth (7%) is related to K^+ and temperature. These four PC scores are used as derived variables under the names PCA1, PCA2, PCA3 and PCA4 in the following analyses of the results.

3.2. Presence of crayfish

Crayfish were found in 112 of the 155 samples. *Austropotamobius pallipes* was present in 29 cases (11 reaches) and the introduced *Pacifastacus leniusculus* in 83 (27 reaches). In all the years of the study, only one species occurred in any given reach. The data can therefore be classed into one of these three cases: no crayfish (case 0),

Table 1
Eigenvalues, percentage of variance explained, cumulative percentage and weights of each normalized variable for each principal component extracted in the principal components analysis. Significant relationships between components and variables are indicated: $n = 94$; * $P \leq 0.01$; ** $P \leq 0.001$

Component	1	2	3	4
Eigenvalue	7.26	1.77	1.60	1.17
%Var.Expl.	45.4	11.0	10.0	7.3
Cum. %	45.4	56.0	66.4	73.1
Conductivity	*0.314	0.050	0.080	–0.014
sqrt Ca^{+2}	**0.332	–0.051	–0.157	0.191
log Cl^-	**0.327	–0.083	–0.174	0.043
log Hardness	**0.337	–0.053	–0.123	0.172
HCO_3^-	*0.283	0.125	–0.056	0.013
sqrt K^+	0.098	**0.349	*–0.316	**–0.543
log Mg^{+2}	**0.341	–0.091	0.022	0.085
log Na^+	*0.328	–0.195	–0.139	–0.050
log NH_4^+	0.096	0.028	**0.621	–0.237
NO_2^-	0.237	–0.251	0.218	–0.153
sqrt NO_3^-	0.154	**0.425	**–0.351	–0.180
pH	0.085	**0.411	0.082	**0.430
log PO_4^{+3}	0.053	**–0.496	–0.127	*–0.265
log SiO_2	–0.180	**–0.333	**–0.377	–0.088
log SO_4^{-2}	*0.311	–0.097	0.139	0.115
Temperature	0.166	0.148	0.246	**–0.489

A. pallipes (case 1) or signal crayfish (case 2). These last two can be classed as ‘presence’ (case 1 + 2).

3.3. Relationship between water chemistry and crayfish presence

The mean, minimum and maximum values for the variables in each of the three cases mentioned above can be used to define a large part of the abiotic factors of the habitat dimensions of the realized ecological niche of crayfish in the rivers of Biscay (Fig. 4 and Table 2). The size and shape of the resulting polygons are comparable. Areas represent total mineralization (left column, including variables grouped by the cluster analysis) and nutrient status of habitat (right column, variables included as defined in cluster analysis). Thus, considering the areas of the polygons, *P. leniusculus* inhabits 2.5 times more mineralized waters than the native species *(A. pallipes)*, but appears to be associated with low concentrations of K^+. The general conditions of the sites without crayfish are intermediate. *A. pallipes* occurred at sites characterized by low concentrations of SO_4^{-2} and relatively softer waters. However, K^+ concentrations were greater. The distributions of both crayfish species occurred at low NO_2^- concentrations. The concentrations were very low for the native species, suggesting that *A. pallipes* is especially sensitive to NO_2^-. *A. pallipes* was also found in reaches with low PO_4^{-3} concentrations.

The question arises as to what variables distinguish sites with or without crayfish. This has been investigated by comparing the values of the variables for each case. Only Mg^{+2} and SO_4^{+2} separated cases without (case 0) or with crayfish, regardless of species (case 1 + 2; Fig. 5). The mean concentration of Mg^{+2} was 5.6 ± 2.5 mg.l^{-1} (samples where crayfish were not captured) and 8.6 ± 5.2 mg.l^{-1} (the rest of samples, either with native or introduced species) (Kruskal-Wallis test=11.19; P=0.0008). In the case of SO_4^{+2}, the values are 40.2 ± 38.7 mg.l^{-1} (without crayfish) and 74.3 ± 65.5 mg.l^{-1} (with crayfish) (K-W test=11.1636; P=0.0008).

No statistically significant differences at $P \leq 0.01$ were found between cases without crayfish and cases with the presence of native or signal crayfish for conductivity, temperature, pH, HCO_3^-, K^+, NH_4^+, NO_3^- or PCA2, PCA3 and PCA4. This suggests that the ranges of pH, temperature, HCO_3^- and nutrients found in this study are favourable for crayfish. For the remaining variables, one or two (and in the case of SO_4^{-2} all) paired comparisons were significantly different (Table 2). Mean values are in general higher and standard deviations wider in the reaches where *P. leniusculus* is found, except for SiO_2 , NO_2^- and PO_4^{-3}, whose minima are found in the reaches with the native *A. pallipes* (SiO_2) or without crayfish. The PCA1 axis represents mineralization: reaches inhabited by introduced crayfish have more highly mineralized water.

The discriminant functions extracted from all variables according to whether a species was present or not point to five variables as significant predictors of cases: SO_4^{-2}, PO_4^{-3} , Mg^{+2}, NH_4^+ and SiO_2. Two discriminant functions were defined (P-values≤0.01):

First discriminant function:

$$- 0.63 \log(Mg^{+2}) - 0.40 \log(NH_4^+) + 0.60 \log(PO_4^{+3}) - 0.39 \log(SiO_2) + 1.2 \log(SO_4^{-2})$$

Second discriminant function:

$$1.02 \log(Mg^{+2}) + 0.70 \log(NH_4^+) - 0.47 \log(PO_4^{+3}) - 0.10 \log(SiO_2) - 0.56 \log(SO_4^{-2})$$

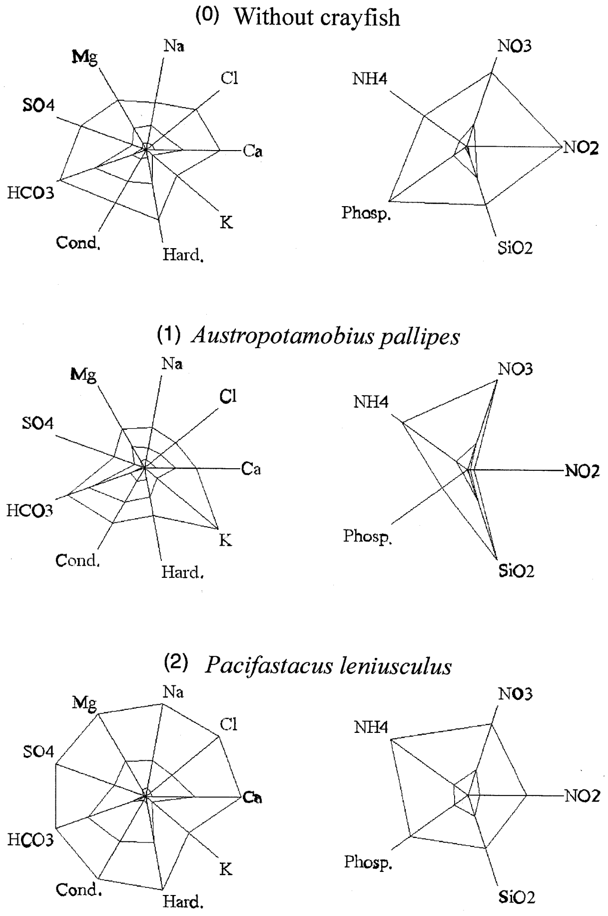

Fig. 4. Representation of dimensions of variables in groups of samples: (0) without crayfish (empty niche); (1) realized niche of *Austropotamobius pallipes*; (2) realized niche of *Pacifastacus leniusculus*. Left column: mineralization variables; right column, nutrient variables. Inside and outside polygons and radius of each spectrum are related to the minimum and maximum values measured for each variable and case (each axis corresponds to the total range variation obtained in this work). Relations between areas (without crayfish: native crayfish: signal crayfish); Mineralization 1:0.8:2.0, Nutrients 1:0.5:0.9.

Table 2
Values of chemical variables (mean ± standard deviation), that differ significantly from fluvial reaches without crayfish (0), with *A. pallipes* (1) or with *P. leniusculus* (2). Values are in mg.l^{-1}, except the first principal component (PCA1), without dimensions

Variable	Case	Value	KW-test	P
Ca^{+2}	0	48.43 ± 21.01	20.826	0.00003
	1	40.00 ± 13.17		
	2	63.07 ± 26.09		
Hardness	0	143.46 ± 59.85	21.159	0.00003
	1	124.85 ± 39.68		
	2	192.59 ± 83.22		
Mg^{+2}	0	5.59 ± 2.52	19.849	0.00005
	1	7.01 ± 2.34		
	2	9.50 ± 1.59		
Na^{+}	0	10.80 ± 4.61	17.942	0.00013
	1	9.37 ± 2.26		
	2	16.32 ± 9.68		
NO_2^{-}	0	0.061 ± 0.118	17.751	0.00014
	1	0.016 ± 0.000		
	2	0.031 ± 0.009		
PO_4^{-3}	0	0.186 ± 0.291	12.621	0.00182
	1	0.072 ± 0.091		
	2	0.175 ± 0.219		
SiO_2	0	5.56 ± 2.29	16.197	0.0003
	1	6.38 ± 3.66		
	2	4.16 ± 2.33		
SO_4^{-2}	0	40.23 ± 38.72	34.279	0.0000
	1	28.97 ± 18.09		
	2	90.11 ± 70.21		
PCA1	0	–0.879 ± 1.936	20.791	0.00003
	1	–1.088 ± 1.800		
	2	0.835 ± 2.632		

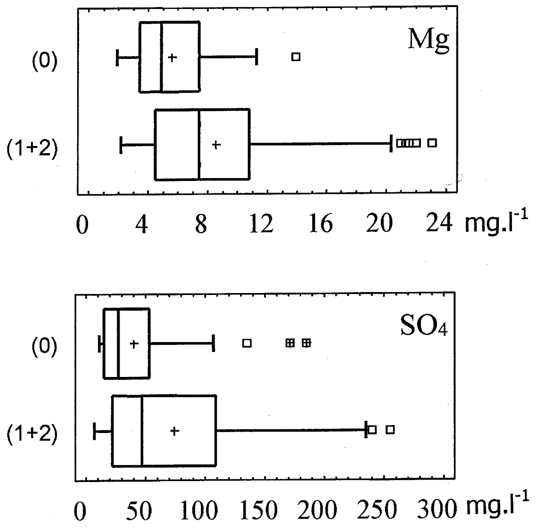

Fig. 5. Plots of values of variables that show statistically significant differences (Kruskal-Wallis; $P<0.001$) within samples without (0) or with crayfish (1+2: either native or signal species). Magnesium (Mg^{+2}) and sulphate (SO_4^{-2}) in mg.l^{-1}.

The first discriminant function separates cases into two partly overlapping groups: one with *A. pallipes* and the other with *P. leniusculus* (Fig. 6). Cases without crayfish are not separated along this axis. The relative magnitude of coefficients in the equation can be used to estimate the weight of each variable in discriminating between native and signal groups. SO_4^{-2} is the most important discriminating variable, followed by Mg^{+2}, PO_4^{+3} and, finally, NH_4^{+} and SiO_2. The second function separates samples with and without crayfish, and is related to Mg^{+2} concentration, followed by NH_4^{+}.

These functions classify 62% of the samples correctly. Ratios of misclassification vary from case to case (Table 3). The cases are best classified for presence of *A. pallipes* (65.5% were correctly classified, 27.6% of cases with native crayfish were predicted as empty and 6.9% were predicted as being inhabited by signal crayfish). Almost 26% of the

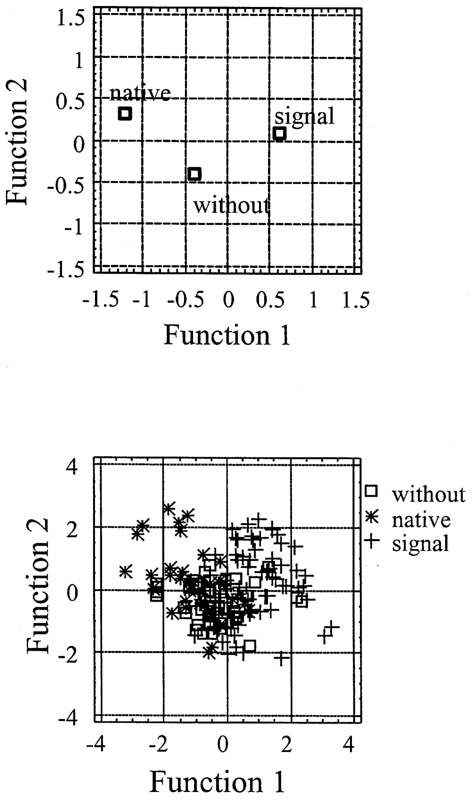

Fig. 6. Discriminant functions and cases with absence or presence of different species of crayfish: above, centroids of each cluster, below, ordered samples.

samples without crayfish were predicted as being inhabited by *A. pallipes*.

4. Discussion

Faunal distributions are the result of interactions between two types of factor: ecological factors (the degree to which a species' niche requirements are fulfilled, considering both abiotic and biotic aspects) and historical factors (whether the species has been able to colonise an area characterized by an acceptable niche either naturally or through human action). In our case, we accept that historical reasons are not influencing crayfish distribution in the streams of Biscay, because native crayfish *A. pallipes* are known to have occupied all the reaches studied in the recent past. Where the crayfish niche became vacant and signal crayfish were introduced more than 12 years ago, breeding populations have had time to establish themselves wherever their niche requirements are met (Rallo et al., 1998). Thus, we believe that crayfish distribution in Biscay depends upon ecological, non-historical factors.

Many studies in lakes have indicated that substratum is the most important variable related to the presence (and also to total abundance) of crayfish (Flint and Goldman, 1977; Kirjvainen and Westman, 1999). Both signal crayfish and native crayfish avoid flat, soft bottoms but can be found (in different degrees of abundance) everywhere when the substratum offers rocks, stones, pebbles, submerged plants, etc. (Guan and Wiles, 1996). This is the case in our fluvial reaches. Thus, physical river conditions in our sampling reaches, such as substratum, vegetation, hydrodynamism, etc., are adequate for crayfish life. Furthermore there are no pests, and the presence of larger predators is balanced and not excessive. Therefore our hypothesis is that water chemistry is the main reason for the absence or presence of one or another species of crayfish.

The figures for chemical and physical variables of water found in this study are within the usual ranges recorded throughout Europe (Berner and Berner, 1987), but with slightly higher average concentrations of HCO_3^-, SO_4^{-2} and associated cations (Fig. 2). Fluvial water variables show values within or near the ranges cited as natural for similar streams with geological substrates dominated by calcareous rocks and influenced by oceanic aerosols but naturally modified because of diapiric salt intrusions (Meybeck, 1986; Meybeck and Helmer, 1989; Chapman, 1996). As in all rivers in the zone (Orive et al., 1989; Rallo, 1992), overall analyses separate mineralization and nutrient components.

Relationships between water chemistry and freshwater fauna are well known for aquatic vertebrates, but not for invertebrates (Light et al., 1995). As fishes and amphibians (Marco et al., 1999), crayfish seem to be sensitive to nitrite concentration (as stated previously by Liu et al., 1995 and

Table 3
Classification of samples by the discriminant functions model in comparison with the real conditions (see text). Cases: (0) without crayfish, (1) with native crayfish and (2) with signal crayfish

		case 0	case 1	case 2
	n samples	43	29	83
Predicted as:	case 0	24 (55.8%)	8 (27.6%)	24 (28.9%)
	case 1	11 (25.6%)	19 (65.5%)	6 (7.2%)
	case 2	8 (18.6%)	2 (6.9%)	53 (63.9%)
Total	correctly classified	24 (55.8%)	19 (65.5%)	53 (63.9%)
	incorrectly classified	19 (44.2%)	10 (34.5%)	30 (36.1%)

Rouse et al., 1995), but although it is generally known that high nitrite concentrations are lethal to aquatic fauna, experimentation would be require to probe this relationship more accurately.

Earlier studies of water chemistry and crayfish fauna have been mostly concerned with lakes and ponds. Referring to the protected species *A. pallipes*, our results are within the ranges cited, although the concentrations of some nutrients are greater (Table 4). The conductivity level is the highest found, but this variable does not seem to be a limiting factor for *A. pallipes* life. This species has been found in ponds with water well above 400 µS.cm^{-1} (Bohl, 1997) and living at more than 1 700 µS.cm^{-1} in a diapiric hole (Rallo and García-Arberas, 2000).

Our results suggest that Mg^{+2} is an important factor associated with crayfish presence in the rivers of Biscay. The mean Mg^{+2} concentration is one of the lowest recorded in the published literature (Table 4). We postulate that we are near the limit of the species' requirements. Ca^{+2} and Mg^{+2} compounds are essential components of the crayfish integument and are required for successful growth and moulting (Jusilla et al., 1995).

Water chemistry conditions in rivers depend on natural conditions (especially geological substrate) that cannot be modified, but in Europe human activities currently play the major role (Gibbs, 1970; Chapman, 1996). Our results show associations between chemical variables that could be caused by runoffs from the basin and nearby land; this was studied especially for the Cadagua river (Rallo et al., 2001). In the present work it is shown that there are several ions with different concentrations in the reaches inhabited by one or other species, or without crayfish. *Pacifastacus leniusculus* appears to be much more tolerant than *A. pallipes* to these chemical fluctuations.

In terms of management, it is very important to find what habitat conditions are most suited to native species and most hinder the establishment of the introduced species. When 'adequate habitat' conditions are accomplished (see epigraph 2.1), the proposed model based on the chemistry of the water allows crayfish presence in our rivers to be predicted from only a few parameters. Sixty-two percent of the cases fit the discriminant functions extracted. The remaining 38% must be explained otherwise, by historical reasons (unsuccessful introductions or reintroductions) or ecological factors (other unknown habitat conditions, hydrology, etc.).

The river reaches where no crayfish were found but which were classified by discriminant analysis as suitable

Table 4
Values of chemical variables from rivers where the native crayfish *Austropotamobius pallipes* were found (average, maximum and minimum values: average, maximum, minimum). Hardness (hard) is measured as $mgCaCO_3$ l^{-1}. Units are mg.l^{-1} except for conductivity (in µS.cm^{-1} at 20 °C) and pH

References		Cond.	Hard	Cl^-	SO_4^{-2}	Ca^{+2}	Mg^{+2}	Na^+	K^+	HCO_3^-	NO_3^-	NH_4^+	PO_4^{+3}	pH
Lilley et al., 1979	aver													
n=?	max	390				67	12.3	12.1	3.9					
	min	60				6	1.8	7.0	0.8					
Arrignon et al., 1983	aver	160		8	2	40	8.0			146	0.6	0	0	7.6
n=1	max													
	min													
Arrignon et al., 1993	aver	335	200	18	7	66	8.5			230	0.6	0	0	8.4
n=3	max	385	215	24	8	78	11.5			240	0.8	0	0	8.4
	min	310	170	14	6	52	5.0			216	0.5	0	0	8.4
Jussila et al., 1995	aver					3	0.9							6.6
n=?	max													
**	min													
Smith et al., 1996	aver													
n=62	max	643		23	45	95	21.7	26.7	6.5		4.2	0.32	0.59	
	min	93		8	8	8	3.0	5.7	0.8		0.6	0.05	0.22	
Troschel, 1997 (Kiba)	aver	267		33							2.6	0.03	0.03	7.6
n=24	max	345		60							3.5	0.04	0.20	7.9
	min	225		10							0.5	<0.01	<0.001	7.0
Troschel, 1997 (Lawa)	aver	277		33							3.0	0.03	0.008	7.2
n=25	max	288		50							5.0	0.07	0.008	7.4
	min	268		20							0.05	<0.001	<0.001	6.8
This work (native)	aver	376	125	13	29	40	6.0	9.4	1.7	128	2.7	0.12	0.07	8.1
n=29	max	607	204	25	87	66	11.4	17.5	12.2	178	9.1	0.73	0.39	8.6
	min	144	47	5	7	15	2.3	4.3	<0.01	32	<0.01	0.005	<0.01	7.6
This work (total)	aver	441	166	18	65	55	7.8	13.5	1.4	128	2.6	0.13	0.17	8.2
n=155	max	891	390	59	255	123	23.0	40.5	12.2	207	9.1	0.86	1.17	8.9
	min	42	26	4	7	8	1.6	3.4	<0.001	29	<0.001	<0.001	<0.01	7.3

** lake or pond.

for one or the other species should be surveyed more intensively in search of unknown factors. Provided that physical habitat conditions allow crayfish survival, reintroduction of the native species might be attempted successfully there. *A. pallipes* has been reintroduced since 1998, and we have very recently recorded successful, promising results. Furthermore, the habitat conditions at six sampling sites now occupied by signal crayfish seem to be adequate for *A. pallipes*. Reintroduction of native crayfish in such sites should be preceded by an attempt to eradicate the introduced species (Holdich et al., 1999).

It is noteworthy that both species have not been found anywhere inhabiting the same river reaches, as reported in some other cases: mixed populations were present for almost 20 years in a lake in England. Coexistence ended when the native species (*A. pallipes*) was eliminated as result of interspecific competition (Holdich et al., 1995). Similar outcomes have been recorded with different native species in America (Lodge et al., 1986) and also in Europe (Söderbäck, 1991, Söderbäck, 1995). All the studies cited involve populations inhabiting lakes or ponds. In our streams, there may have been some species displacements before this study began, but in 5 years we have observed no displacement of boundaries between populations. We have found that a few crayfish populations have disappeared, and major changes in water chemistry have been detected (Rallo et al., 2001).

Looking to the future, we think it unlikely that *A. pallipes* will ever be restored to its former range or abundance because our rivers will not be the same. Even the maintenance of the population in its present locations is a difficult task. The alien crayfish is certainly a very major threat to the native one (Taugbøl and Skudal, 1999). Protection of the remaining populations of the native *A. pallipes* crayfish should be a top management priority. Understanding the habitat requirements of crayfish will enable us to determine suitable areas for reintroducing the native species and allow the strictly-delimited reserves of the alien signal crayfish to be managed to meet fishery demands.

5. Conclusion

The present distribution patterns of the native crayfish (*Austropotamobius pallipes)* and the introduced signal species (*Pacifastacus leniusculus*) in the reaches of the streams of Biscay that offer adequate general habitat conditions depend mainly on water chemistry. Neither species inhabits waters with high NO_2^- concentrations; it appears both species are sensitive to nitrite. Mg^{+2} and NH_4^+, and SO_4^{-2} and Mg^{+2} concentrations seem to be the most important factors separating sites with and without crayfish and sites with one or the other species, respectively. The discriminant model may be used to classify fluvial reaches as to their adequacy for crayfish habitation.

Acknowledgements

This study was supported by the *Departamento de Agricultura (Sección Pesca) de la Exma. Diputación de Bizkaia*. We are especially indebted to E. Alvarez, former Head of this Section, and to the river guard (gamekeepers) of Biscay. Also we are grateful to Alvaro Antón, a predoctoral student in our Department.

References

Alonso, F., Temiño, C., Diéguez-Uribeondo, J., 2000. Status of the white-clawed crayfish, *Autropotamobius pallipes* (Lereboullet, 1858) in Spain: distribution and legislation. Bull. Fr. Pêche Piscic. 356, 31–54.

APHA-AWWA-WPCF, 1998. Standard methods for the examination of water and wastewater. 20th ed. In: Clesceri, L.S., Greenberg, A.E., Eaton, A.D (Eds.), Washington DC.

Arrignon, J.C.V., Roché, B., 1983. Population of the crayfish *Austropotamobius pallipes pallipes* Lereb. in a brook of Corsica, France. Freshwater Crayfish 5, 229–238.

Arrignon, J.C.V., Lamy, G., Roché, B., 1993. Situation d'une population corse d' *Austropotamobius pallipes pallipes* Lereb. Publ. Assoc. Dev. Aquac. 35, 89–98.

Berner, E.K., Berner, R.A., 1987. The global water cycle. Prentice-Hall, Englewood, New Jersey.

Bohl, E., 1997. An isolated population of the white-clawed crayfish (*Austropotamobius pallipes)* in the principality of Liechtenstein. Bull. Fr. Pêche Piscic. 347, 701–712.

Chapman, D., 1996. Water quality assessments. 2nded. UNESCO, WHO, UNEP. E and FN Spon. Chapman & Hall, London.

Diéguez-Uribeondo, J., Temiño, C., Muzquiz, J.L., 1997. The crayfish plague fungus (*Aphanomyces astaci*) in Spain. Bull. Fr. Pêche Piscic. 347, 753–763.

Docampo, L., Rico, E., Sevillano, M., Bikuña, B.G., Rallo, A., 1991. Morphology and quantitative analysis of fluvial erosion systems in the hydrological network of the Basque Country autonomous regions. J. Environ. Manage. 32, 251–266.

Eversole, A.G., Foltz, J.W., 1993. Habitat relationships of two crayfish species in a mountain stream. Freshwater Crayfish 9, 300–310.

Flint, R.W., Goldman, C.R., 1977. Crayfish growth in Lake Tahoe: Effects of habitat variation. J. Fish Res. Board Can. 34, 155–159.

Foster, J., 1995. Factors influencing the distribution and abundance of the crayfish *Austropotamobius pallipes* (Lereboullet) in Wales and the Marches, UK. Freshwater Crayfish 8, 78–98.

García-Arberas, L., Rallo, A., 2000. Survival of natural populations of *Austropotamobius pallipes* in rivers in Biscay, Basque Country (North of Iberian Peninsula). Bull. Fr. Pêche Piscic. 356, 17–30.

Gibbs, R.J., 1970. Mechanisms controlling world water chemistry. Science 170, 1088–1090.

Granjean, F., Harris, D.J., Southy-Grosset, C., Crandall, K.A., 2000. Systematics of the European endangered crayfish species *Austropotamobius pallipes* (Decapoda: Astacidae. J. Crust. Biol. 20, 524–531.

Guan, R., Wiles, P.R., 1996. Growth, density and biomass of crayfish *Pacifastacus leniusculus,* in a British lowland river. Aquat. Living Resour. 9, 265–272.

Gobierno Vasco, 1995. Red de Vigilancia de la Calidad de las Aguas y del estado ambiental de los Ríos de la CAPV. Dpto. Urbanismo, Vivienda y Medio Ambiente. Servicio de Publicaciones, Vitoria.

Hobbs, H.H., Hall, E.T., 1974. Crayfishes (Decapoda: Astacidae). In: Hart, C.W., Fuller, S.L.H (Eds.), Pollution ecology of freshwater invertebrates. Academic Press, New York.

Hogger, J.B., 1988. Ecology, population biology and behaviour. In: Holdich, D.M., Lowery, R.S (Eds.), Freshwater crayfish. Biology, management and exploitation. Croom Helm, London, pp. 114–144.

Holdich, D.M., Gydemo, R., Rogers, W.D., 1999. A review of possible methods for controlling nuisance populations of alien crayfish. In: Gherardi, F., Holdich, D.M (Eds.), Crayfish in Europe as alien species. Balkema, Rotterdam, pp. 245–270.

Holdich, D.M., Rogers, W.D., Reader, J.P., Harliouglu, M., 1995. Interactions between three species of freshwater crayfish (*Austropotamobius pallipes, Astacus leptodactylus* and *Pacifastacus leniusculus*. Freshwater Crayfish 10, 46–56.

Jolliffe, I.T., 1986. Principal component analysis. Springer Verlag, New York.

Jussila, J., Henttonen, P., Huner, J.V., 1995. Calcium, magnesium and manganese content of the noble crayfish *Astacus astacus* (L.) branchial carapace and its relationships to water and sediment mineral contents of two ponds and one lake in central Finland. Freshwater Crayfish 10, 230–238.

Kirjavainen, J., Westman, K., 1999. Natural history and development of the introduced signal crayfish, *Pacifastacus leniusculus*, in a small, isolated Finnish lake, from 1968 to 1993. Aquat. Living Resour. 12, 387–401.

Lachat, G., Laurent, P.J., 1987. The habitats of *Astacus astacus* L. and *Austropotamobius pallipes* Lereboullet in the Morvan. Freshwater Crayfish 7, 61–68.

Laurent, P.J., 1988. *Austropotamobius pallipes* and *A. torrentium*, with observations on their interaction with other species in Europa. In: Holdich, D.M., Lowery, R.S (Eds.), Freshwater crayfish. Biology, management and exploitation. Croom Helm, London, pp. 341–364.

Light, T., Erman, D.C., Myrick, C., Clarke, J., 1995. Decline of the Shasta crayfish (*Pacifastacus fortis* Faxon) of Northeastern California. Conserv. Biol. 9, 1567–1577.

Lilley, A.J., Brooker, M.P., Edwards, R.W., 1979. The distribution of the crayfish *Austropotamobius pallipes* (Lereboullet) in the upper Wye catchment, Wales. Nat. Wales 16, 195–200.

Liu, H., Avault, J.W., Medley, P., 1995. Toxicity of ammonia and nitrite to juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens). Freshwater Crayfish 10, 249–255.

Lodge, D.M., Hill, A.M., 1994. Factors governing species composition, population size and productivity of cool-water crayfishes. Nord. J. Freshwater Res. 69, 111–136.

Lodge, D.M., Kratz, T.K., Capelli, G.M., 1986. Long-term dynamics of three crayfish species in Trout Lake, Wisconsin. Can. J. Fish. Aquat. Sci. 50, 1279–1288.

Lowery, R.S., Holdich, D.M., 1988. *Pacifastacus leniusculus* in North America and Europe, with details of the distribution and introduced and native crayfish in Europe. In: Holdich, D.M., Lowery, R.S (Eds.), Freshwater crayfish. Biology, management and exploitation. Croom Helm, London, pp. 283–326.

Manly, B.F.J., 1994. Multivariate statistical methods. 2nd ed. Chapman & Hall, London.

Manugistics, Inc., 1997. Statgraphics Plus for Windows (vers 4.1). Rockville, MD, USA.

Marco, A., Quilchano, C., Blaustein, A.R., 1999. Sensitivity to nitrate and nitrite in pond-breeding amphibians from the Pacific northwest. USA. Environ. Toxicol. Chem. 18, 2836–2839.

Matthews, M.A., Reynolds, J.D., Keatinge, M.J., 1993. Macrophyte reduction and benthic community alteration by crayfish *Austropotamobius pallipes* (Lereboullet). Freshwater Crayfish 9, 289–299.

Meybeck, M., 1986. Composition chimique naturelle des ruisseaux non pollués en France. Sci. Géol. Bull. Strasbourg 39, 3–77.

Meybeck, M., Helmer, R., 1989. The quality of rivers: from pristine stage to global pollution. Palaeogeogr. Palaeocl. Palaeoecol. 75, 283–309.

Nyström, P., 1999. Ecological impact of introduced and native crayfish on freshwater communities: European perspective. In: Gherardi, F., Holdich, D.M (Eds.), Crayfish in Europe as alien species. Balkema, Rotterdam, pp. 63–86.

Orive, E., Basaguren, A., Bikuña, B.G., Cacho, M., 1989. A comparative study of water mineralization and nutrient status in the main water courses of Biscay (Basque Country. Water Res. 23, 705–710.

Orive, E., Rallo, A., 1997. Bizkaiko Ibaiak/Ríos de Bizkaia. Instituto de Estudios Territoriales. Diputación de Bizkaia, Bilbao, pp. 258.

Rallo, A., 1992. Caracterización hidrobiológica de la red fluvial de Alava y Gipuzkoa. Gobierno Vasco, Dpto. Medio Ambiente, Vitoria.

Rallo, A., García-Arberas, L., 2000. Population structure and dynamics and habitat conditions of the native crayfish *Austropotamobius pallipes* in a pond: a case study in Basque Country (Northern Iberian peninsula. Bull. Fr. Pêche Piscic. 356, 5–16.

Rallo, A., García-Arberas, L., Antón, A., 1998. Caracterización de la red hidrográfica de Bizkaia por su aptitud como hábitat para las especies nativa e introducidas de cangrejos fluviales. Informe Técnico. Diputación de Bizkaia, Bilbao.

Rallo, A., García-Arberas, L., Antón, A., 2001. Relationships between changes in habitat conditions and population density of an introduced population of signal crayfish (*Pacifastacus leniusculus*) in a fluvial system. Bull. Fr. Pêche Piscic. 361, 643–657.

Rico, E., Rallo, A., Sevillano, M., Arretxe, M.L., 1992. Comparison of several biological indices based on river macroinvertebrate benthic community for assessment of running water quality. Ann. Limnol.-Int. J. Limnol. 28, 147–156.

Rohlf, F.J., 1997. NTSYSpc. Numerical taxonomy and multivariate analysis system ver.2.0. Exeter Software, New York.

Rouse, D.B., Kastner, R.J., Reddy, K.S., 1995. Toxicity of ammonia and nitrite to hatching redclaw crayfish, *Cherax quadricarinatus*. Freshwater Crayfish 10, 298–303.

Smith, G.R.T., Learner, M.A., Slater, F.M., Foster, J., 1996. Habitat features important for the conservation of the native crayfish *Austropotamobius pallipes* in Britain. Biol. Conserv. 75, 239–246.

Söderbäck, B., 1991. Interspecific dominance relationships and aggressive interactions in the freshwater crayfish *Astacus astacus* (L.) and *Pacifastacus leniusculus* (Dana). Can. J. Zool. 69, 1321–1325.

Söderbäck, B., 1995. Replacement of the native crayfish *Astacus astacus* by the introduced species *Pacifastacus leniusculus* in a Swedish lake: possible causes and mechanism. Freshwater Biol. 33, 291–304.

Sokal, R.R., Rohlf, F.J., 1981. Biometry. 2nd ed. Freeman and Co., New York.

Taugbøl, T., Skurdal, J., 1999. The future of the native crayfish in Europe: How to make the best of a bad situation? In: Gherardi, F., Holdich, D.M (Eds.), Crayfish in Europe as alien species. A.A. Balkema, Rotterdam, pp. 271–294.

Troschel, H.J., 1997. Distribution and ecology of *Austropotamobius pallipes* in Germany. Bull. Fr. Pêche Piscic. 347, 639–648.

Westman, K., 1985. Effects of habitat modifications on freshwater crayfish. In: Alabaster, J.S (Ed.), Habitat modification and freshwater fisheries. London, Butterwoths. EIFAC Symposium.