SURVIVAL OF NATURAL POPULATIONS OF *AUSTROPOTAMOBIUS PALLIPES* IN RIVERS IN BIZKAIA, BASQUE COUNTRY (NORTH OF IBERIAN PENINSULA).

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Reçu le 27 octobre 1997 *Received 27 October, 1997*
Accepté le 29 décembre 1998 *Accepted 29 December, 1998*

ABSTRACT

Some relict populations of the native crayfish *Austropotamobius pallipes* have been located in rivers in Bizkaia, (Basque Country, Spain), and its population numbers and dynamics, and habitat conditions have been studied for three years. The first descriptive results are given in this paper. The native crayfish populations must be considered residual because of the disrupted area distribution and highly fluctuating demography of the species.

Up to now, the species has been located in more than thirty fluvial areas of relatively high slope and shallow and good quality water. Population characteristics (sex ratio, length and weight relations and length frequency classes) are studied in nineteen cases. Maximal relative population numbers are about 100 captures per hour ; these values are correlated to variables of conductivity, hardness, and concentrations of nitrates, nitrites, magnesium, potassium and ammonium. The degree of mineralization must reach a minimum level and, within the values found in the studied rivers, its increase favours the population of crayfish.

Management measures to conserve native crayfish must include the protection and improvement of their habitat, prevention of access to it and to the commencement of a genetic study to palliate the phenomenon of endemism. It would also be highly recommendable to begin experimental restocking of riverbeds now without crayfish fauna and with apparently optimum conditions for the establishment of populations of autochthonous crayfish.

Key-words : crayfish, native species, population, rivers, ecology, Spain.

SURVIVANCE DES POPULATIONS D'*AUSTROPOTAMOBIUS PALLIPES* DANS LES RIVIÈRES DU PAYS BASQUE (NORD DE LA PÉNINSULE IBÉRIQUE).

RÉSUMÉ

Un certain nombre de populations d'écrevisses autochtones d'*Austropotamobius pallipes* a été localisé dans des rivières de Biscaye (Pays Basque, Espagne), et l'on s'est livré, durant trois ans, à une étude portant aussi bien sur leurs dynamiques de peuplement que sur leurs conditions d'habitat. Cet article en est une première étude descriptive. Les populations d'écrevisses autochtones doivent être considérées comme résiduelles en raison d'une distribution très éclatée dans la zone dans laquelle on les rencontre, ainsi que de leur démographie qui est des plus fluctuantes.

A ce jour, les espèces ont été localisées dans une trentaine de rivières présentant une pente et une profondeur relativement importantes, ayant une eau d'assez bonne qualité. Les caractéristiques de population (sexe-ratio, rapports de longueur et poids et classes de fréquence de longueur) sont étudiées dans dix-neuf cas. Les nombres relatifs de population tournent au maximum légèrement au dessus de 100 captures par jour ; les valeurs sont en corrélation avec les variables de conductivité, dureté et concentrations de nitrates, nitrites, magnésium, potassium et ammonium. De ce fait, le degré de minéralisation doit atteindre un niveau minimum et, à l'intérieur des valeurs constatées pour les rivières étudiées, son accroissement favorise la population d'écrevisses.

Les mesures administratives destinées à la conservation de l'écrevisse autochtone doivent inclure la protection et la sauvegarde de l'habitat, l'accès en étant restreint et une étude génétique étant initiée, visant à pallier le phénomène de l'endémisme. Il est vivement recommandé par ailleurs de commencer des expériences de repeuplements de rivières dont la faune écrevisse est absente et présentant des conditions apparemment optimales pour l'établissement de populations d'écrevisses autochtones.

Mots-clés : écrevisse, espèces autochtones, population, rivière, écologie, Espagne.

INTRODUCTION AND OBJECTIVES

Austropotamobius pallipes is a native European species, protected by EC, which played a very important ecological role in fluvial systems, not only in the trophic chain but also in the ecosystem equilibrium (MOMOT, 1995). It is observed as a general fact that when crayfish disappeared, river vegetation increased so much that in some cases dystrophic situations arose. Besides this, an important recreational recourse is lost : crayfish dishes are greatly appreciated in our country.

Some relicted populations of the native crayfish of *Austropotamobius pallipes* have been located in rivers in Bizkaia, Basque Country, Spain, where it was thought that the species had been rendered extinct by aphanomycosis and by habitat deterioration in a short period on 1978-80.

So, our work aims are :

1. to find out where native populations are now living in Bizkaia ;
2. to characterize the habitat conditions of these fluvial zones ;
3. to evaluate crayfish stocks and conditions ;
4. to work out the relationships between habitat and population conditions.

METHODS

Rivers where native crayfish lived before their catastrophic disappearance, and probably live at present (a hypothesis based on an earlier survey of river gamekeepers and on habitat conditions) were visited in late spring and early summer (June to early August) in 1994, 95 and 96. Six rivers were specially chosen in order to perform an intensive study. In these sites fluvial conditions were measured : river slope, width and depth, banks and vegetation, substrate, temperature, pH, conductivity and chemicals dissolved in water. The biotic index (BMWP) (ALBA-TERCEDOR and SÁNCHEZ-ORTEGA, 1988 ; RICO *et al.*, 1992) was also calculated to evaluate the biological habitat quality. Standard apparatus and methods were used. Throughout the text data are given as average ± standard deviation (s.d.). For security reasons in order to protect the endangered species, the geographical rivers names are omitted and labels are given as River 1 (R1), River 2 (R2), *etc.* when required.

Crayfish were caught by hand, at night, at definite times and on specific river section lengths, so that population numbers could be referred to effort rates (captures per hour). Each specimen was measured (length and weight), its sex recorded and then put back into the river. The body length of the crayfish was measured to the nearest 1 mm in two ways : total length (TL), from the tip of the rostrum to the end of the telson, and cephalotorax length (CL), from the tip of the rostrum to the end of the carapace. Crayfish were also weighted with a dynamometer to the nearest 1 g.

The data matrix (samples, physical and chemical conditions, population numbers and associated values : sex, length and weight measures) was analysed by statistical methods (descriptive statistics, multivariate analysis, *etc.*) using computer programs (StatGraphics Plus, NTSYS ; ROHLF, 1992). Growth rates are estimated by the comparison of the average total length of the size classes determined by BHATTACHARYA's method (BHATTACHARYA, 1967).

RESULTS AND DISCUSSION

Presence

During the three years of the study (1994-1996), 90 chosen rives or different fluvial sections were visited (Figure 1). We found native crayfish in 32 places (36.0 % of total visited), and no crayfish (neither native, nor alien species) in the rest. In some of them the population disappeared while the study was going on. Sites were sampled annually in the six rivers included in the intensive study, so that population numbers and condition temporal changes could be recorded. In other rivers, when populations seemed to be relatively large (above 10 individuals captured per hour), sites were also sampled annually.

Habitat conditions

All the fluvial zones where native crayfish are now living are small rivers located upstream. The average widths of the wet riverbed are low : for the intensive study width is 1.6 ± 0.6 m, and the depth never passes 20 cm ; the average slope is 8.8 ± 4.8 %. The most frequent substrate is predominantly stony or bare rock, but with patches of sand and even mud. The waters are alkaline, (pH 8.2 ± 0.25), with conductivity and hardness of 372 ± 127 µSiemens/cm and 13.4 ± 3.6 French Degrees, respectively. Temperatures recorded when visiting the sites varied between 16 and 24 ºC, with an average of 17.8 ± 2.1 ºC. The principal chemical components showed the following concentrations ; the high values of standard deviations proved that water composition (especially potassium and ammonium) are very variable among rivers :

Sulphate	30.8	±	13.7	mg/l
Chloride	14.3	±	5.4	mg/l
Bicarbonate	140.22	±	30.29	mg/l
Calcium	42.02	±	12.23	mg/l
Magnesium	6.84	±	2.53	mg/l

Potassium	2.44	±	2.96	mg/l
Sodium	10.34	±	3.53	mg/l
Nitrate	3.12	±	2.52	mg/l
Nitrite	0.02	±	0.01	mg/l
Ammonium	0.17	±	0.19	mg/l

Biotic indices (131.4 ± 41.6) indicate that the running water is of very good, good or medium quality (in this last case because of organic charge, for the most part).

All these values present normal distribution, except for temperature, potassium and ammonium concentrations, which were normalised when required. All these values are within the ranges cited in other papers published on this matter (ARRIGNON and ROCHÉ, 1983 ; LILLEY *et al.*, 1979 ; SMITH *et al.*, 1996) excepting ammonium, which is much higher in our case.

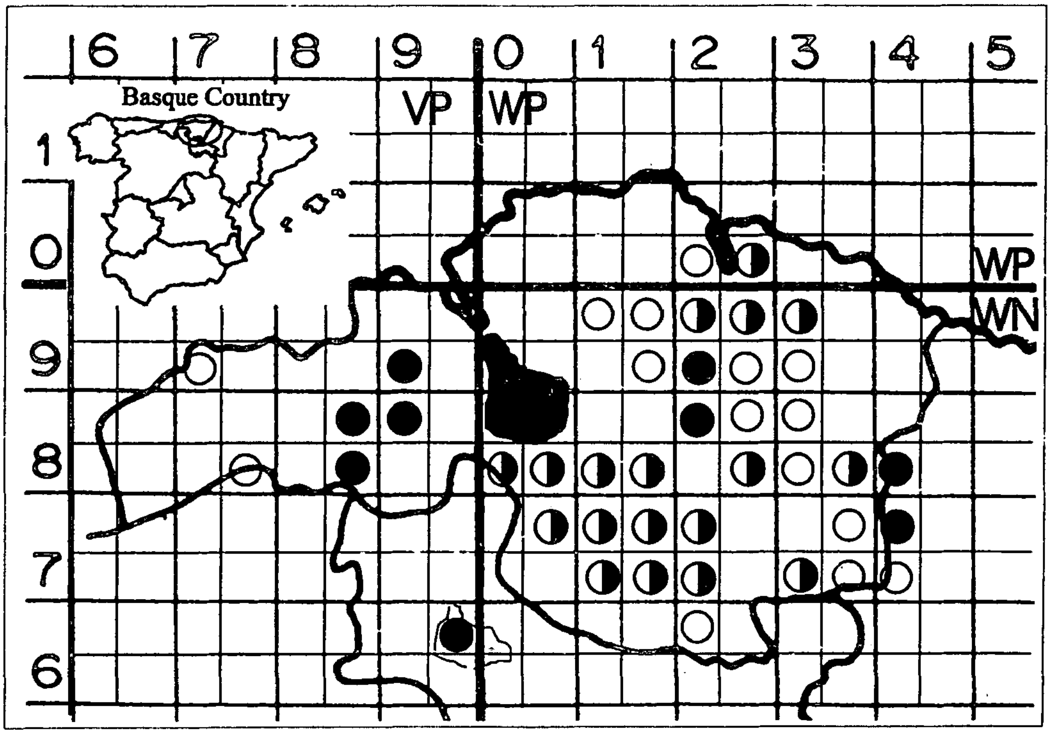

Figure 1

Geographical distribution of *Austropotamobius pallipes* in Bizkaia : present situation. Sites in UTM. Black circles : presence ; empty circles : absence. Half black : either places with and without in the same UTM square or disappearance over time.

Figure 1

Distribution géographique d'*Austropotamobius pallipes* en Biscaye : situation actuelle. Sites en UTM. Cercles noirs : présence ; cercles vides : absence. Demi-noirs : à la fois endroits avec et sans dans le même carré UTM ou disparition au cours du temps.

The relationships between samples (from different places or dates and physical and chemical variables of habitat) were determined through multivariate analysis. The similarities between river points are studied by cluster analysis (Figure 2), that allows the identification of groups of similar objects. Three cases (R6_96, R1_96 and R5_96) are separated from all the others, that are grouped first by spatial conditions (group A = river R1 except R1_96 ; B = river R2 ; C = river R4 ; D = river R3, and E = two rivers, R6 and R5, from the same basin, except samples from 1996), and then by time (the most similar are cases from 94 and 95).

In order to look deeper into the similarity of cases due to habitat conditions (and not only to time and space factors) data were put through a principal components analysis, so that cases are spatially ordered in relation to principal axes defined by linear combinations of habitat conditions. The first three principal axes extracted (Table I) proved to be significant and explained 78 % of the total variance of the sample. The first one associates bicarbonate and calcium concentrations and total solids on the one hand (negative axis) with ammonium and magnesium on the other (positive), and explains the separation of R1_96 and R5_96 from all the other cases. The second component is related to conductivity, hardness and concentration of chloride, that takes away R6_96, and the third one to organic load measured as QDO. Those cases were also clearly separated by cluster analysis : these are the reasons.

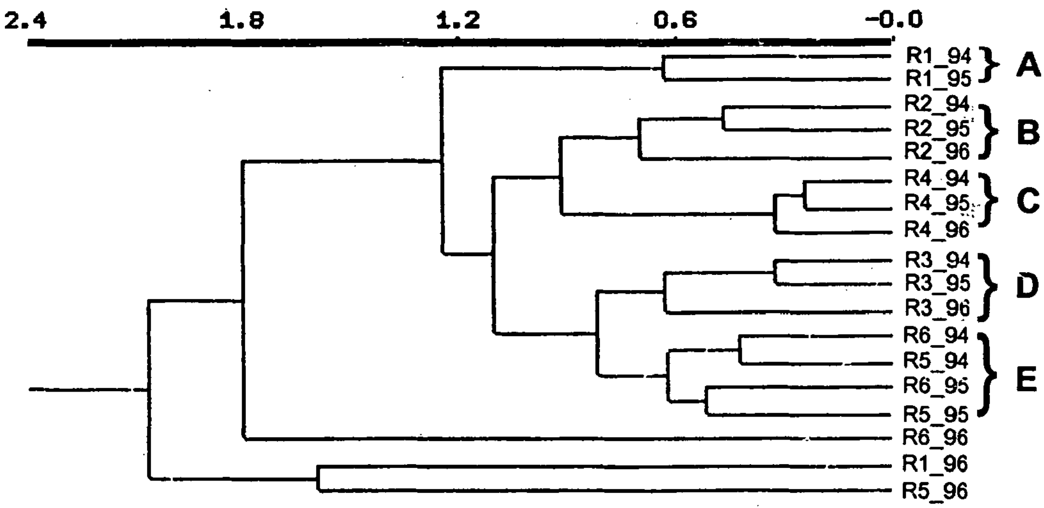

Figure 2

Dendrogram of cases based on habitat conditions. Six sites ; three years are represented. (coeff. = r de PEARSON ; agglomerative algorithm : UPGMA ; fit of results to original data : correlation of matrices r = 0.8893, MANTEL t-test = 6.962).

Figure 2

Dendrogramme des cas basés sur les conditions d'habitat. Six sites ; trois années sont représentées. (coeff. = r de PEARSON ; algorithme agglomératif : UPGMA ; ajustement des résultats aux données d'origine : corrélation de matrices r = 0.8893, MANTEL t-test = 6.962).

Crayfish stocks and relation to habitat conditions

The evaluation of relative population numbers measured by catch effort (Figure 3) is represented only for the nineteen cases where populations seemed to be relatively high (rivers of the intensive study are marked by an asterisk). Population sizes are not large : the maximum reached was over 100 capture/hour, but this quantity must be considered important taking into account the preceding remarks about the size of the rivers or brooks, where native crayfish populations are found. Also this stock is large when compared to other evaluations : the numbers mentioned by ALMAÇA (1989) in Portugal are between 22 and 74 specimen/hour and those found by SMITH *et al.* (1996) in Southern Britain, 8-31 specimen/hour (as average).

Table I

Principal component analysis : significance of axes and weight of the variables.

Tableau I

Analyse des composantes principales : signification des axes et des variables de poids.

Principal component	Eigenvalue	Percent	Cumulative percent	Expected	Axis significance
1	6.984119	34.9206	34.9206	17.9887	yes
2	5.564068	27.8203	62.7409	12.9887	yes
3	3.055915	15.2796	78.0205	10.4887	yes
4	1.758120	8.7906	86.8111	8.8220	not

Principal component axis :	1	2	3
Variables			
Temperature	0.077	0.042	0.693
pH	0.205	0.242	0.667
O_2	0.545	-0.115	0.663
QDO	0.113	0.527	-0.880
Conductivity	-0.022	0.955	0.210
Hardness	0.057	0.952	0.109
Chloride	-0.221	0.909	0.149
Sulphate	-0.451	0.601	0.212
Nitrate	0.700	0.453	0.089
Nitrite	0.735	0.161	0.040
Phosphate	-0.332	0.547	-0.488
Silicate.	-0.457	-0.207	-0.485
Calcium	-0.847	0.412	0.206
Magnesium	0.864	0.417	-0.153
Sodium	-0.540	0.670	-0.112
Potassium	0.557	0.793	-0.157
Bicarbonate	-0.925	0.168	0.235
Ammonium	0.910	0.332	-0.148
Total solids	-0.869	0.267	0.300
Biotic Index	0.717	0.048	0.203

The relative population numbers of crayfish have proved to be positively correlated (to a significance level of 95 % ; r of PEARSON ; n = 18) with the following variables : conductivity, hardness and concentrations of nitrates, nitrites, magnesium, potassium and ammonium. All these facts confirm that the degree of mineralization must reach a minimal level and, within the values found in the rivers studied, its increase favours the population of crayfish. The water also has a medium degree of concentration in nutrients (measured by nitrogen compounds), that in our case, on account of the slope, does not cause the oxygen concentration to decline.

Nowadays, we are carrying out studies in order to detect possible differences between the abiotic conditions of groups of cases defined by the presence or absence of native or alien crayfishes.

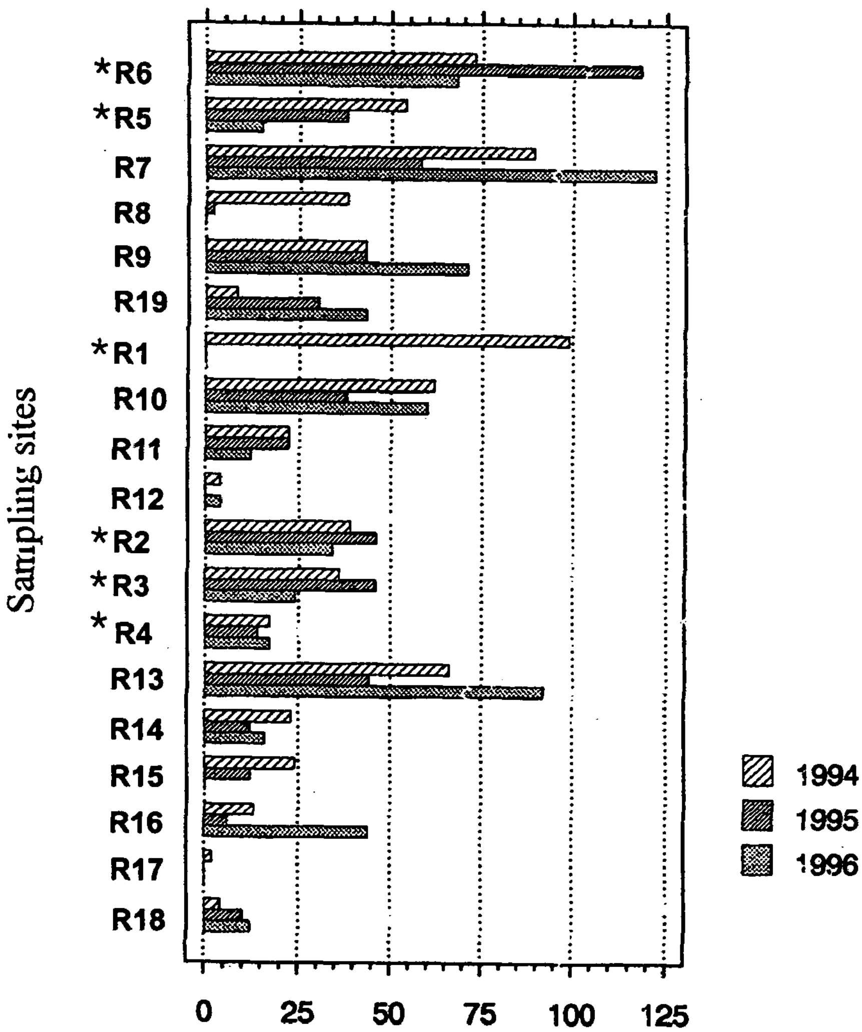

Figure 3
Relative population number estimated by catch's effort rates. Nineteen cases ; three years are represented.

Figure 3
Nombre de population relatif estimé par taux d'effort de captures. Dix-neuf cas ; trois années sont représentées.

Population structure : sexes, sizes and shapes

In most cases of studied crayfish populations there were more females than males so that the sexes proportions are not adjusted to the hypothesis 1 : 1 (Figure 4). By applying a χ^2 test to check this hypothesis, we found that the likelihood of its being true is 14.98 % for the overall data in 1994 (N, number of crayfish studied, = 696), and lower for 1995 and 1996 (0.38 %, in a sample of N = 276 and 4.77 %, N = 408, respectively), so the hypothesis must be rejected. This seems to reflect the actual situation of the population, the manual method of capture and the season given. This preponderance of females has also been detected in other populations (ARRIGNON *et al.*, 1988 ; ARRIGNON *et al.*, 1993 ; SMITH *et al.*, 1996). BERNARDO *et al.* (1997) found an average sex ratio slightly in favour of females, despite one of the two studied populations was sampled during the reproductive period when part of the females might stay in refuges, thus avoiding capture.

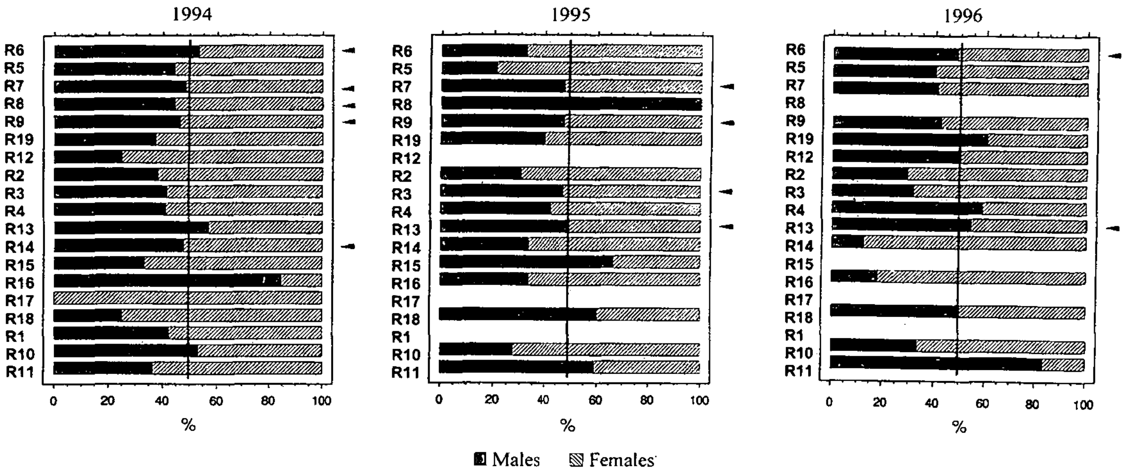

Figure 4
Sex ratios as percentages. Nineteen cases ; three years are represented.

Figure 4
Sexe ratio en pourcentages. Dix-neuf cas ; trois années sont représentées.

Body sizes of crayfish are studied as total length (TL) from rostrum to telson. Maximum TL found are 111 mm (male) and 116 mm (female), that are longer than those cited for other populations (ALMAÇA, 1989 ; ARRIGNON and ROCHÉ, 1983 ; BERNARDO *et al.*, 1997 ; TROSCHEL, 1997). The distribution of length and weight frequencies is different in males and females (analysis made using data from the three years : length χ^2 = 54.13, P = 0% ; weight χ^2 = 133.11, P = 0%). Males are about 1 g heavier than females (averages : W_m = 9.007 ± 0.351 g ; W_f = 8.004 ± 0.230), but average length is almost equal in both sexes (56.04 ± 17.98 mm in males ; 56.34 ± 17.56 mm in females). In this, our results differ from those by ALMAÇA (1987) who found females significantly longer than males.

The distribution of length frequencies (as TL, Figure 5) enables us to distinguish four size groups (Table II), which can be interpreted as age groups. The first (length 2.0 ± 1.1 cm), is that of age 0+, which is difficult to capture. Most crayfish in the river populations belong to size groups II and III (size : 4.1 ± 0.7 and 6.3 ± 0.8 cm ; ages 1+ and 2+, respectively), and a minority is four years old or more (class IV, age 4+, with average length 8.5 ± 0.8 cm). Size overlaps between year classes and the attribution of the biggest crayfish to an age class by size is uncertain (as LAURENT, 1988). Mean growth rate is practically constant, at a mean value 2.2 cm/year, in our case (Figure 6).

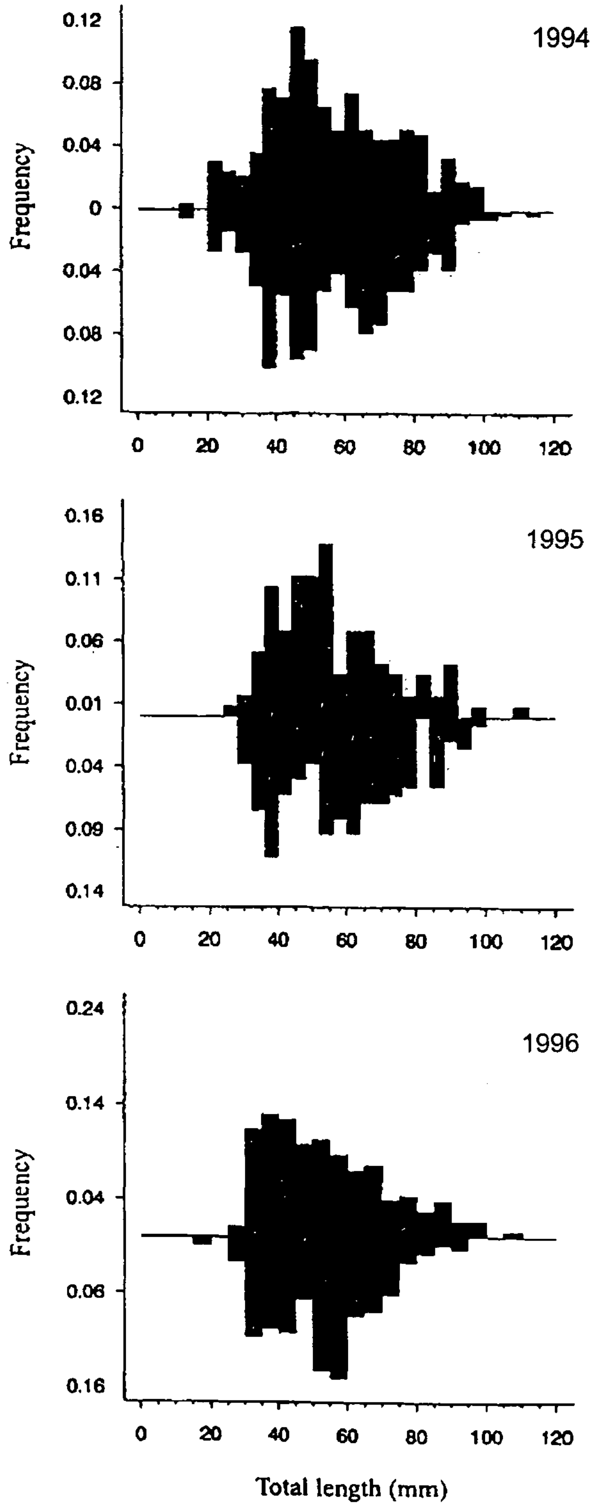

Figure 5

Frequency analysis of body lengths in three years. Above the axis : males ; below : females.

Figure 5

Analyse de fréquence des tailles du corps sur trois ans. Au-dessus de l'axe : les mâles ; en-dessous, les femelles.

Table II

Size groups determined by BATTACHARYA's method : average total-length, standard deviation, number and percentage of individuals belonging to each size group and separation index between groups (differences are significant when the index is greater than 2)

Tableau II

Groupes de taille déterminés par la méthode de BATTACHARYA : moyenne de la taille totale, écart standard, nombre et pourcentage d'individus appartenant à chaque groupe de taille et index de séparation entre les groupes (les différences sont significatives lorsque l'index est supérieur à 2)

	1994			
size classes	x ± ds	N	%	S.I.
I	2 ± 1.1	66	9.5	-
II	4.6 ± 0.8	341	49.1	2.72
III	7.4 ± 0.8	236	34.0	3.65
IV	8.8 ± 0.7	51	7.4	2.06

	1995			
size classes	x ± ds	N	%	S.I.
II	3.8 ± 0.5	88	32.1	-
III	5.9 ± 1.0	142	51.8	2.72
IV	8.3 ± 0.8	44	16.1	2.64

	1996			
size classes	x ± ds	N	%	S.I.
II	3.8 ± 0.5	164	40.6	-
III	5.6 ± 0.6	160	39.3	3.17
IV	8.5 ± 0.8	83	20.4	3.94

The shape of specimens has been studied by establishing morphometric relationships of the measures TL, cephalotorax length (CL) and weight. The relationship between total length (TL) and cephalotorax length (CL) is adjusted to the linear model TL = 2.86 + 2.03 x CL (r^2 = 98.97 ; number of cases = 1381 ; at 95 %). Testing TL by covariance analysis in order to extract the CL influence, significant differences in TL according to year and sex are detected (Figure 7). The following regression equations valid for cephalotorax lengths between 8 and 55 mm were thus obtained :

Year	**Males**			**Females**		
		r^2	N. cases		r^2	N. cases
1994	TL = 3.63 + 1.98 x CL	99.1	329	TL = 1.84 + 2.12 x CL	98.5	368
1995	TL = 3.27 + 1.99 x CL	98.7	114	TL = 1.08 + 2.14 x CL	98.6	160
1996	TL = 4.33 + 1.92 x CL	97.6	184	TL = 2.71 + 2.02 x CL	97.2	224

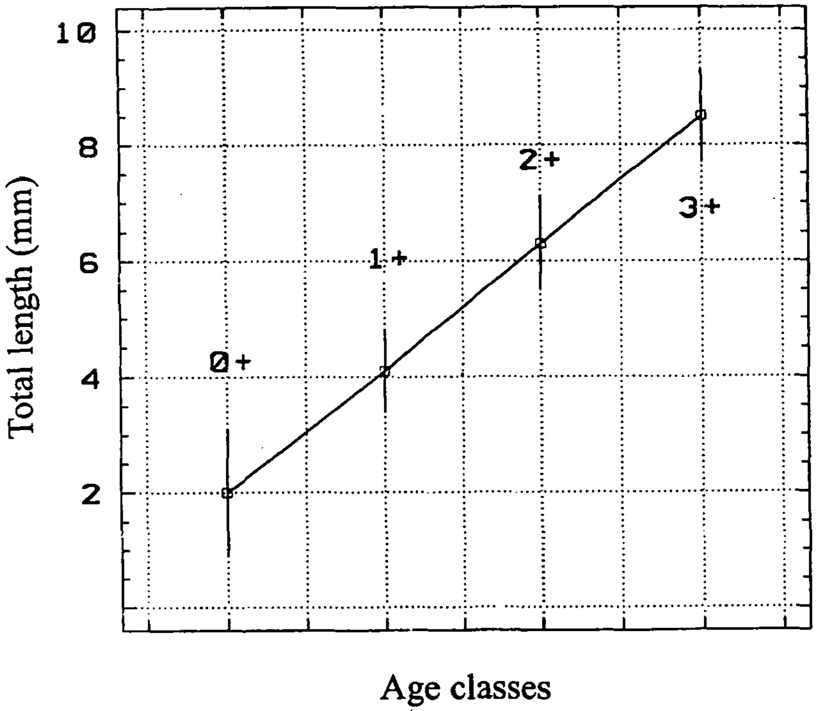

Figure 6
Growth estimates : evolution of the average length of size classes.

Figure 6
Estimation de croissance : évolution de la longueur moyenne des classes de taille.

In general terms, total length is about double of cephalotorax length (less in males, 1.93 ± 0.04 mm ; more in females, 2.09 ± 0.06 mm), plus a constant of 3.77 ± 0.56 in males and 1.86 ± 0.84 in females. These values differ significantly according to sexes (tested by Student's t-test at 95%). As showed by comparison of slopes, females TL referred to CL is greater, so that they have relatively longer abdomens. From 1994 to 1996, a trend of diminishing TL/TC might be detected for both sexes (Figure 7).

It is also interesting to examine the relationship between total length (mm) and weight (g), and whether it varies according to sex and year or not. As generally is in the case of animals, the relationship is : $W = 0.0002 \times TL^{2.592}$ (r^2 = 91.47 ; number of cases = 1,317). At a signification level of 95 %, weight differences not related with TL are detected between groups defined by sex, but not if classified by year (Figure 8) (ANCOVA analysis ; n = 1,317 cases). So, two regression models are proposed :

Males	r^2	N. cases	**Females**	r^2	N. cases
$W = 0.0001 \times TL^{2.761}$	92.7	604	$W = 0.0003 \times TL^{2.450}$	91.0	713

The TL influence extracted as covariate, weight differences in averages are a little greater than those above calculated : males weight is 9.156 ± 0.125 g and females 7.878 ± 0.115 g. That shows that, independently of size, males are heavier than females, surely because of carapace and chelipeds. The same is probed by the exponent being greater in males than in females.

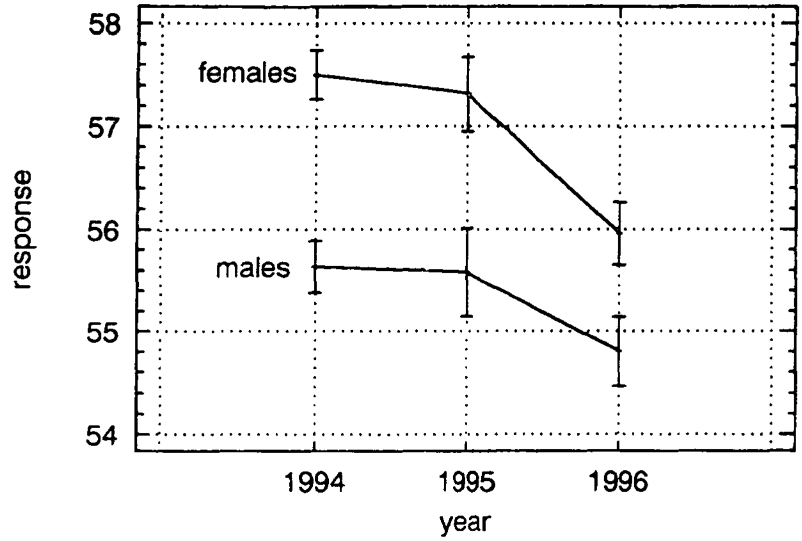

Figure 7
Differences in total length values in mm not explained by cephalotorax length variability (extracted by covariance analysis) according to sex and year.

Figure 7
Différences des valeurs de la longueur totale en mm qui ne sont pas exprimées par la variabilité de la longueur du céphalothorax (obtenues sur l'analyse de la covariance) selon le sexe et l'année.

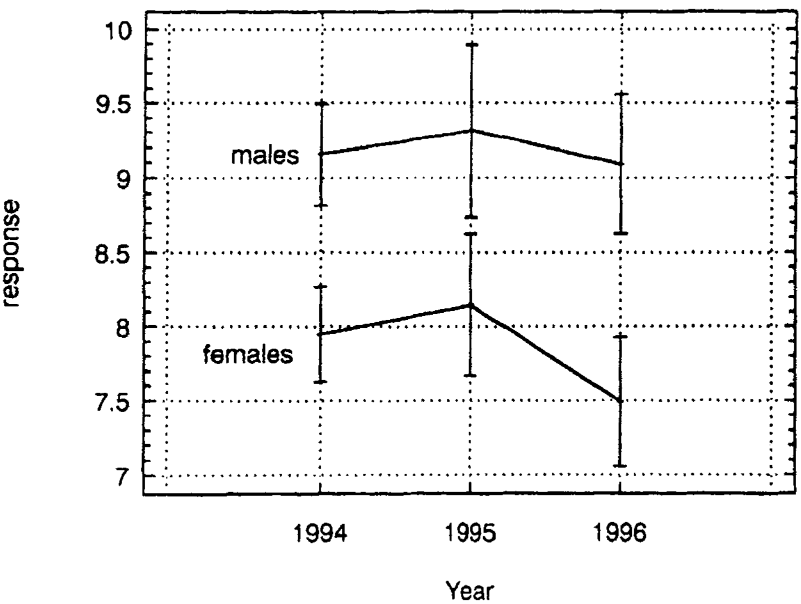

Figure 8
Differences in weight values in g not explained by total length variability (extracted by covariance analysis) according to sex and year.

Figure 8
Différences des valeurs du poids en g qui ne sont pas exprimées par la variabilité de la longueur totale (obtenues sur l'analyse de la covariance) selon le sexe et l'année.

Global conclusions and recommendations

In short, the data and commentaries above are a first description of the situation of most cases of the populations of native crayfish in Bizkaia´s rivers. It may be accepted that they are residual populations, with a highly fluctuating demography, reduced to a few of specific sites with a highly fragmented distribution, and that they are in danger of extinction. Their habitat is characterized by a certain degree of conductivity and nutrients concentration.

Management measures for native crayfish conservation must include the protection and improvement of their habitat, prevention of access to it and the beginning and carrying out a genetic study to palliate the possible phenomenon of endemism. It would also be highly recommendable to start with experimental restocking of riverbeds now without crayfish fauna and apparently with the optimum in conditions for the establishment of populations of autochthonous crayfish.

ACKNOWLEDGEMENTS

This study was supported by the Departamento de Agricultura (Sección Pesca), Exma. Diputación de Bizkaia. We are specially indebted to E. ALVAREZ, Head of this Section, and to the river guard (gamekeepers) of Bizkaia. We wish also to acknowledge the observations of the referees.

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