ESTIMATION OF NOBLE CRAYFISH (*ASTACUS ASTACUS* L.) POPULATION SIZE IN THE VELIKA PAKLENICA STREAM (CROATIA)

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Reçu le 6 janvier 2004 — *Received January 6, 2004*
Accepté le 14 juin 2004 — *Accepted June 14, 2004*

ABSTRACT

Astacus astacus is one of four native European crayfish species living in Croatian freshwaters. It is naturally distributed in rivers belonging to the Black Sea Basin. To some rivers of the Adriatic Sea Basin, however, it had been introduced by man. According to the information from local people it is most likely that *A. astacus* has been introduced into the Velika Paklenica Stream during the first half of the 20th century. Our study stream, draining the Velebit Mountain, is situated in Paklenica National Park, Middle Adriatic. The fieldwork of our study was performed during July 2000 and 2002, respectively. To estimate the population size we used mark-recapture techniques (Jolly-Seber Method, Schnabel Method and Schumacher and Eschmeyer Method). Crayfish were trapped with baited LiNi traps that were set along the stream banks at 10 m intervals and left there overnight. Caught animals were individually marked by piercing holes into the uropods and telson and then released. In July 2000 we caught 233 crayfish in total, from which 70 specimens were trapped more than once. In July 2002 we caught 296 crayfish, from which 86 were trapped more than once. Differences in the population size between the two years are discussed.

Key-words: *Astacus astacus*, estimation of population size, mark-recapture, Croatia.

ESTIMATION DE LA TAILLE DE LA POPULATION D'ÉCREVISSE À PIEDS ROUGES (*ASTACUS ASTACUS* L.) DANS LE FLEUVE VELIKA PAKLENICA (CROATIE)

RÉSUMÉ

Astacus astacus est une des quatre espèces autochtones d'écrevisses de Croatie. Elle est naturellement présente dans les fleuves du bassin de la Mer Noire, mais elle vit aussi dans les fleuves du bassin Adriatique où sa présence est d'origine anthropogène. Selon l'information obtenue auprès des autochtones, cette espèce a été introduite pendant la première moitié du XXe siècle. Le fleuve Velika Paklenica est situé dans le Parc National Paklenica, drainant les eaux de la montagne Velebit, Adriatique centrale. Notre recherche a été menée en juillet 2000 et en juillet 2002. Pour estimer la taille de la population nous avons utilisé la technique du marquage-recapture (la méthode Jolly-Seber, la méthode de Schnabel et la méthode de Schumacher et Eschmeyer). Les écrevisses ont été capturées avec des nasses LiNi, placées le long du bord du fleuve, à 10 m d'intervalles, et laissées en place pendant la nuit. Les écrevisses ont été marquées individuellement, avec des trous dans les uropodes et le telson, et relâchées ensuite. En juillet 2000 nous avons capturé

Article available at http://www.kmae-journal.org or http://dx.doi.org/10.1051/kmae:2004009

233 écrevisses, dont 70 spécimens ont été capturés plus d'une fois. En juillet 2002 nous avons capturé 296 écrevisses, dont 86 spécimens ont été capturés plus d'une fois. Nous analysons ici la différence entre les tailles des populations de ces deux années.

Mots-clés : *Astacus astacus*, estimation de la taille de la population, marquage-recapture, Croatie.

INTRODUCTION

Four native European crayfish species from the family Astacidae live in Croatia; the noble crayfish *Astacus astacus* (Linnaeus), the narrow-clawed crayfish *Astacus leptodactylus* (Eschscholtz), the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet) and the stone crayfish *Austropotamobius torrentium* (Schrank). Since *A. leptodactylus* and *A. pallipes* are highly structured taxa, the latest suggestion is to consider them as species complexes (HOLDICH, 2002). Stone crayfish, white-clawed and noble crayfish are protected by Croatian Law, Law of Nature Conservation (NARODNE NOVINE, 30/94) and Rule Book on Protection of Crayfish (Crustacea, Astacidae) (NARODNE NOVINE, 76/98).

Astacus astacus is distributed in freshwater habitats that belong to the Black Sea Basin, but it also occurs in some rivers belonging to the Adriatic Sea Basin (MAGUIRE, 2002; MAGUIRE and GOTTSTEIN-MATOČEC, 2004). According to the information from local inhabitants it is most likely that a noble crayfish population had been introduced into the Velika Paklenica Stream during the first half of the 20th century (MAGUIRE *et al*., 2002).

The purpose of this study was to analyse crayfish size and to estimate population abundance of *A. astacus* from the Velika Paklenica Stream by mark-recapture techniques in July 2000 and 2002, respectively.

MATERIAL AND METHODS

Velika Paklenica Stream is situated in the Paklenica National Park, on southern slopes of the Velebit Mountain (Figure 1). The source of this karstic stream is located at 1 200 m above sea level. It partly runs through a deciduous forest, dominated by hornbeam (*Carpinus betulus*) and Downy oak (*Quercus pubescens*) forest. The lower part of the stream usually dries out during the summer months.

For our study we have chosen a locality called Anića Luka, at 270 m above sea level. The stretch was approximately 100 m long with lots of shaded patches. The bottom consisted of stones and pebbles, with sand deposits and layers of leaf litter in places where the water current was slower. Average water temperature in the first study year (19-25 July) was 18ºC while in the second year (9-15 July) it was 22ºC, average pH was 8.05 and total hardness (expressed as $CaCO_3$) was 175 mg/l.

Animals were caught with 12 LiNi traps (WESTMAN *et al*., 1978). These baited traps were set along both banks of the stream in approximately 10 m intervals and were left there overnight. For each trapped crayfish we recorded sex, weight (using Pesola balance) and total length (using Vernier calliper). Then the animal was marked by piercing a hole in the telson or/and uropodes (GUAN, 1997). After marking, each crayfish was released into the stream at the same site where it had been caught.

The fieldwork of our study was performed for six consecutive nights in July 2000 and for six consecutive nights in July 2002. To estimate the population size we used mark-recapture techniques (Jolly-Seber Method, Schnabel Method and Schumacher and Eschmeyer Method) summarized by KREBS (1989). The total length and weight of caught

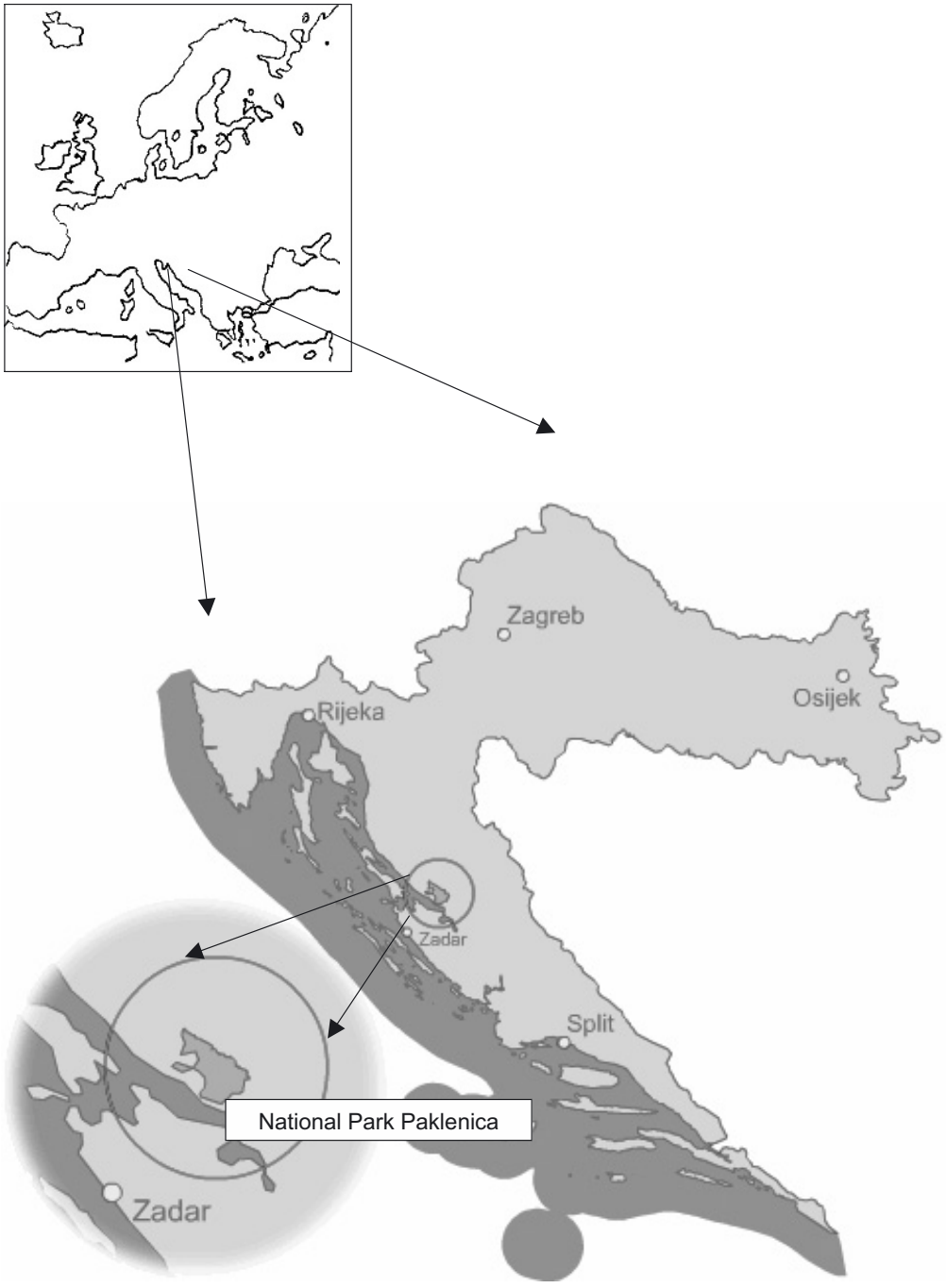

Figure 1
Position of the National Park Paklenica in Croatia.

Figure 1
Localisation du Parc National Paklenica en Croatie.

crayfish were analysed by standard descriptive statistics. To show the size distribution in the catch during six consecutive nights in 2000 and 2002 for males and females separately, we introduced size classes. Size classes were defined arbitrarily (Table I).

Table I
Size classes.

Tableau I
Classes de taille.

Total length/cm	Size class
< 6.0-6.9	1
7.0-7.9	2
8.0-8.9	3
9.0-9.9	4
10.0-10.9	5
11.0-11.9	6
> 12.0	7

Correlation was calculated between the total length and weight for males and females separately, and t-test was used to verify if the sizes of males and females differed between the two study years. Correlation between sampling year and total crayfish length and weight was calculated as well as correlation between sampling year and number of caught females and males. To verify if the number of caught males and females differed significantly between the two sampling years t-test was used. Statistical analyses were performed using program STATISTICA 5.0.

RESULTS

In the year 2000 research was conducted between July 19 and 25. Mean water temperature was 18°C. A total of 233 crayfish, longer than 5.2 cm total length, were caught (from which 70 specimens were trapped more than once), 123 females and 110 males (Figure 2). The sex ratio was 1.1 : 1. Females and males differed significantly in their weight ($t = 3.946$, $p = 0.0001$, $df = 108$) and total length ($t = 3.024$, $p = 0.0029$, $df = 157$) (Table II).

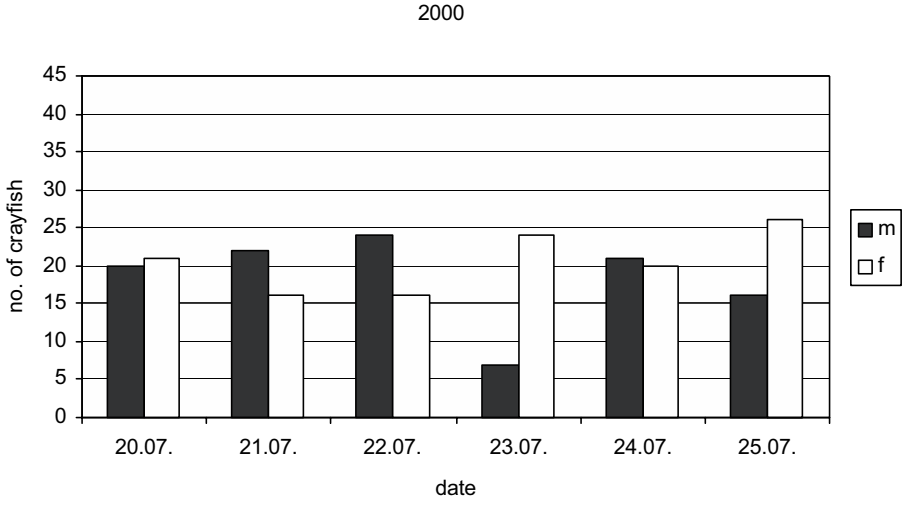

Figure 2
The number of males and females caught per night in 2000.

Figure 2
Nombre de mâles et des femelles capturés par nuit en 2000.

Table II
Description of weight and total length for females and males in 2000.

Tableau II
Description du poids et de la longueur totale des femelles et des mâles en 2000.

Year 2000	N	Mean	Minimum	Maximum	Std. Dev.
FEMALES					
Weight/g	52	24.538	11.000	60.000	10.243
Total length/cm	80	9.250	7.400	12.700	1.159
MALES					
Weight/g	58	47.086	5.000	142.000	40.025
Total length/cm	79	10.138	5.200	15.000	2.353

In both sexes weight and total length were in strong positive correlation (females $n = 52$, $r = 0.96$, $p < 0.05$; males $n = 58$, $r = 0.95$, $p < 0.05$). The size distribution, based on the size classes, in the catch during six consecutive nights for males and females, separately, is shown in Figures 3 and 4.

Estimation of the population size based on the mark-recapture techniques was done using different methods. Results are shown in Table III:

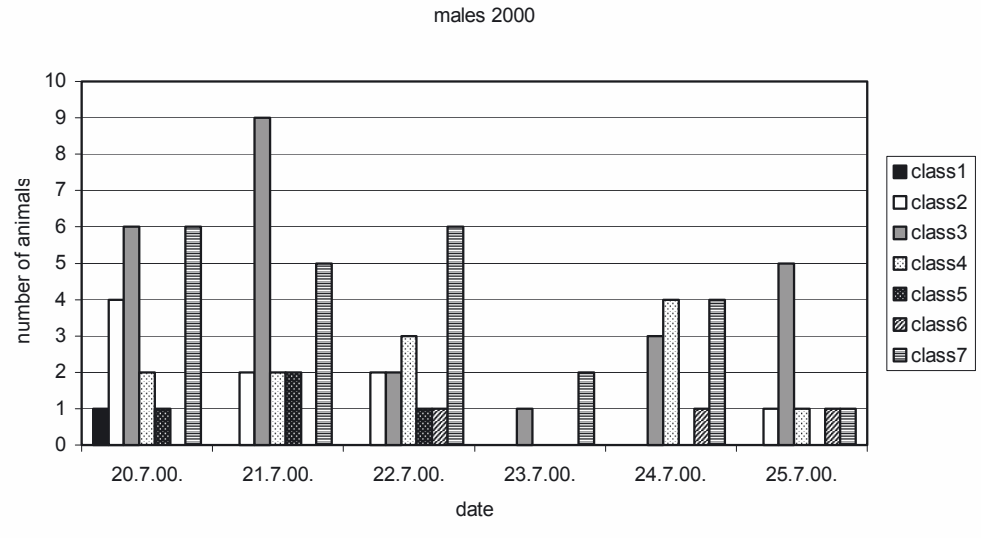

Figure 3
Distribution of size classes in the catch per night in 2000 for males.

Figure 3
Distribution en classes de taille des mâles capturés par nuit en 2000.

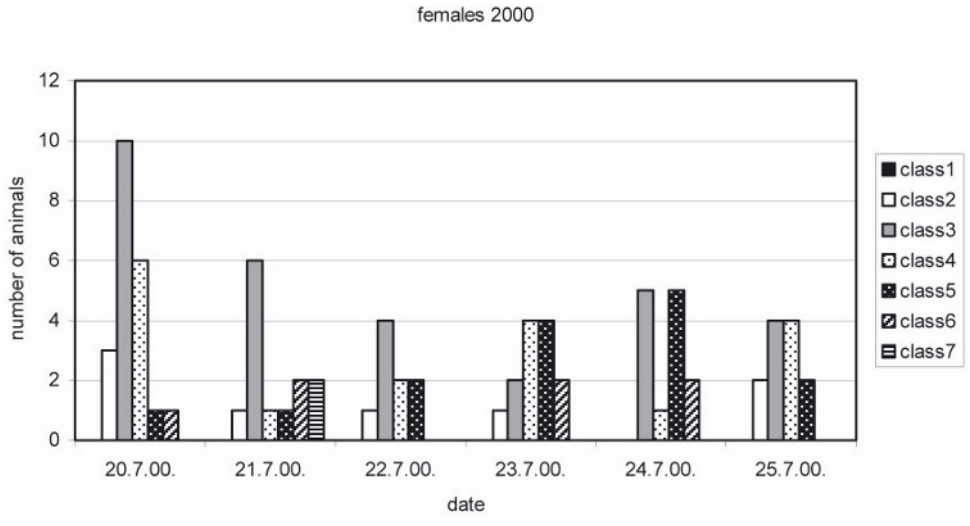

Figure 4
Distribution of size classes in the catch per night in 2000 for females.

Figure 4
Distribution en classes de taille des femelles capturées par nuit en 2000.

Table III
Estimated population size of *Astacus astacus* in the Velika Paklenica Stream in 2000, using three different methods.

Tableau III
Estimation de la taille de la population d'*Astacus astacus* dans le fleuve Velika Paklenica en 2000, obtenue à l'aide de trois méthodes différentes.

Method	Estimated population size	95% C.L.
Jolly-Seber	121.8	63.1-181.6
Schnabel	236.8	180.8-342.9
Schumacher-Eschmeyer	244.8	192.4-336.4

In the year 2002 research was conducted between July 9 and 15. Mean water temperature was 22.2°C. A total of 296 crayfish, longer than 6.5 cm total length, were caught (from which 86 were trapped more than once), 113 females and 183 males (Figure 5). The sex ratio was 0.62 : 1. Females and males differed significantly in their weight ($t = 4.314$, $p = 0.00002$, $df = 207$) and total length ($t = 3.852$, $p = 0.00016$, $df = 207$) (Table IV).

In both sexes weight and total length were in strong positive correlation (females $n = 90$, $r = 0.91$, $p < 0.05$; males $n = 119$ $r = 0.95$, $p < 0.05$). The size distribution, based on the size classes, in the catch during six consecutive nights for males and females, separately, is shown in Figures 6 and 7.

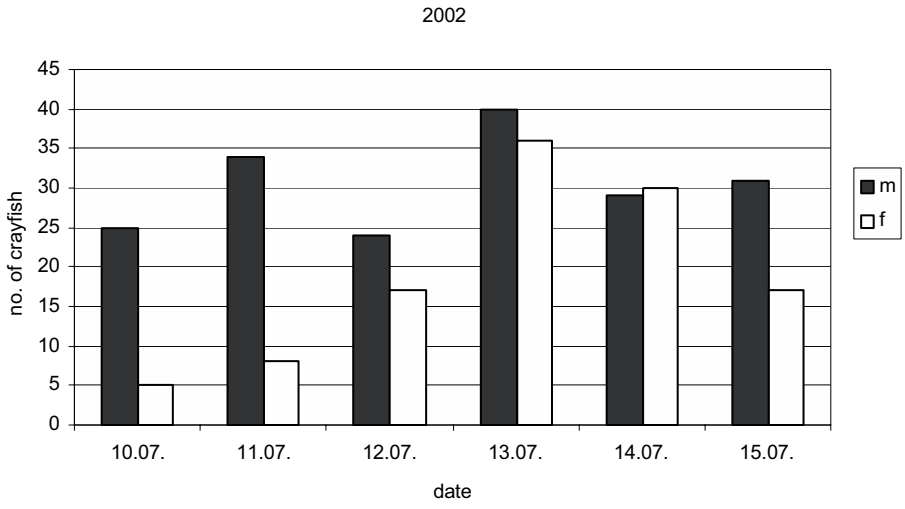

Figure 5
The number of males and females caught per night in 2002.

Figure 5
Nombre de mâles et de femelles capturés par nuit en 2002.

Table IV
Description of weight and total length for females and males in 2002.

Tableau IV

Description du poids et de la longueur totale des femelles et des mâles en 2002.

Year 2002	N	Mean	Minimum	Maximum	Std. Dev.
FEMALES					
Weight/g	90	22.880	7.000	95.000	12.746
Total length/cm	90	8.591	6.590	12.700	1.129
MALES					
Weight/g	119	36.137	10.000	142.000	26.955
Total length/cm	119	9.422	6.475	14.570	1.795

Estimation of the population size based on the mark-recapture techniques was done using different methods and the results are shown in Table V.

When female size was compared between the two years they differed significantly in their total length ($t = 3.705$, $p = 0.00029$, $df = 166$), but not in their weight ($t = 0.800$, $p = 0.424$, $df = 140$), while males differed significantly in both total length ($t = 2.557$, $p = 0.011$, $df = 194$) and weight ($t = 2.220$, $p = 0.027$, $df = 174$).

When we compared the total number of trapped crayfish between the two years we found no statistically significant difference ($t = -1.545$, $p = 0.153$, $df = 10$), but when we compared separately the number of caught males and females between the two years, we

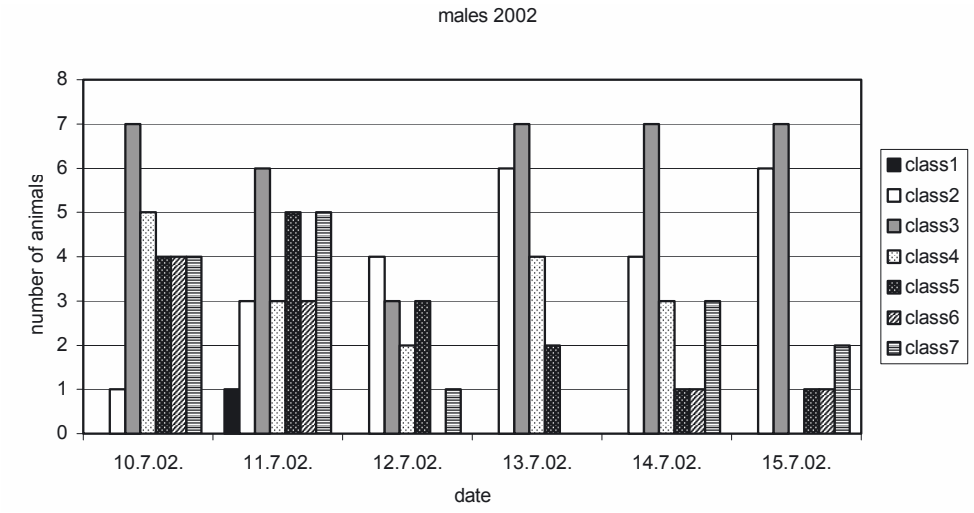

Figure 6
Distribution of size classes in the catch per night in 2002 for males.

Figure 6
Distribution en classes de taille des mâles capturés par nuit en 2002.

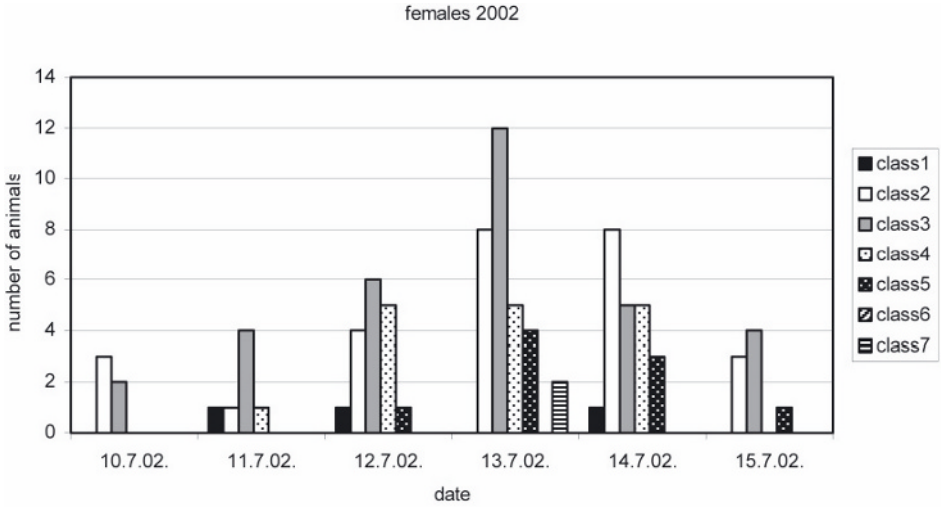

Figure 7
Distribution of size classes in the catch per night in 2002 for females.

Figure 7
Distribution en classes de taille des femelles capturées par nuit en 2002.

found that the number of males was significantly higher in the second year ($t = -3.479$, $p = 0.0059$, $df = 10$), while the number of females was not significantly different ($t = 0.319$, $p = 0.756$, $df = 10$). The same result was confirmed using Pearson's correlation between year and number of males ($n = 12$, $r = 0.74$, $p < 0.05$) and year and number of females ($n = 12$, $r = -0.10$, $p < 0.05$). We also examined correlations between the year of capture and crayfish size (weight and total length) for females and males, separately. For females there was no significant correlation between the year of capture and their weight ($n = 142$, $r = -0.07$, $p < 0.05$) while there was a significant negative correlation between the year of

Table V

Estimated population size of *Astacus astacus* in the Velika Paklenica Stream in 2002, using three different methods.

Tableau V

Estimation de la taille de la population d'*Astacus astacus* dans le fleuve Velika Paklenica en 2002, obtenue à l'aide de trois méthodes différentes.

Method	Estimated population size	95% C.L.
Jolly-Seber	247.5	137.7-349.9
Schnabel	689.6	590.5-828.7
Schumacher-Eschmeyer	846.3	606.2-1402.2

capture and their total length ($n = 142$, $r = -0.22$, $p < 0.05$). For males both correlations were statistically significant (year – weight : $n = 176$, $r = -0.17$, $p < 0.05$; year – total length : $n = 176$, $r = -0.17$, $p < 0.05$).

DISCUSSION

The noble crayfish is a native species in Croatia. It is treated as a threatened species and it is protected by Croatian Law (NARODNE NOVINE 30/94; 76/98). There has been no continuity in the study of this species in Croatia (ŠOŠTARIĆ, 1888; CAR, 1901; ENTZ, 1914; KARAMAN, 1929; KARAMAN, 1961; ALBRECHT, 1982; OBRADOVIĆ, 1987; DELIĆ, 1993; BRUSINA, 1995; MAGUIRE, 2002), but it has been used as food until today although the law forbids it. In Europe it probably has been an object of trade, commerce and zoological study for more than 2000 years (SKURDAL and TAUGBØL, 2002). At a European level *Astacus astacus* is considered to be a vulnerable species and special care is recommended in its management (Bern Convention and EC's Habitat Directive) and it is listed on the IUCN Red List (SKURDAL and TAUGBØL, 2002).

Today's pattern of the distribution of the noble crayfish in Europe is the consequence of many different factors such as changes of freshwater habitats, introduction of alien competitive crayfish species and diseases they brought with them. It is hard to reconstruct its original pattern of distribution because it has been changed during history due to the transplantation by people (SKURDAL and TAUGBØL, 2002). The situation is similar in Croatia, but there are no written documents about these transplantations and one could get the information just from local inhabitants. *A. astacus* is wide spread in water bodies that belong to the Black Sea Basin (MAGUIRE, 2002; MAGUIRE and GOTTSTEIN-MATOČEC, 2004). Croatian rivers belong to the Black Sea Basin as well as to Adriatic Sea Basin. A range of mountains separates the two basins and no natural connection between them exists. It is most likely that men had introduced populations of *A. astacus* into some rivers on the Adriatic coast. Inhabitants from the National Park Paklenica told us that *A. astacus* had been introduced into the Velika Paklenica Stream between the two World Wars. It is not known, however, if any other crayfish species (e. g. the white-clawed crayfish that is naturally spread in the Adriatic Basin) existed there before (MAGUIRE *et al*., 2002).

Summer temperatures (18ºC in the first year and 22ºC in the second year) were optimal for *A. astacus* (CUKERZIS, 1988). ABRAHAMSSON (1983) considered that trappability is mainly related to food searching but might also fluctuate with a change in other factors such as light conditions, water temperature, etc. As temperature influences locomotion activity (KIVIVUORI, 1977) and trappability is greater at higher temperatures

(ACKEFORS, 1999), we conclude that probably fewer crayfish were caught in 2000 because the water temperature was lower.

The population in the stream is completely isolated. During research we noticed no signs of diseases or parasites within the population and the crayfish seemed to be in a good condition. The study stretch of the stream offers plenty of hiding places into which crayfish can retreat during the daylight hours and moulting periods. It therefore offers good habitat quality for the noble crayfish. The presence of crayfish, among other factors, is strongly dependent on the structure of the stream bottom (NIEMI, 1977; BOHL, 1987; LAURENT, 1988; FOSTER, 1995; BYRNE *et al*., 1999) and higher population density is expected in places where greater morphometric and structural variability of bottom exists (BOHL, 1997). As the stream is situated in a karstic region, the water is rich in calcium (70 mg/l) and pH value was around 8. Both parameters are in ranges within which noble crayfish can complete successfully their life cycle (BOHL, 1987; CUKERZIS, 1988).

Sampling with baited traps is the method most commonly used in research on crayfish (WESTMAN and PURSIAINEN, 1982). However, BROWN and BREWIS (1978) found that the use of trapping for estimation of the populations size underestimates population size by factor three. As in our research we used LiNi traps, mesh size 14 mm, and they are size selective (HOGGER, 1988), we caught only crayfish larger than 5.2 cm (Table II), so the estimation of the population size was actually estimation of the proportion of the population larger than 5.2 cm. It is known that noble crayfish females reach sexual maturity at a size which ranges from 6.2 cm total length in localities with early maturity or slow growth to 8.5 cm total length in localities with late maturity or fast growth. Males become mature at a size of 6.0-7.0 cm total length (SKURDAL and TAUGBØL, 2002). Therefore the juvenile part of the population has not been studied which was also the case in studies by NIEMI (1977), WESTMAN and PURSIAINEN (1978, 1982), SKURDAL *et al*. (1992), LAPPALAINEN and PURSIAINEN (1995), WESTMAN *et al*. (1995), BOHL (1997), FRUTIGER *et al*. (1999), GRANDJEAN *et al*., (2000), RALLO and GARCÍA-ARBERAS (2000).

For the estimation of population size we used mark-recapture techniques (Jolly-Seber Method, Schnabel Method, and Schumacher and Eschmeyer Method). Two of the methods (Schnabel and Schumacher and Eschmeyer) presume closed population while Jolly-Seber Method presumes an open population (e.g. migration, death and birth). Our population is isolated and crayfish were trapped during a short-term period, insufficient for detection of birth/death, and the marking method does not significantly influence survival rate (GUAN, 1997). Another important assumption for use of mark-recapture methods is that immigration or emigration during the study was negligible. If random or directional movements occurred, unmarked individuals would enter the study area while marked and unmarked animals would exit. That means that number of marked individuals in the study area would decrease. During our research the number of marked individuals actually increased over time from the second to last sampling, so we conclude that immigration and emigration were not significant, the same as in the work from RABENI *et al*. (1997). Taking all that into consideration, we think that methods of Schnabel and Schumacher and Eschmeyer are more applicable for estimation of our population. SEBER (cf. KREBS, 1989) also pointed out Schumacher and Eschmeyer method as the most robust and useful ecological model. The estimation of population size by mark-recapture methods was frequently used in other studies (BROWN and BOWLER, 1977; WESTMAN and PURSIAINEN, 1982; SKURDAL *et al.*, 1992; MOMOT, 1993; LAPPALAINEN and PURSIAINEN, 1995; WESTMAN *et al*., 1995; GHERARDI *et al*. 1996; BYRNE et al. 1999; CRUZ-HERNANDEZ *et al*. 1999; FRUTIGER *et al*. 1999; GRANDJEAN *et al*., 2000; RALLO and GARCÍA-ARBERAS, 2000; RENTZ and BREITHAUPT, 2000).

All three methods gave the higher estimation of population size for the second sampling year. The wide 95% confidence limits associated with the population estimates

of the crayfish longer than 52 mm, especially in the second sampling year, could be explained either by the insufficient trapping intensity or low number of marked crayfish or the low recapture rate of marked crayfish (LAPPALAINEN and PURSIAINEN, 1995; BYRNE *et al*., 1999; FRUTIGER *et al*. 1999). Furthermore, taking into account that only the crayfish longer than 5.2 cm were marked, it seems reasonable to conclude that the studied *A. astacus* population is much bigger. Assuming a normal, pyramid shaped age structure of the *Procambarus clarkii* population, FRUTIGER *et al*. (1999) estimated that the trappable fraction of population (animals > 7.5 cm body length) would account for around 15-20%, while the large fraction of small, not trappable animals, represents more than 80% of the total population, representing the immense recruitment reservoir that guarantees a high resilience of the population. According to ABRAHAMSSON (1966) individuals less than 3 years old (< 7.5 cm total length) represented 75% of the *A. astacus* population examined with electrofishing in Sweden. In a small lake in the Lithuania, CUKERZIS (1988) observed that *A. astacus* individuals which were sexually immature (< 7.0 cm total length) constituted 90% of the population. In order to estimate the percentage of the younger crayfish fraction in our population some supplementary trapping should be done, including different sampling methods (e.g. electrofishing, enclosure, hand catching or usage of specially designed traps), because it is known that the problem of different degrees in catchability may be overcome if different sampling method are combined (BYRNE et al. 1999; GRANDJEAN *et al*., 2000).

In the research done by BROWN and BOWLER (1977) there was always a higher number of trapped males than females regardless of the season. In our research we found no statistically significant difference in the number of trapped males and females, which corresponds to the results obtained by SKURDAL *et al*. (1992). In the first year of study the sex ratio was 1.1 : 1 (females : males). In the second year it was 0.64 : 1. This difference is explained by the fact that research was conducted earlier in 2002 than in 2000: July 10^{th}-15^{th} and 20^{th}-25^{th}, respectively. Females that accomplished hatching and moulting did not appear in the traps early in July at our study stretch. Figure 7 clearly shows that only smaller females (size class 1, 2, 3) were trapped in the period from July 10^{th}-12^{th}, whereas bigger females (size class 4, 5, 6, 7) started to appear in the traps from July 12^{th} onwards. Males were larger than females. Both total length and weight differed significantly between the two sexes, due to sexual dimorphism mainly caused by the allometric growth in male chelipeds (ABRAHAMSSON, 1966; SKURDAL and TAUGBØL, 2002).

The comparison of total length for both sexes between the two sampling years has shown a statistically significant difference. Larger specimens were caught in the first year of study. The decrease in the crayfish size could mean that the population is regenerating and growing or that it is a stunted population (KULESH *et al*., 1999).

Also, when we compared the number of caught males and females separately between the two sampling years, we found no statistically significant difference for females, but the number of caught males differed significantly. The higher number of males was recorded in the second year. As described by MOMOT (1993) the density of the younger male age groups significantly increases with an increase in the mortality of the older male age groups or, in other words, recruitment of younger males decreases with an increase in the density of sexually mature males. Females display a corresponding, but not statistically significant trend (MOMOT, 1993). We therefore conclude that the higher number of smaller males trapped in 2002 is probably due to the regeneration of the population.

CONCLUSION

The studied population of *Astacus astacus* in the Velika Paklenica Stream is of anthropogenic origin. The analysis showed a normal distribution of crayfish size (weight and total length) and a statistically significant difference between female and male size.

These data indicate that population is stable. Caught crayfish showed no sign of diseases and parasites and were in a good condition. The structure of the stream bottom, offering many hiding places, is a good habitat for the animals. The estimation of population size together with analysis of crayfish size structure indicates that the population may be growing. In order to obtain more data on the population dynamics that could help us to determine stream carrying capacity, further research is needed.

ACKNOWLEDGEMENTS

This work was supported by the National Park “Paklenica”. We would like to thank to Dr. Gordan Lukač, Duje, Petra, Nika, Anamaria, Goran and Zoran for the help during the fieldwork.

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