Ecological Notes on *Austropotamobius pallipes italicus* (Faxon, 1914) (Decapoda, Astacidae) in a Karstic Spring of the Neretva Delta (Croatia)

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ABSTRACT

Our research on a population of *Austropotamobius pallipes italicus* (Faxon. 1914) in the protected karstic limnocrenic spring Modro oko (Dalmatia. southern Croatia) was carried out between February and October 1995. Data were collected on the density of crayfish population, the depth at which specimens were caught, water salinity, water temperature, alkalinity, concentrations of nitrite, ammonia and phosphate and the presence of chlorophyll *a*. We also examined submersed vegetation as a potential microhabitat for this species. The aim of our research was to discover a possible correlation between some of these parameters and the number of crayfish. Small negative correlation was found between population density and water salinity and population density and concentration of phosphate. A positive correlation was found between the number of crayfish and the water temperature and the number of crayfish and the depth at which they were caught. The water temperature was relatively stable throughout the year.

Key Words: *Austropotamobius pallipes italicus*, water salinity, karstic limnocrenic spring, Neretva Delta, Croatia.

I. INTRODUCTION

Little is known on the ecology of the populations of *Austropotamobius pallipes italicus* within the region of Dalmatia (Southern Croatia). There is also a controversy about the taxonomic status of this species. In the 1960s, Karaman (1961, 1962) studied crayfish in the Neretva delta region. By examining the external morphological characters, he determined *Austropotamobius italicus italicus* (Faxon, 1914) as a distinct species. Ten years later, Bott (1972) claimed that the taxonomic characteristics of these animals are subspecific and that it is therefore not possible to describe them as a distinct species. He stated that crayfish living in the river Neretva belong to the subspecies *A. pallipes italicus*. In the 1990s, results of starch gel horizontal electrophoresis, used for enzymes analyses of *A. pallipes sensu lato*, seemed to show that the subspecies *A. pallipes italicus* is in fact a distinct species named *A. italicus* (Faxon, 1914) (Santucci et al. 1997). However, further research at a molecular level is needed. It is hoped that new molecular techniques for studying genetic variability in crayfish will finally show their real taxonomic status. One of the perspectives is based on the analysis of mitochondrial DNA variation by RFLP (Restriction Fragment Length Polymorphism) (Souty-Grosset et al. 1997) and the other on RAPD (Random Amplified Polymorphic DNA) (Souty-Grosset et al. 1998).

A closely related species, the white-clawed crayfish *Austropotamobius pallipes pallipes* (Lereboullet, 1858), is indigenous to Western Europe. Although it is still widespread in France, populations are in decline in Britain and Spain. It is seriously threatened throughout its range by the oomycete fungus *Aphanomyces astaci* Schikora, over-fishing, habitat destruction, water acidification, pollution, competition with introduced foreign crayfish species and extreme climatic conditions (Carral et al. 1993, Grandjean et al. 1997).

For the investigated area there is neither data about the outbreak of crayfish plague, nor the existence of exotic crayfish species. In the 1960s, the subspecies *A. pallipes italicus* still inhabited the River Neretva (Karaman 1961). In the meantime it has disappeared from the river because of intensification of anthropogenic pressures like intensive drainage for agriculture, and air and soil pollution caused by the growth of industry and increased traffic and agriculture (Curić 1994). Nowadays, in Croatia the subspecies is protected by law and still exists in the Neretva delta region within the protected limnocrenic springs of Modro oko and Malo Modro oko.

II. MATERIALS AND METHODS

Study site

Research on the population of *A. pallipes italicus* in the karstic spring Modro oko (with legislative status of a protected landscape) in the delta of the River Neretva (Southern Croatia) was conducted between February and October 1995 (Fig. 1). The spring area is 1.45 km^2 and the maximum depth is 22.5 m (Štambuk-Giljanović 1998). The dominant trees around the spring were *Alnus* and *Salix*. The spring is supplied with water through a series of underwater springs, the biggest of which is situated at the bottom. Modro oko also has a brackish waterside spring. The water within Modro oko is influenced by water from Vrgoračko polje (= solution lake; this type of a lake is a specific karstic phenomenon) (Hutchinson 1957). This is due to the fact that there exists a direct underground connection between the two waterbodies. Generally, rainfall is the main factor regulating the water level within Lake Vrgoračko and all other lakes and springs in the Neretva Valley.

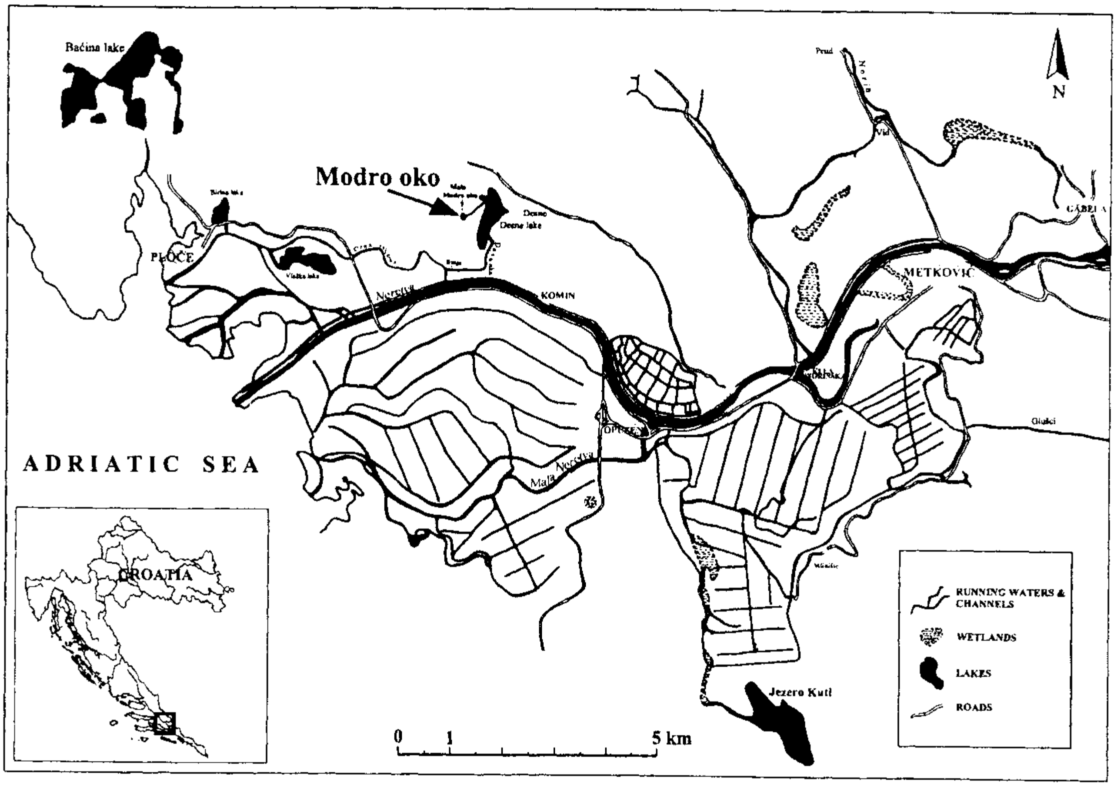

Figure 1. The study area in the delta of the River Neretva (Croatia).

Field methods

In the littoral region, the crayfish were caught once per month by using traps, hand nets, and also by electro-fishing (approximately 500 V). The depth at which the crayfish were caught was always recorded. SCUBA diving was used to observe their presence in the deeper parts of the spring. The specimens were then preserved in 75% ethanol for later reference and further research (stomach content). The crayfish were identified to species by using standard taxonomic references (Bott 1972, Froglia 1978, Karaman 1961, 1962, Laurent and Forest 1979).

The following physico-chemical parameters were determined: water salinity (using a refractometer Biomarine-INC Aqua Fauna, USA), water temperature (using Oxy Guard – Handy Mk II, Belgium), alkalinity (mg $CaCO_3$/l), nitrite

(NO_2^-), ammonia (NH_4^+), phosphate (PO_4^{3-}), and *a* according to APHA (1985). Water samples were obtained once per month from the spring's surface (0-1 m depth) in the morning (10 a.m.). Temperature was also measured once per month in the morning (10 a.m.) at a depth of 0-1 m. All aquatic macrophytes were identified to species level using standard taxonomic references (Javorka and Csapody 1991). The Pearson correlation test was used to quantify the relationship between the number of crayfish and the physico-chemical parameters of the water (significance level 0.05) (Zar 1996).

III. RESULTS

The subspecies *A. pallipes italicus* still exists in the Neretva delta region in protected springs with limnic water, namely in Modro Oko and Malo Modro oko (Fig. 1). All crayfish of this research were caught in the littoral region (maximal depth 1.5 m) of Modro oko within the aquatic vegetation shaded by *Alnus* and *Salix* canopies. Three species of submersed macrophytes (*Hippuris vulgaris* L., *Myriophyllum verticilatum* L., *Potamogeton perfoliatus* L.) and one species of aquatic moss (*Fontinalis antipyretica* Hedw.) were found in the investigated area.

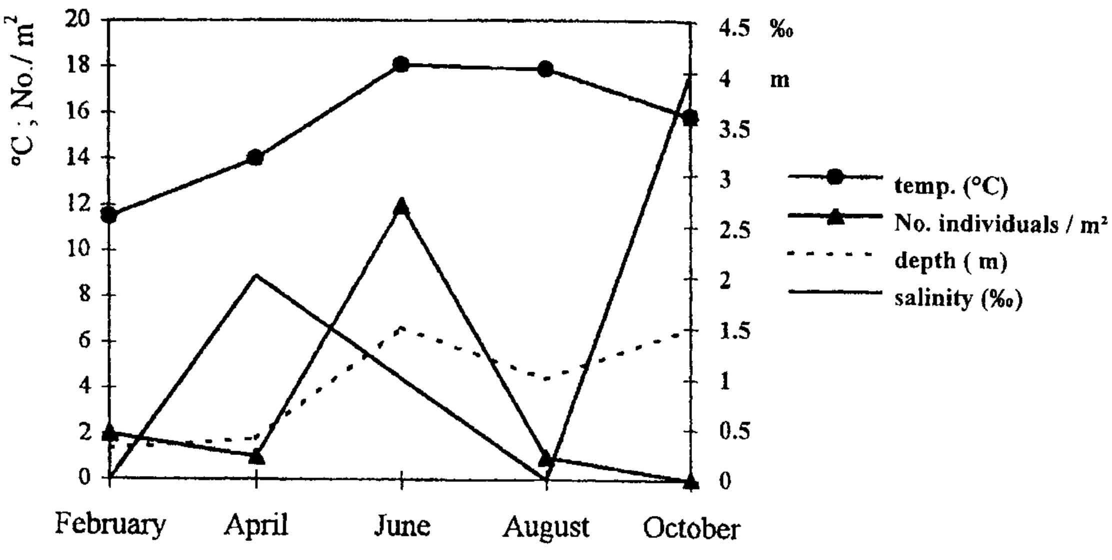

Figure 2. Relationship between crayfish numbers per m² and temperature, water depth and salinity.

The highest water salinity was recorded in October (Table I). At the same time the abundance of crayfish was at its lowest within the littoral region; but specimens were recorded at the bottom of the spring (22.5 m) using SCUBA equipment (Fig. 2). Although the water temperature was generally stable throughout the year, a slight increase in temperature during the summer months appeared to increase the number of crayfish within the littoral region (Fig. 2). The rise of alkalinity in June seemed to correspond with an increase in the number of crayfish contrary to the later rise of alkalinity. This latter phenomenon is probably due to the higher water salinity, which in turn causes crayfish to migrate into the deeper parts of Modro oko (Fig. 3). The concentrations of the ions NO_2^-, NH_4^+ and PO_4^{3-} and of chlorophyll *a* were generally very low (Fig. 4 and Fig. 5).

Table I. Crayfish density, physico-chemical parameters and chlorophyll *a* measured in 1995 in Modro oko.

	February	April	June	August	October
number of individuals per m^2	2.00	1.00	12.00	1.00	0.00
depth (m)	0.30	0.40	1.50	1.00	1.50
temperature (°C)	11.50	14.00	18.10	17.90	15.90
salinity (‰)	0.00	2.00	1.00	0.00	4.00
alkalinity ($CaCO_3$) (mg/l)	--	140.00	179.00	200.00	210.00
NO_2^- (mg/l)	0.03	0.01	0.01	0.01	--
NH_4^+ (mg/l)	--	0.05	0.02	0.05	--
PO_4^{3-} (mg/l)	0.03	0.02	0.00	0.00	0.01
chlorophyll *a* (µg/l)	0.11	0.39	0.24	--	--

The results of the Pearson correlation test showed that there is no significant correlation between crayfish number and physico-chemical parameters, but a small positive correlation exists between the number of crayfish and water temperature ($r = 0.44$) and also between the depth at which animals were caught and their number ($r = 0.43$). Small negative correlation was recorded between the number of crayfish and water salinity ($r = -0.25$) and also between the number of crayfish and concentration of phosphate ($r = -0.43$).

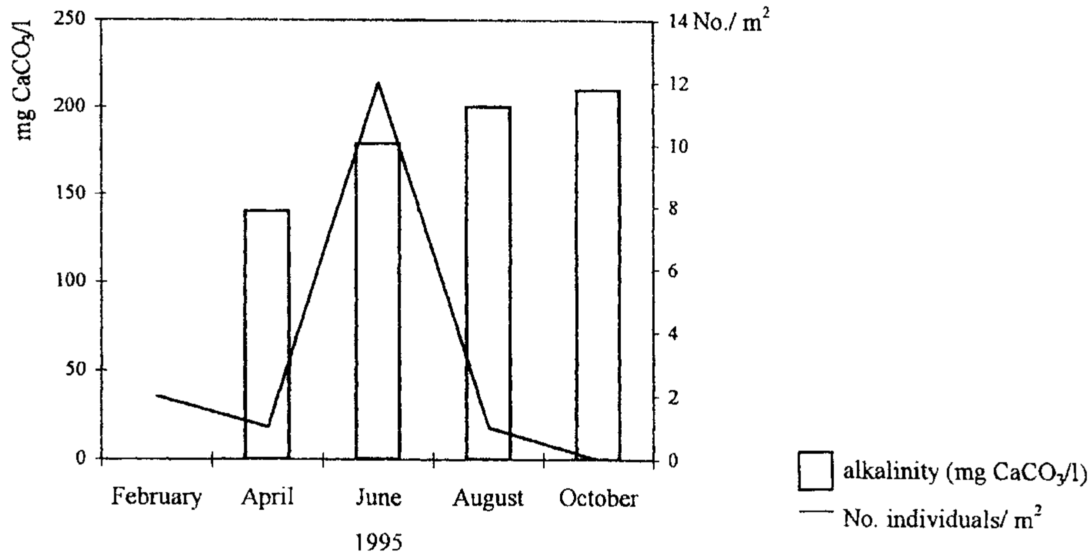

Figure 3. Relationship between crayfish numbers/m² and the concentration of $CaCO_3$.

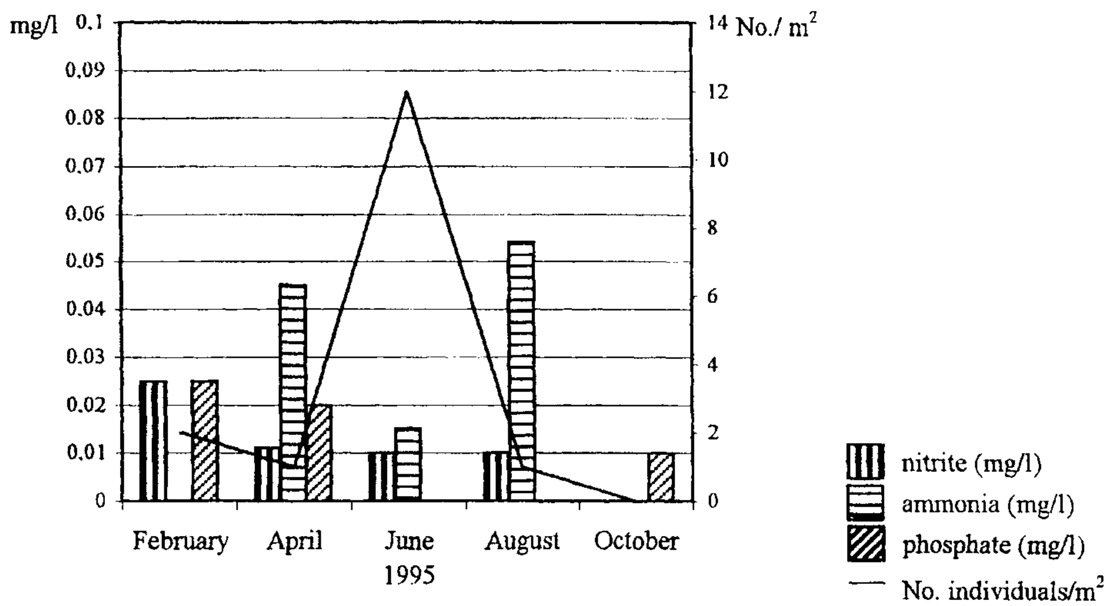

Figure 4. Relationship between crayfish numbers/m² and the quantity of nitrite (NO_2^-), ammonia (NH_4^+) and phosphate (PO_4^{3-}).

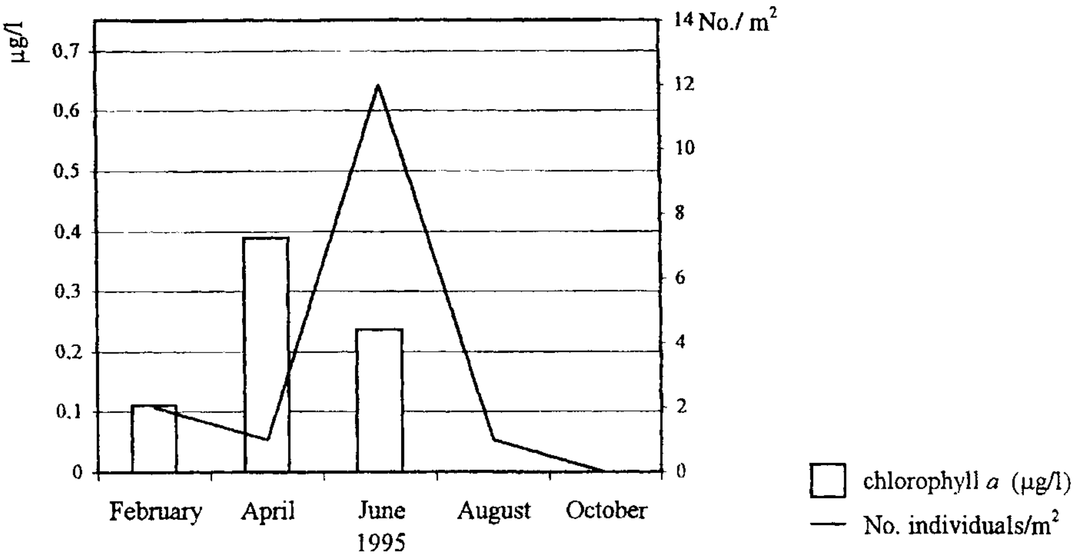

Figure 5. Relationship between crayfish numbers and the quantity of chlorophyll *a*.

IV. DISCUSSION

The population density of *Austropotamobius pallipes* is affected by the availability of shelter and the abundance of food (Foster 1995). In the investigated area the aquatic macrophytes and riparian vegetation offer not only a potential shelter but also a probable source of food for the crayfish. Even though omnivorous the species can feed solely on plants. In some Irish lakes *Chara desmacantha* dominated in the crayfish diet. An English population in Northumbria even existed on the moss *Fontinalis antipyretica* as there were insufficient quantities of other food sources (Matthews et al. 1993).

The composition of aquatic macrophytes gives also information about the microhabitat characteristics and about the trophic stage of an aquatic ecosystem. Through grazing pressure on macrophytes crayfish play an overall positive role in the aquatic ecosystems with respect to eutrophication (Hessen et al. 1993). The main discriminatory factor for determination of the trophic stage of a waterbody is the quantity of nutrients (especially phosphate and ammonia) (Carbiener et al. 1990, Boavida and Wetzel 1998). Taking into account the low concentrations of nutrients and chlorophyll *a* recorded all around the year, Modro oko belongs to the ultraoligotrophic ecosystems. *A. pallipes* tolerates well eutrophication (de Bikuña et al. 1989) and can also be found in mesotrophic lakes (Matthews et al. 1993). In Croatia, all lakes and springs in which this species was found thus far were of a low trophic stage (ultraoligotrophic), for instance Lake Vrana on the island of Cres (Gottstein and Kerovec 1998) and the limnocrenic spring Modro oko.

Even though Modro oko is supplied with brackish water from a side spring on its surface, there is no salty water stratification within its deeper parts. The brackish water cannot penetrate into the deeper parts due to the large amount of ground water seeping into Modro oko through strong springs located at its bottom. This effect causes a constant mixing of waters in deeper parts and may explain the vertical distribution of crayfish during the year: the salinity increase on the surface may cause a decrease in the number of crayfish within the littoral region. The high salinity probably forces the crayfish from the littoral region into deeper parts of the limnocrenic spring and might be one of the factors regulating crayfish population density and their vertical distribution.

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