

The signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) [Crustacea: Decapoda: Astacidae], in the Brugneto Lake (Liguria, NW Italy). The beginning of the invasion of the River Po watershed?

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Abstract

The recent finding of the signal crayfish, *Pacifastacus leniusculus* (Dana, 1852) in the Brugneto Lake (Trebbia River basin, Liguria, NW Italy) raises new management problems. In this paper, the results of trapping carried out between November 2005 and October 2006, the population structure and the diseases of *P. leniusculus* inhabiting Brugneto Lake are analysed. The total sex ratio observed was 1:1.03 (males/females) and it did not differ significantly from the theoretical 1:1 value, whereas the sex ratio recorded during winter differed significantly from the theoretical value. The CPUE (Catch Per Unit Effort) calculated was significantly related both to mean seasonal temperature and water-level variations. Regarding diseases only the presence of chitinoclastic bacteria on exoskeleton were found. Branchiobdellidae (Annelida, Clitellata) were not observed either on the exoskeleton or in the branchial-chambers, neither were ectocommensals, nor melanisation caused by bacterial infections on gill filaments. Fortunately, melanised spots on the legs usually caused by an infection of *Aphanomyces astaci* Schikora were not found.

Key words: *Pacifastacus leniusculus*, population structure, diseases, captures, Brugneto Lake, Italy

Introduction

Biodiversity has important social and cultural values (Gherardi et al. 2003): unfortunately biological resources are threatened by several factors, often of anthropogenic origin. The introduction of alien species is recognized as one of the greatest biological threats to our planet's ecological well-being (Mooney and Hobbs 2000, Pimentel et al. 2000), and practically no habitat escapes from this risk. Freshwater ones are probably the most affected: lotic and lentic environments consist of a dynamic mosaic of spatial elements and ecological processes arrayed hierarchically (Ward et al. 2002) and their

survival is due to the maintenance of delicate equilibriums (Amoros and Bornette 2002, Malmqvist 2002). The introduction of allochthonous species represents the main threat for freshwater catchments: fish, shellfish and crustaceans are easily transferred for food purposes, restocking and ornamental or aquacultural purposes. These introduced organisms are often able to become established in the wild by reaching high densities and having a large impact on native species or ecosystems (Light 2003).

Freshwater crayfish are important components of European aquatic fauna, and they can be considered as key-species in the habitat they colonised (Holdich and Gherardi 1999, Souty-Grosset et al. 2006): being the biggest freshwater macro-

invertebrates (Holdich 2003), they are either predators and prey (Peris et al. 1994, Beja 1996, Elvira et al. 1996, Parkin et al. 2001, Renai and Gherardi 2004), they act as ecosystem engineers (Statzner et al. 2003) and also convert particulate organic matter in noble proteins (Reynolds 1979). On the other hand, crayfish are also highly valued from a cultural, recreational and economical point of view, and their fisheries and culture are very widespread (Edsman 2004, Jussila and Mannonen 2004, Taugbøl 2004). The reasons for the historical introductions of alien crayfish stocks were mainly because of the perceived need to replace the impoverished native crayfish fauna, to fill a vacant niche and to control aquatic water vegetation, whereas accidental introductions have often been due to escapes from aquacultural facilities (Henttonen and Huner 1999).

In Italy the introduction of alien crayfish is widespread, producing significant changes in aquatic communities (Gherardi et al. 1999). Stable breeding populations of *Procambarus clarkii* (Girard) and *Orconectes limosus* (Rafinésque) are known in Northern and Central Italy, while there is little information about *Astacus leptodactylus* (Eschscholtz) and *Pacifastacus leniusculus* (Dana, 1852) (Gherardi et al. 1999, Souty-Grosset et al. 2006). The occurrence of signal crayfish *P. leniusculus* in the Italian freshwater habitat was first recorded by Machino (1997) and Füreder and Machino (1999) in the Adige River basin (Trentino Alto Adige, NE Italy): the recent finding of *P. leniusculus* in the Brugneto Lake (Trebbia River basin, Liguria, NW Italy) (Capurro et al. 2006) raises new management problems for the lake, especially as *P. leniusculus* is regarded as an invasive species (Guan and Wiles 1998, Guan and Wiles 1999, Huber and Schubart 2005, Stenroth and Nyström 2003, Vorbürger and Ribi 1999, Westman et al. 2002).

The purpose of this paper is to relate captures of crayfish to two environmental features, i.e. water temperature and level variations. In addition, the population structure and occurrence of diseases of *P. leniusculus* established in the Brugneto Lake were analysed.

Material and Methods

Study Site

The Brugneto Lake (44°32'N, 9°12'E) lies in the Trebbia watershed (Liguria, NW Italy) at 770 m above sea level, approximately 40 km northeast of Genoa. This lake, having a volume of 25 million

m³ and a maximum depth of 77 m, is the most important artificial water reservoir in Liguria (Arillo and Mariotti 2002). The lake, characterized by a muddy steep bottom, does not provide many available shelters, therefore the signal crayfish has to burrow in more compact clayey strata (M. Capurro, research in progress). The Brugneto Lake effluent streams flow into the Trebbia River. This one rises in the Ligurian Apennines at an altitude of 1406 m, and flows 115 km north-northeast across the northern Apennines and the Po lowland to enter the Po River, just west of Piacenza. Dense populations of endangered in Europe native crayfish *Austropotamobius pallipes* (Lereboullet) inhabit the Apennine effluents of the Trebbia basin (Salvidio et al. 2002).

Pacifastacus leniusculus was probably introduced into the lake around 2002, released from thoughtless aquarists or as bait by anglers (Capurro et al. 2006), and its population has markedly increased. We selected an area as a sampling station that is thought to be the initial point of release of *P. leniusculus* and where individual density seems to be the highest in the lake (Capurro et al. 2006).

Field sampling

Pacifastacus leniusculus specimens were sampled biweekly from November 2005 to October 2006. Along the shores of the above-mentioned area, we identified ten sampling stations 30 m long. Then, for each sampling session, we randomly selected four stations and we set five traps for each one. Two-way traps baited with cat food (diameter 15 cm; length 60 cm; mesh size 1 cm) were thrown from the shore at intervals of approximately 5-6 m, between 5 and 8 m depth. We based the trap spacing on the effective trapping radius presented in previous study (Abrahamsson and Goldman 1970). Each trap was set in the afternoon and then visited two days later, to exploit also the diurnal activity of signal crayfish (Lozán 2000, Strempel 1974).

Crayfish trapped were immediately frozen after capture and then thawed for laboratory analysis. Each specimen was sexed and CL (carapace length in mm, from the apex of the rostrum to the mid-dorsal posterior edge of the carapace), recorded using a vernier caliper to the nearest 0.1 mm, was used as a size indicator. For female specimens we observed whether they carried eggs or hatchlings on the pleopods or not and if spermatophores were present. For each crayfish

sampled we evaluated the disease condition at a macroscopical level, also analysing branchial-chambers and gill filaments by stereomicroscopy in the laboratory. Because samples were often small for individual months, data were seasonally pooled. So we calculated the mean cumulated seasonal Catch Per Unit Effort (CPUE), expressed as number of individuals trapped/number of traps used, and these values were related to mean water temperature (T) and to water level variations (WLV) in the same period. Temperature was measured every day at 5 m depth; water level, measured daily too, was expressed as altitude of water surface above sea level (Physical features were recorded by AMGA by means of electronic instruments). The possible presence of *P. leniusculus* in the main tributaries and in the effluent of the lake, the Brugneto Stream, was also investigated. The presence/absence of the crayfish was verified by manual survey conducted during the day (1 hour) in spring/summer 2006: we could not use baited traps owing to streams morphology, characterised by small depth. The presence/absence of *A. pallipes* was noted too.

Results

A total of 439 *P. leniusculus* individuals (212 males and 227 females) were captured throughout the sampling period. The CL of male individuals ranged between 22 and 72 mm, whereas CL of female individuals ranged between 22 and 66 mm; length-frequency distribution (Figure 1) revealed that individuals between 48 and 64 mm CL values were caught more frequently in the traps. Although the majority of large individuals were males, there was no statistically difference in size between sexes (Student t test: $t=0.563$; $P>0.05$) and the medians were also equal (Kolmogorov-Smirnov test: $D=0.091$; $P>0.05$). The total observed sex ratio was 1:1.03 (males/females) and it did not differ significantly from the theoretical 1:1 value ($\chi^2=0.083$; $P>0.05$). Figure 2 shows the seasonal sex ratio changes. In winter the sex ratio showed a relative decline of females, while from Spring to Autumn it did not deviated from 1:1 (Figure 2).

Mean seasonal water temperature increased from winter to summer and decreased from autumn to winter (Figure 3). The peak of water level variations recorded in spring is related with the snowmelt and the rainfalls in late winter and early spring (Figure 4).

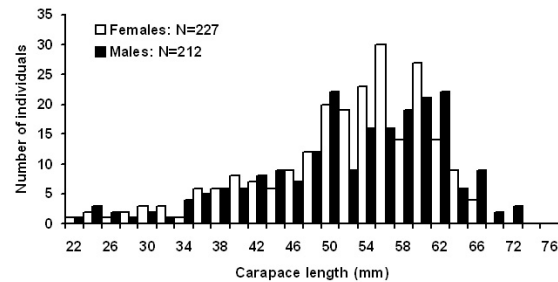


Figure 1. Size (carapace length) frequency distributions of male and female *Pacifastacus leniusculus* collected and measured during the whole study

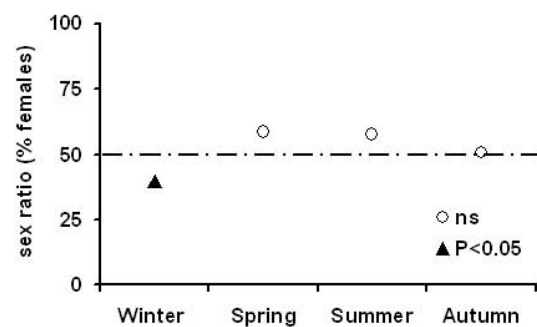


Figure 2. Seasonal sex ratio, as percentage of females, in *Pacifastacus leniusculus*

The CPUE was significantly related to the mean seasonal temperature (Figure 3) and to water level variations (Figure 4). The correlation between water temperature and CPUE was significant ($r=0.821$; $P<0.05$) and the equation of the regression line was: $\text{Log CPUE} = -0.623 + 5.985 \text{ Log T}$; the correlation between water level variations and CPUE was significant too ($r= -0.885$; $P<0.05$) and the equation for regression line was: $\text{Log CPUE} = 776.35 + -6.226 \text{ Log WLV}$. The number of trapped crayfish decreased from winter to spring, during which we registered the lowest CPUE values, and then it increased from summer to autumn.

During the study period 21 berried females were trapped, ranging between 40.5 and 64.7 mm in CL: the first berried female was trapped in December 2005, the last one in July 2006. The number of pleopodal eggs ranged from 1 to 327. In June 2006 a female carrying only one second stage juvenile was captured. During sampling spermatophores were found on only 9 females, the first trapped in December 2005 and the last one in February 2006.

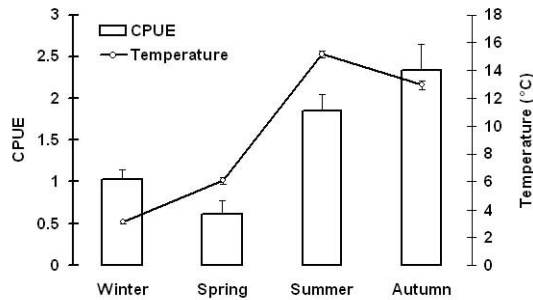


Figure 3. Seasonal Catch Per Unit Effort (CPUE) of *Pacifastacus leniusculus*, expressed as number of individuals trapped/number of traps used, related to mean/seasonal water temperature of Brugneto lake. Vertical bars indicate \pm SE of means

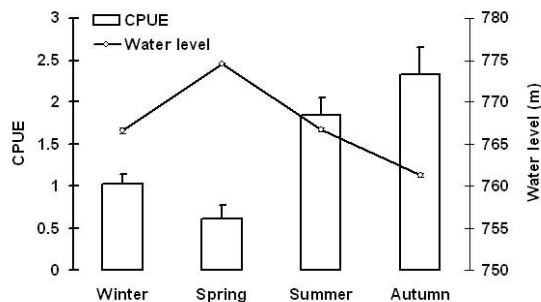


Figure 4. Seasonal Catch Per Unit Effort (CPUE) of *Pacifastacus leniusculus* related to mean/seasonal water level variations of Brugneto lake. Vertical bars indicate \pm SE of means

Regarding diseases, only the presence of chitinoclastic bacteria on the exoskeleton (carapace and chelipeds) of nine males (CL ranges from 50 to 71 mm) and four females (CL ranges from 49.7 to 61 mm) were observed. Branchiobdellidae (Annelida, Clitellata) were not seen either on the exoskeleton or in the branchial-chambers, neither were ectocommensals or melanisation caused by bacterial infections on the gill filaments. Our macroscopic investigations did not show black melanised spots on the legs, usually caused by an infection of *A. astaci* (Diéguez-Uribeondo 2006).

No *A. pallipes* individuals were captured with baited traps in the lake. *P. leniusculus* were not found in the Brugneto tributaries or in Brugneto Stream, whereas *A. pallipes* was found only in a little tributary of Brugneto Stream (Fosso Ciappa).

Discussion

Pacifastacus leniusculus cannot be considered as an r-selected species such as *Procambarus clarkii* is (Lindqvist and Huner 1999); however its reproduction efficiency (Guan and Wiles 1999), feeding ecology (Guan and Wiles 1998) and behavioural and ecological characteristics (Nyström and Strand 1996, Söderbäck 1995, Vorburger and Ribí 1999, Westman et al. 2002) make it an highly invasive species. Signal crayfish can co-exist with some indigenous crayfish species (ICS) in Europe, but in the long run it usually out competes them (Souty-Grosset et al. 2006). The colonisation of Lake Brugneto by *P. leniusculus* seems to be in its early stages, and fortunately it has not yet been found in the tributaries or in the lake effluent; nevertheless its rate of spread (Abrahamsson 1981, Holdich et al. 1995, Guan and Wiles 1997, Kirjavainen and Westman 1999), activity behaviour (Lozán 2000, Bubb et al. 2002) and the lack of obstacles (artificial or natural) along the shores of the lake, will probably allow a rapid colonisation of the whole lake. Unfortunately there is no previous quantitative data about the presence of *P. leniusculus* inhabiting Brugneto Lake; however some fishermen have occasionally caught this species since 2002 (Capurro et al. 2006). The growth of *P. leniusculus* has been studied using many different methods, e.g. use of a technique based on lipofuscin, a neuronal age pigment, indicates that some *P. leniusculus* individuals can live for 20 years (Souty-Grosset et al. 2006). Typically signal crayfish will attain a maximum carapace length of 50 to 70 mm, with a corresponding weight of 60 to 110 g (Lewis 2002). In the present study a maximum carapace length of 66 and 72 mm for females and males was registered, respectively. Comparing the carapace length values with growth curves of *P. leniusculus* valued from several populations (Lewis 2002), it might be supposed that the signal crayfish population inhabiting Brugneto Lake could be more than 7-8 years old. Furthermore the native habitat of *P. leniusculus* ranges from small streams to large rivers and lakes (Mason 1963; Miller 1970), so it should have no problem in occupying the small tributaries and the effluent of the lake, where a small population of *A. pallipes* is still present. Other populations of the white-clawed crayfish are present in the Trebbia watershed (Salvidio et al. 2002), and the risk that signal crayfish could replace these populations is a possibility that should to be avoided.

The results of trapping during the study period provided evidence on the size-selectivity of traps and the seasonal variations of trappability (Abrahamsson 1981, Brown and Brewis 1977, Reynolds and Matthews 1993). The size distribution of trapped *P. leniusculus* in the Brugneto Lake is similar to that recorded by Westman et al. (2002) for a population inhabiting a Finnish lake. However, the present data are missing juveniles (0+) of both sexes, identified by the above-mentioned authors as sized less than 20 mm CL. This may be due to the fact that the catch of juveniles in a lake requires different methods, e.g. hydraulic diver-operated dredge sieve (Westman et al. 2002), since large individuals somehow prevent smaller ones from entering the traps (Kirjavainen and Westman 1999). However, the use of baited traps, set by the lakeshore or from a boat, is the only suitable method in the Brugneto Lake, since it seem to be the only effective one in turbid conditions (Peay 2004). As the traps used for the present study were all placed at the same depth, their range could not cover all habitats, and the size of the trappable population was probably underestimated.

The lower catchability during winter and spring with respect to summer and autumn could be related to the minor activity of crayfish after breeding and later, for females only, after spawning (Gherardi et al. 1997, Kirjavainen and Westman 1999, Grandjean et al. 2000 and therein). The deviation of the sex ratio from 1:1 recorded in winter could be related to the females being less vulnerable to capture as in this period of the year they remain in the burrows brooding their eggs. The increasing number of trapped individuals registered from late summer to autumn could be related to the foraging and the growing activity for the following mating season (Mason 1970).

Previous studies have shown that there is a strong correlation of activity of crayfish with water temperature (Abrahamsson 1981, Kirjavainen and Westman 1999, Riggert et al. 1999, Barbaresi and Gherardi 2001, Bubb et al. 2002). In the present study we confirmed this relation. Mean seasonal temperature during the trapping period appeared to influence crayfish catchability: the lower the temperature on the trapping, the smaller the CPUE. Such relationship was the one expected in an aquatic ectotherm species (Bubb et al. 2004).

Our results seem to provide evidence of a correlation between CPUE and the water level variations too, even if we could suppose that these

variations influenced more the catchability than the activity of crayfish. At the same time we must remark that we threw the traps from the shores and so, when the water level increased, we were not able to reach the sites previously occupied by crayfish. However, the increase of water level occurred at the same time as the post-breeding period of lower crayfish activity: so we are not able to completely separate the effects of these two factors.

The period of presence of ovigerous females found in our study, was similar to that reported by Nakata et al. (2004) and also for North America and Europe (Lewis 2002).

The presence of pathogens or parasites, except for a few cases of chitinoclastic bacteraemias were not observed in the present study. However, more careful analysis will be carried out in future, since Souty-Grosset et al. (2006) mentioned that *P. leniusculus* is a chronic carrier of crayfish plague in Europe, and possibly of other parasites, e.g. *Psorospermium* sp., *Thelohania contejeani* (Henneguy), and Branchiobdellidae (Diéguez-Uribeondo et al. 1993, Ohtaka et al. 2005, Diéguez-Uribeondo 2006).

Management actions will be necessary to contain the growth of the signal crayfish population and to avoid its spread outside the lake, above all in the Trebbia River that flows into the Po River, the most important Italian freshwater course. The rate of spread of *P. leniusculus* can vary from 0.2 km year⁻¹ to 2.8 km year⁻¹ (Peay and Rogers 1999 and therein). Therefore it is reasonable to suppose a time of about 7 year for the signal crayfish population to reach the Po River, which is only 115 km from the Brugneto Lake. However, such spread could be faster as the importance of juveniles and smaller size classes is poorly understood. In fact, Bubb et al. (2004) underlined the fact that although the smallest age class is unlikely to be capable of making substantial active movements, they could be passively transported downstream, since the passive drift of many macroinvertebrates is recognised as important element for species' dispersal ability (Bilton et al. 2001).

Teleost fishes are the only natural enemies of the signal crayfish present in Brugneto Lake. The importance of predation as a process structuring the distribution and abundance of crustaceans is difficult to assess (Blake et al. 1994); however predatory fish can have strong effects on the local distribution of crayfish, even if this influence on prey density and distribution are very variable, from trivial to overwhelming (Sih et al. 1985,

Englund 1999). In Brugnato Lake, different teleost fish species prey on *P. leniusculus*: indeed, among the components of stomach content of *Perca fluviatilis* (L.), *Anguilla anguilla* (L.) and *Ictalurus melas* (Rafinésque) the remains of *P. leniusculus* have been found (I. Borroni pers. comm.). Eels are, however, extremely rare in the lake, whereas catfish is present only as juveniles individuals (I. Borroni pers. comm.). Perch is probably the most important predator for *P. leniusculus* in Brugnato Lake, since its influence is confirmed for other crayfish species too (Appelberg 1987, 1990; Appelberg and Odelström 1988, Svensson 1993, Nyström 2005); *Cyprinus carpio* (L.) and species of bass (*Micropterus salmoides* Lacépède) is present in Brugnato Lake) prey on crayfish (Garvey et al. 1992, Rabeni 1992, Lewis 1999), but their impact on *P. leniusculus* in Brugnato Lake has not been yet verified. Exploitation could be another method to prevent the future complete colonisation of the lake and of the entire watershed, although a more complete knowledge is necessary to plan any management proposal.

However, these efforts could be in vain since, compared to other European states (Ireland, Norway, Sweden, Finland, Spain, France, Poland), in Italy, as well as in Germany and Austria, the import of all live crayfish from abroad is not banned by customs legislation (Souty-Grosset et al. 2006).

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