J. Limnol., 2013; 72(2): 343-360
DOI: 10.4081/jlimnol.2013.e28

Distribution and morphometry of native and alien crayfish in Trentino (Italy)

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ABSTRACT

To fill the existing gaps in the knowledge of the crayfish species distribution in Trentino region (North-East Italy), we surveyed fourteen lakes, six ponds and sixty-nine creeks and streams in 2010-2012. We recorded four populations of the invasive alien species Orconectes limosus *in four lakes and twenty-one of the native* Austropotamobius pallipes *complex from four lakes, four ponds and thirteen creeks; three of these populations went extinct during the three years of our survey. Crayfish populations were monitored in spring, summer and early autumn, to assess the distribution, density and seasonal dynamics of the two species. Statistical analysis of morphometric data showed differences in body size and growth rates among sampling sites, possibly related to local environmental factors, with higher growth rates in males of both species*; A. pallipes *grew faster in creeks than in lakes. The extinction of twenty-two native populations of* A. pallipes *complex in the last century was likely due to habitat modifications, in particular to the loss of riparian habitat, in few cases to overfishing, and, more recently, to the spread of alien species and the related transmission of their parasite* Aphanomyces astaci. *Some populations of* A. pallipes *complex were infested with the parasite* Thelohania *sp., and only one population with ectosymbiotic Branchiobdellida. In Trentino, small creeks with well developed riparian vegetation and good hydromorphological conditions, flowing through mountain slopes, can represent potential refuge and recruitment areas for of* A. pallipes *complex, without significant management intervention, although a sustainable management of piedmont water bodies would allow preserving or increasing the number and density of the relict populations.*

Key words: Austropotamobius pallipes *complex,* Orconectes limosus*, invasive alien crayfish, crayfish distribution, crayfish morphometry.*

Received: November 2012. Accepted: February 2013.

INTRODUCTION

Crayfish play an important role in the natural balance of freshwater ecosystems, influencing the distribution and abundance of other aquatic organisms through their omnivory and bioturbatory behaviour (Nyström and Strand, 1996; Usio and Townsend, 2004). From the 20th century, the European populations of crayfish have been significantly decreasing, due to environmental deterioration (*e.g.* water pollution, habitat fragmentation and modifications, poor river basin management), overfishing, and the introduction of alien species from other continents (Jay and Holdich, 1981; Holdich and Lowery, 1988; Evans and Edgerton, 2002; Gherardi *et al*., 2002; Füreder *et al*., 2006; Renai *et al*., 2006; Scalici and Gibertini, 2007). Europe hosts six native species belonging to the genera *Astacus* and *Austropotamobius*, and seven alien species of the genera *Procambarus*, *Pacifastacus*, *Orconectes*, and *Cherax*, introduced from the United States and Australia (DAISIE, 2012). The American crayfish, unlike the European ones (Brusconi *et al*., 2008), are *r*-selected organisms and are able to spread rapidly in new habitats, adapting to different ecological conditions (Schulz and Smietana, 2001). Moreover, they often carry and transmit the oomycota *Aphanomices astaci* (Schikora 1906), lethal for European crayfish (Diéguez-Uribeondo *et al*., 1997a, 1997b; Kozubíková *et al*., 2006; Cammà *et al*., 2010). Thus, in several European countries, the populations of indigenous crayfish have been replaced by alien ones (Alderman, 1996; Desprez-Loustau *et al*., 2007). The only alien crayfish present in Trentino region (North-East Italy) is *Orconectes limosus* (Rafinesque 1817), while *Procambarus clarkii* (Girard 1852) is widespread in the south-east and south-west neighbouring Veneto and Lombardia regions, and *Pacifastacus leniusculus* (Dana 1852) is present with scattered populations in the northern part of the neighbouring South Tyrol region (Morpurgo *et al*., 2010).

Three native crayfish species are present in Italy: the *Austropotamobius pallipes* species complex (Lereboullette 1858) *sensu* Souty-Grosset *et al*. (2006) is the most widespread one (see below), whereas *Astacus astacus* (Linnaeus 1758) and *Austropotamobius torrentium* (Schrank 1803) are present with only few and small populations in the provinces of Bolzano, Belluno and Udine for the former (Füreder, 2007; Aquiloni *et al*., 2010), and in the Province of Udine for the latter (De Luise, 2006). The native species present in Trentino belongs to the *A. pallipes* complex (called hereafter *A. pallipes*). The IUCN (2012) Red List of threatened species classifies the *A. pallipes* complex as *endangered A2ce*, meaning that an irreversible population size reduction greater than 50% has been observed over the last 10 years due to a decline in habitat suitability and to the effects of introduced species (competitors and parasites). *A. pallipes* complex is also listed the European habitat directive (92/43/EEC) (European Commission, 1992), Annexes II and V (animal and plant species of community interest in need of strict protection and whose conservation requires the designation of special areas of conservation, and whose taking in the wild and exploitation may be subject to management measures, respectively). In Trentino, this species is protected by the decree n. 23-25/Leg 2009 (Trento Province Regulation, 2009).

A. pallipes is widely present in Central-Western Europe, occupying 18 countries, spanning from Montenegro as per East, Scotland as per North, Spain as per West, peninsular Italy as per South (Souty-Grosset *et al*., 2006). In Italy, it is present in all regions except Sicily and small islands, and part of Puglia and Calabria (Aquiloni *et al*., 2010, Bertocchi *et al*., 2010). *O. limosus,* widespread in Central Europe, mostly occurs in France, Germany, Switzerland, Czech Republic, Poland and Lithuania, and it is rapidly spreading down the Danube (Souty-Grosset *et al*., 2006). *O. limosus* was probably introduced accidentally in Italy, in association with batches of fish imported from Poland where the species was first introduced in 1890 (Aquiloni *et al*., 2010); in Italy, it was first recorded in 1991 in Iseo lake (Delmastro, 1992; Gherardi *et al*., 1999), and it is now widespread in Northern Italy, particularly in the Po river valley, and in central Italy with few populations in Lazio region (Aquiloni *et al*., 2010).

Recent studies carried out in South Tyrol (north of Trentino) showed a progressive decrease of native crayfish with twelve populations recorded in 2002, seven in 2003 and only five in 2006 (Füreder *et al*., 2003; Sint *et al*., 2007). The extinction rate in Trentino, adjacent to South Tyrol and with similar climatic and geographical settings, could be similar. An extensive and detailed knowledge of the distribution of the different crayfish species is essential to prevent and control the spread of the invasive alien species and to preserve native populations. This paper, therefore, represents a first contribution to: i) assess the presence and fine-scale distribution of native and alien species of freshwater crayfish in Trentino; ii) improve the knowledge of the morphometry and population characteristics of the recorded species; iii) assess the status of native populations, the major threats to their conservation and the characteristics of their preferred habitat and refuge areas.

METHODS

Study area

Research was conducted in Trentino region (North-East Italy), in the catchments of seven Alpine rivers and streams: Adige, Avisio, Brenta, Chiese, Fersina, Noce and Sarca (Fig. 1). The survey area lies between 45.971888° and 46.200610° latitude North and from 10.652472° to 11.628384° longitude East, with elevation ranging between 153 and 1199 m asl (Supplementary Tab. 1). The sub-continental climate ensures high precipitation, with cold winters and warm summers. Streams are characterised by the typical alpine pluvio-nival flow regime with minimum discharge in winter and maximum in spring/early summer. Most of the rivers in Trentino are impacted by extensive hydropower exploitation (Zolezzi *et al*., 2009) causing changes of the flow regime along each river. The main geological setting varies from sedimentary (limestone and dolomite in the Chiese, Sarca, Adige and lower course of the Noce catchments) to igneous formations (granite and porphyry in the Avisio, Fersina, Brenta and upper course of the Noce catchments). The mountain slopes frequently preserve the natural forest ecosystems due to the irregular morphology of the territory which limits human exploitation of the valley bottoms. The two major lakes in the investigated area (Caldonazzo and Levico) are important touristic resources for the region, with moderate organic pollution (Perini *et al.*, 2009). Some of the smaller lakes (Madrano and Canzolino) still suffer from civil and agricultural wastes input, occurred during the first part of the last century, despite the restoration actions designed to improve water quality (M. Tolotti, personal communication). Madrano and Canzolino lakes are connected to each other, and Canzolino lake is also connected to Costa lake by a partly artificial canal with intermittent flow, and this setting affected the spread of the alien species.

The investigated sites were chosen for their different ecological and environmental conditions: from the near to natural brooks flowing through mountain woods to the modified rivers of the plains, and from clear water lakes to eutrophic ponds. A total of fourteen lakes, six ponds and sixty-nine stream and river reaches were surveyed (Fig. 1; Supplementary Tab. 1).

Sampling methods

Field work was carried out from April 2010 to September 2012 during the crayfish active season (generally from April to October). Some sites (including all lakes and ponds, and all sites where the invasive species was recorded) were investigated more than once, in the same year or in different years. We conducted some initial trials by coupling visual assessment conducted by day and baited traps set at night. The baited traps were seldom successful in collecting crayfish, even when crayfish were recorded as present by visual inspection. Moreover, because baited traps have been shown to be selective for size and sex (Pratten, 1980; Dorn *et al*., 2005; Price and Welch, 2009) and in some instances ineffective for sampling crayfish in streams (Rabeni *et al.*, 1997), and because trapping, as a passive sampling method, integrates density over an unspecified area (Dorn *et al*., 2005), we chose to use the active sampling method, *i.e.* to visually assess the presence, density, and population composition as sex and size, as already reported in several studies (Pratten, 1980; Streissl and Hödl, 2002; Ďuris *et al*., 2006; Scalici *et al*., 2008; Pilotto *et al*., 2008; Arce and Alonso, 2011). For safety reasons, small brooks and running water habitats were surveyed in daytime, whereas lakes were investigated during the night when the crayfish are most active. Time of sampling was recorded by two researchers on each occasion; each researcher spent one hour searching for crayfish and collecting them with hand nets. In creeks, because we sampled when the crayfish are not active, we searched as exhaustively as possible by hand in all refuges (stones and leaf litter on the bottom of watercourses, holes along the river banks). For lakes, we sampled all the crayfish which were moving outside the shelters. Each specimen was sexed, weighed with an electronic scale (±0.2 g linearity), and sized with a digital caliper (±0.02 mm accuracy). Total carapace lenght, and the length of both chelae were recorded, and the mean chelae length was used for the analysis, with the exception of damaged or regenerated chelae which were excluded. In 2010, we measured only the cephalic length. We recorded missing chelae, presence of damaged body portions, soft specimens, and egg or juveniles-bearing individuals. Visually diagnosable diseases were registered, especially the infestation by the parasitic protozoan *Thelohania* sp., which in the late stages of infection is easily recognisable in crayfish by the typical opaque white colour of the ventral surface of abdominal muscles, in contrast to the translucent coloration of normal uninfected muscles (Sumari and Westman, 1969). Historical distribution data of crayfish in Trentino were collected from literature and by interviewing local residents.

The sampling equipment was sterilised after each use with sodium hypochlorite solution to prevent possible disease diffusions between water bodies. All visited sites were georeferenced and ecological data were recorded for most of the sites where crayfish were present and in some of the sites where crayfish were absent: stream depth and flow, predominant habitat type, environmental characteristics (shading, presence and type of refugia such as boul-

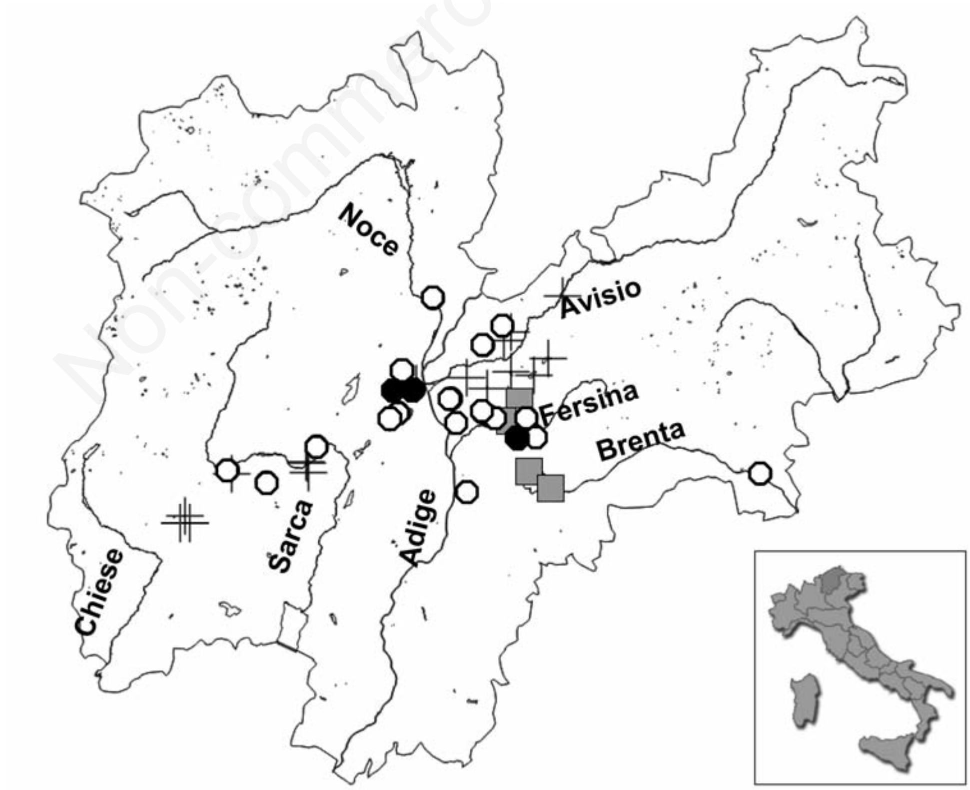

Fig. 1. Survey area in Trentino, divided into subcatchments (Adige, Brenta, Fersina, Avisio, Noce, Chiese, Sarca). Sites are classified based on the presence/absence of the two crayfish species. Crosses represent extinct populations of *A. pallipes* (historical data); circles represent *A. pallipes* populations recorded in 2010-2012 and still existing (white circles) or extinct in 2010-2012 (black circles); grey squares represent *O. limosus* populations recorded in 2010-2012.

ders, cobble, woody debris, tree roots, presence of submerged and emerged vegetation). In addition, the fluvial functionality index (FFI) (Siligardi *et al*., 2007) was calculated for each creek. Physico-chemical water parameters (temperature, oxygen concentration, pH, conductivity and turbidity) were measured on most occasions (with the exception of several dates in 2010) using a WTW Oxi 3310 dissolved oxygen meter [Wissenschaftlich-Technische Werkstätten (WTW) GmbH, Weilheim, Germany], a WTW pH 3310 pH meter (WTW GmbH), a WTW Cond 3310 conductivity meter (WTW GmbH), and a WTW Turb 430 turbidity meter (WTW GmbH).

Data analysis

Morphometric and physico-chemical data collected in 2010 had several gaps and were not used for statistical analysis; 2010 data were used as presence/absence to assess the present distribution of the species. For data collected in 2011 and 2012, non-parametric methods were applied to test group differences. For *A. pallipes*, we always conducted two separate analyses for creeks and lakes, due to different sampling times (day *vs* night, respectively). For *O. limosus*, only one analysis was conducted, since this species was only collected in lakes. For both species, in the few cases where the same site was surveyed twice in the same year, we used only data from the first visit in order to avoid overestimating the differences. For each species, a Mann-Whitney U test was used to test for significant differences in total carapace length, chela length and weight between sexes for all collection sites and months pooled together. To assess seasonal trends, we also tested the same differences separately for each month. A Kruskal-Wallis non parametric ANOVA followed by a Multiple Comparison test was run (separately for each species) to test for significant differences in total carapace length and weight among the different sampling sites with the two sexes pooled together. The relationship between carapace length and weight, and carapace length and chela length in both sexes of the two species was assessed with simple regression analyses, and the significance of the differences between sexes for regression lines was assessed with an ANCOVA analysis. Population abundance was calculated as catch per unit effort (CPUE) for each sampling occasion, based on the sampling time employed by each researcher and the number of researchers. Catch per unit effort was then expressed as n. crayfish min^{-1}. For each species, a chi-squared test was calculated to assess significant differences in number of males and females for all data pooled together. Sex ratio for each population and each sampling date was calculated as number of males/number of females, and differences in sex-ratio among populations were tested with a Kruskal-Wallis non parametric ANOVA test. We used a multiple regression analysis to assess the effects of physico-chemical parameters on CPUE, and we run a principal components analysis (PCA) on a matrix based on environmental data to assess which settings to compare the characteristics of the sites with and without crayfish. All statistical analyses were performed using the softwares STATISTICA ver. 9.1 (StatSoft Inc., Tulsa, OK, USA) (StatSoft, 2010), R ver. 2.15.0. (R Development Core Team, 2012), and PRIMER 6 ver. 6.1.12 (PRIMER-E Ltd., Lutton, UK) (PRIMER-E, 2009).

RESULTS

Distribution

Water temperature ranged between 16.0°C at the end of June in the lakes at higher elevation and 26.7°C in the lakes at the lowest elevation at the beginning of August, and between 10.2°C at the end of July and 19.6°C at the end of August in streams and creeks. Other environmental parameters ranged as follows: i) in lakes: pH, 8.00-8.62; dissolved oxygen, 122.0-66.3 mg L^{-1}; conductivity, 175.5-349.0 μS cm^{-1}; and turbidity, 3.90-11.50 NTU; ii) in streams and creeks: pH, 7.94-8.31; dissolved oxygen, 35.7-94.3 mg L^{-1}; conductivity, 295.0-775.0 μS cm^{-1}; and turbidity, 0.27-10.00 NTU.

Physico-chemical variables did not affect the CPUE, since the results of the multiple regression were not significant (P=0.513, adjusted-R^2=0.393).

Crayfish populations were recorded in 25 of the 89 investigated sites (Fig. 1; Tab. 1, Supplementary Tab. 1). The physico-chemical and environmental characteristics of the sites are listed in Supplementary Tab. 2. The results of PCA (axes 1 and 2 explaining together 66% of the variance) show that crayfish were mostly present in the sites with shading and a substrate provide appropriate refugia (Fig. 2; Supplementary Tab. 2).

Eleven investigated water bodies are seasonal (Cortesano, Monreale, S. Giuliana, Merdar, Vignola, Roncone lake emissary, Valgrande, Diga, Tignerone creeks, and Bellasio ponds; Supplementary Tab. 1) or strongly artificial (Brenta river station 1 near the river source, Trambario stream, channel connecting Canzolino and Costa lakes; Supplementary Tab. 1), and therefore lacked the adequate habitat for crayfish. Four lakes were characterised by the presence of the American species *O. limosus*, wheras four lakes, three ponds and thirteen streams hosted the native species *A. pallipes,* although three of these populations went extinct during the three years of our survey (Fig. 1; Supplementary Tab. 1). Populations of one of the two species were found in the connected lakes of Madrano (*O. limosus*), Canzolino (*O. limosus*) and Costa (*A. pallipes*) during the first sampling campaign in 2010, but the native species disappeared by 2011. Similarly, *A. pallipes* populations recorded in Terlago pond and creek in 2011

Tab. 1. List of sampling sites and relevant information regarding the crayfish, their life cycle and infestation. Data refer to the entire sampling period (2010-2012).

Site	Site code	Date	M	F	Tot.	Sex ratio	CPUE	Mean weight (gr)	Mean CL (mm)	Mean CheL (mm)	Species	Number of individuals with specific characteristics	
												e, j, mM, mF	r/mM, r/mF, The
Costa l.	CoL	26-Jun-10	6	3	9	2.00	0.30		49.5		*A. p.*	0	1r/mM, 1r/mF
	CoL	21-Oct-10	0	0	0	0	0				*A. p.*	0	0
	CoL	28-Jun-11	0	0	0	0	0				*A. p.*	0	0
Gardolo c.	GaC	22-May-10	1	1	2	1.00	0.03		19.5		*A. p.*	0	0
	GaC	20-Oct-10	3	4	7	0.75	0.12		30.6		*A. p.*	0	1r/mM, 2r/mF
	GaC	12-Jul-11	24	35	59	0.69	0.12		30.1	20.4	*A. p.*	5mF; 1mM; 1ex	3r/mM, 12r/mF, 3The
Restel l.	ReL	23-Jun-11	23	17	40	1.35	0.17	7.5	30.2		*A. p.*	0	6r/mM, 5r/mF
Santo Lamar l.	SLL	30-Aug-11	8	11	19	0.73	0.16	3.4	23.0	13.3	*A. p.*	0	0
	SLL	20-Sep-11	7	8	15	0.88	0.13	5.1	25.9	17.2	*A. p.*	0	0
Fornei c.	FoC	4-Oct-11	13	26	39	0.50	0.16	8.8	30.4	20.2	*A. p.*	0	2r/mM, 15r/mF
	FoC	7-Aug-12	10	17	27	0.59	0.09	13.1	34.5	25.4	*A. p.*	0	2r/mM, 4r/mF
Carpine c.	CaC	22-Jun-10	2	2	4	1.00	0.07		45.3		*A. p.*	2mFe	1r/mM, 2r/mF
	CaC	19-Oct-10	12	2	14	6.00	0.23		51.3		*A. p.*	0	5r/mM, 1r/mF
	CaC	6-Jul-11	15	6	21	2.50	0.06	11.7	33.4		*A. p.*	0	2r/mM, 3r/mF
Farinella c.	FaC	3-Jul-10	11	4	15	2.75	0.20		41.6		*A. p.*	1mM; 1ex	0
	FaC	19-Oct-10	3	1	4	3.00	0.06		46.3		*A. p.*	0	1r/mF
	FaC	19-Aug-11	12	39	51	0.31	0.17	10.4	32.2	21.9	*A. p.*	0	4r/mM, 17r/mF
Nero c.	NeC	2-Jul-10	8	4	12	2.00	0.11		46.7		*A. p.*	4ex	0
	NeC	19-Oct-10	0	1	1	0.00	0.02		51.7		*A. p.*	0	1r/mF
	NeC	23-Jun-11	1	1	2	1.00	0.03	7.6	26.1		*A. p.*	0	1r/mM
	NeC	6-Jul-11	6	6	12	1.00	0.03	9.3	32.1		*A. p.*	1mF	1r/mM, 1r/mF
Santa Colomba c.	SCC	3-Jul-10	5	10	15	0.50	0.13		41.5		*A. p.*	1ex	0
	SCC	18-Oct-10	3	4	7	0.75	0.06		37.0		*A. p.*	0	0
	SCC	21-Jul-11	10	20	30	0.50	0.15	6.6	28.4	17.6	*A. p.*	0	2r/mM, 14r/mF
	SCC	19-Aug-11	6	14	20	0.43	0.08	9.6	32.1	20.2	*A. p.*	1mM	4r/mF, 1The
Valsorda c.	VaC	26-Jul-11	2	8	10	0.25	0.08	11.1	32.9	22.3	*A. p.*	1mF	1r/mM, 5r/mF, 1The
	VaC	28-Sep-11	6	8	14	0.75	0.04	6.7	26.4	17.0	*A. p.*	0	1r/mM, 2r/mF
Terlago c.	TeC	30-Aug-11	29	41	70	0.85	0.58	4.1	21.7	13.9	*A. p.*	0	5r/mM, 13r/mF
Grigno c.	GrC	25-Aug-11	6	8	14	0.75	0.12	10.1	31.6	20.0	*A. p.*	0	3r/mF
Santo Cembra l.	SCL	11-Jul-12	20	27	47	0.74	0.24	7.3	34.5	22.1	*A. p.*	0	7r/mM, 4r/mF
Andogno c.	AnC	13-Sep-12	12	11	23	1.09	0.13	2.8	19.4	12.3	*A. p.*	0	4r/mM, 1r/mF
Folon c.	FlC	4-Jul-12	9	7	16	1.29	0.09	11.3	32.0	20.1	*A. p.*	0	4r/mM, 1r/mF
Rango c.	RaC	13-Sep-12	9	17	26	0.53	0.14	1.9	15.9	9.6	*A. p.*	1mM	4r/mM, 8r/mF
Caldonazzo l.	CaL	15-Jun-10	0	0	0	0.00	0				*O. l.*	0	0
	CaL	12-Aug-10	0	0	0	0.00	0				*O. l.*	0	0
	CaL	28-Jun-11	12	10	22	1.20	0.04	20.6	41.6	31.0	*O. l.*	0	1r/mM, 1r/mF
	CaL	2-Aug-11	0	1	1	0.00	0.01	28.5	32.4	49.4	*O. l.*	0	0

To be continued on next page

Tab. 1. *Continued from previous page.*

Site	Site code	Date	M	F	Tot.	Sex ratio	CPUE	Mean weight (gr)	Mean CL (mm)	Mean CheL (mm)	Species	Number of individuals with specific characteristics	
												e, j, mM, mF	r/mM, r/mF, The
Levico l.	LeL	21-Jun-10	10	7	17	1.43	0.15		50.0		*O. l.*	5ex	0
	LeL	21-Oct-10	16	5	21	3.20	0.18		47.6		*O. l.*	0	2r/mM
	LeL	26-Jun-11	11	30	41	0.37	0.23	5.1	26.5		*O. l.*	1mFe	1r/mM, 2r/mF
	LeL	2-Aug-11	20	26	46	0.77	0.13	4.5	25.2	15.7	*O. l.*	0	0
Canzolino l.	CnL	23-Jun-10	18	14	32	1.29	0.64		41.5		*O. l.*	0	0
	CnL	20-Oct-10	6	4	10	1.50	0.08		45.5		*O. l.*	0	1r/mM, 1r/mF
	CnL	22-Jun-11	20	21	41	0.95	0.11	5.4	27.3		*O. l.*	1mFj	0
	CnL	4-Aug-11	26	20	46	1.30	0.19	4.1	24.7	14.2	*O. l.*	0	4r/mM, 5r/mF
Madrano l.	MaL	23-Jun-10	16	6	22	2.67	0.34		41.3		*O. l.*	0	0
	MaL	20-Oct-10	10	2	12	5.00	0.10		41.9		*O. l.*	0	2r/mM
	MaL	22-Jun-11	29	11	40	2.64	0.11	8.0	30.7		*O. l.*	0	0
	MaL	4-Aug-11	19	21	40	0.90	0.33	8.7	31.4	20.0	*O. l.*	0	6r/mF

M, male; F, female; CPUE, catch per unit effort; CL, total carapace length; CheL, chela length; e, females with eggs; j, females with juveniles; ex, exuviae; mM, male moulted individual; mF, female moulted individual; r/mM, male individual with regenerated or missing chela; r/mF, female individual with regenerated or missing chela; The, the individual infested with Theolania*; l., lake; c., creek;* A. p., Austropotamobius pallipes; O. l., Oronectes limosus.

was extinct in 2012. *O. limosus* was found in Caldonazzo lake only from June 2011.

We collected 407 specimens (227 males and 180 females) of *O. limosus* and 652 specimens (287 males and 365 females) of *A. pallipes* over the entire sampling period (2010-2012). The abundance of the different populations and a summary of the abundance for the two species in the two habitats (lakes and creeks) are listed respectively in Tabs. 1 and 2.

Number of males and females collected was significantly different in *A. pallipes* only for creek populations (Chi-squared test: $P<0.001$) with a higher number of females, and did not differ significantly in *O. limosus* (Tab. 2). The sex ratio of *A. pallipes* did not significantly differ among the different populations of creeks or lakes, nor did it differ among populations of *O. limosus* (Kruskal-Wallis test, $P>0.05$). Sex-ratio changed seasonally and annually in the two species showing highest abundance of males in autumn 2010 (Tab. 1). Coupling behaviour was observed in *O. limosus* in October 2010. Only two females with eggs were found during the entire sampling campaign for *A. pallipes* in June 2010, and one female with eggs and one with juveniles for *O. limosus* in June 2011. The total carapace length of these specimens measured 42.36 and 47.92 mm in *A. pallipes* and 37.14 and 23.25 mm in *O. limosus*. Soft individuals were recorded in July and August in *A. pallipes* populations (2.1%), whereas no soft specimens of *O. limosus* were observed.

Individuals of *A. pallipes* affected by the *Thelohania* sp. were found in three creeks in July 2011: Gardolo (3 individuals, representing 1.77% of all the individuals collected in that site), Valsorda (1 individual, 0.24%) and S. Colomba (1 individual, 0.40%) (Tab. 1). Infestation with ectosymbiotic Branchiobdellida (Anellida, Clitellata) was recorded only for the population of *A. pallipes* from Grigno creek.

Density of crayfish populations expressed as CPUE varied over dates and sites. For *A. pallipes,* the population from Terlago creek was the most abundant (CPUE=0.58) and the one from Nero creek the least abundant (CPUE=0.02) in creeks, whereas in lakes the most abundant was the now extinct population in Costa lake (CPUE=0.3), and the least abundant the one from Santo Lamar lake (Tab. 1). Abundances varied seasonally, with a peak in late spring/early summer. An exception was observed in the Carpine creek population that peaked in October (Tab. 1). For *O. limosus*, the most abundant population was recorded in Canzolino (CPUE=0.64), and the least abundant in Caldonazzo lake (CPUE=0.01) (Tab. 1); the same seasonal trend was observed for the Canzolino and Madrano populations with an abundance drop in October 2010 and a progressive increase in June and August 2011 (Tab. 1). The abundance of the Levico population did not change significantly through seasons and between the two years.

Morphometry

The summary of morphometric measurements for the two species in the two habitats (lakes and creeks) is listed in Tab. 2. Populations of *A. pallipes* collected in creeks did not show overall significant sex-related differences in total carapace length, chela length, and weight, although for the sampled period (June-October), total carapace length differed significantly between sexes in July (Mann-Whitney P=0.017) and September (P=0.042); chela length and weight differed significantly between sexes only in September (P=0.044 and P=0.013, respectively). Males and females of *A. pallipes* collected in lakes differed significantly in weight (P=0.004), and only in June in total carapace length (P=0.006). In creeks, size was on average larger in males, the opposite occurred in lakes; males were on average heavier than female and with larger chelae in both habitats and even more so in creeks (Tab. 1). Populations of *O. limosus* (which were collected only in lakes) showed significant sex-related differences only in chela length, particularly in August (P<0.001 in both cases), with larger chelae recorded in males (Tab. 2).

Both species differed significantly overall in total carapace length and weight among different sampling sites (*i.e.* among different populations) (Kruskal-Wallis P always <0.05). For *A. pallipes*, the three lake populations always significantly differed from one other in total carapace length, and two of them differed also in weight (Tab. 3). The creek populations were less variable, with the exception of those from two creeks in the Sarca and one in the Adige catchment (Tab. 3), which differed more from the rest of the creek populations. The mean heaviest populations in the two habitats were those from Restel lake and Fornei creek, the largest from Santo Cembra lake and Nero creek; the lightest and smallest populations were those from Santo Lamar lake and Rango creek (Tab. 2). All four lake populations of *O. limosus* differed significantly from one other in weight and total carapace length (Tab. 4). The heaviest population was from Caldonazzo, the largest from Levico, and the lightest and smallest from Canzolino lake (Tab. 1). In *A. pallipes*, the

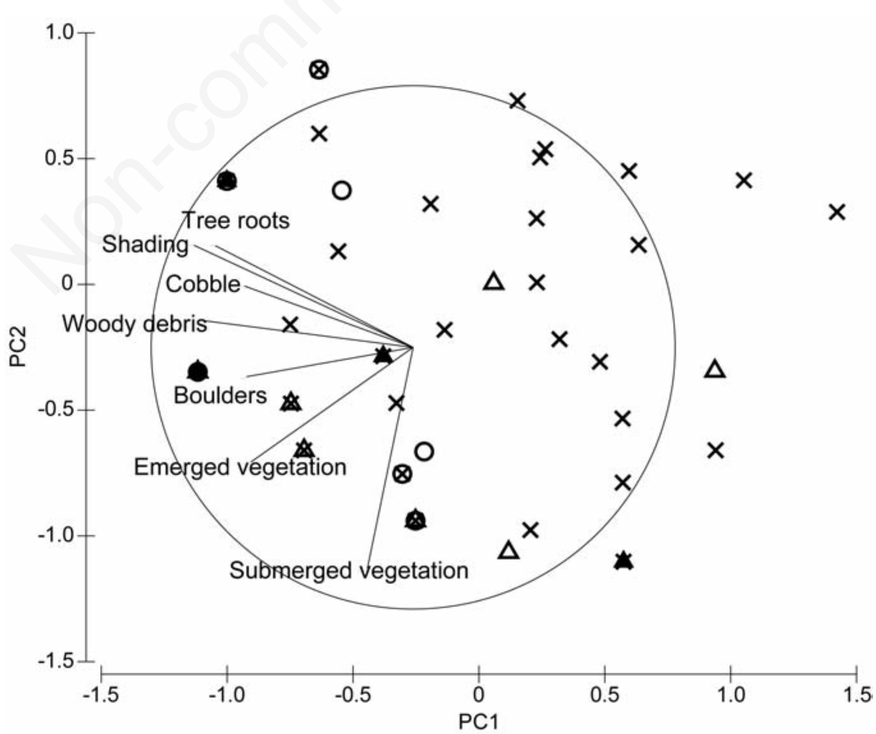

Fig. 2. Principal component analysis of sampling sites characterised by environmental characteristics. ✕ represents sites without crayfish; ▲ represents lakes with *O. limosus*; Δ represents lakes and ponds with *A. pallipes*; ○ represents streams with *A. pallipes*.

Tab. 2. Abundance and morphometric measurements of *Austropotamobius pallipes* and *Orconectes limosus*. Values are calculated over the first visit (2011-2012), unless specified otherwise.

Species	Abundance (2010-2012)			M/F (2010-2012)	Abundance			M/F	
	M	F	Tot.		M	F	Tot.		
A. p. lakes	66	68	134	0.9	51	55	106	0.7	
A. p. creeks	221	297	518	0.8	147	224	371	0.7	
A. p. total	287	365	652	0.8	198	279	477	0.7	
O. l.	227	180	407	1.3	77	76	153	0.9	
	Mean CL			Mean W			Mean CheL		
	M	F	Tot.	M	F	Tot.	M	F	Tot
A. p. lakes	26.8	28.0	27.5	7.2	7.1	7.1	19.0	16.8	17.6
A. p. creeks	32.4	30.0	31.1	7.6	5.5	6.5	22.5	18.9	20.5
O. l.	28.9	29.0	29.0	7.9	7.6	7.7	18.3	14.7	16.5

M/F, median sex ratio; M, males; F, females; Tot, total number of individuals; A. p., Austropotamobius pallipes; O. l., Orconectes limosus*; CL, total carapace length; W, weight; CheL, chela length.*

Tab. 3. Multiple comparison of *A. pallipes* weight and total carapace length among sampling sites utilising the Kruskal-Wallis test. Only significant P values are listed. Data only refer to the first visit (2011-2012). Site codes are the same as in Tab. 1.

	Measurements	GaC	AnC	CaC	FoC	FaC	FlC	NeC	RaC	SCC	VaC	TeC	GrC	SCL	SLL
AnC	W														
	TCL	0.000													
FoC	W		0.000												
	TCL		0.000												
FoC	W		0.000												
	TCL		0.000												
FaC	W	0.005	0.000												
	TCL		0.000												
FlC	W		0.001												
	TCL		0.001												
NeC	W		0.017												
	TCL		0.004												
RaC	W	0.002		0.000	0.000	0.000	0.000	0.000							
	TCL	0.000		0.000	0.000	0.000	0.000	0.000							
SCC	W								0.001						
	TCL														
VaC	W		0.008						0.000						
	TCL		0.003			1.000			0.000						
TeC	W			0.000	0.000	0.000	0.004	0.108			0.049				
	TCL	0.000		0.000	0.000	0.000	0.001	0.005			0.005				
GrC	W		0.003						0.000			0.016			
	TCL		0.007						0.000			0.009			
SLL	W													0.002	
	TCL													0.000	
ReL	W													0.965	0.000
	TCL													0.029	0.002

W, weight; TCL, total carapace length.

Tab. 4. Multiple comparison of *O. limosus* weight and total carapace length among sampling sites utilising the Kruskal-Wallis test. Only significant P values are listed. Data only refer to the first visit (2011-2012).

		Caldonazzo lake	Canzolino lake	Levico lake
Canzolino lake	W	0.000		
	TCL	0.000		
Levico lake	W	0.000		
	TCL	0.000		
Madrano lake	W	0.044		
	0.000			
	0.000			
	TCL	0.032	0.000	0.000

W, weight; TCL, total carapace length.

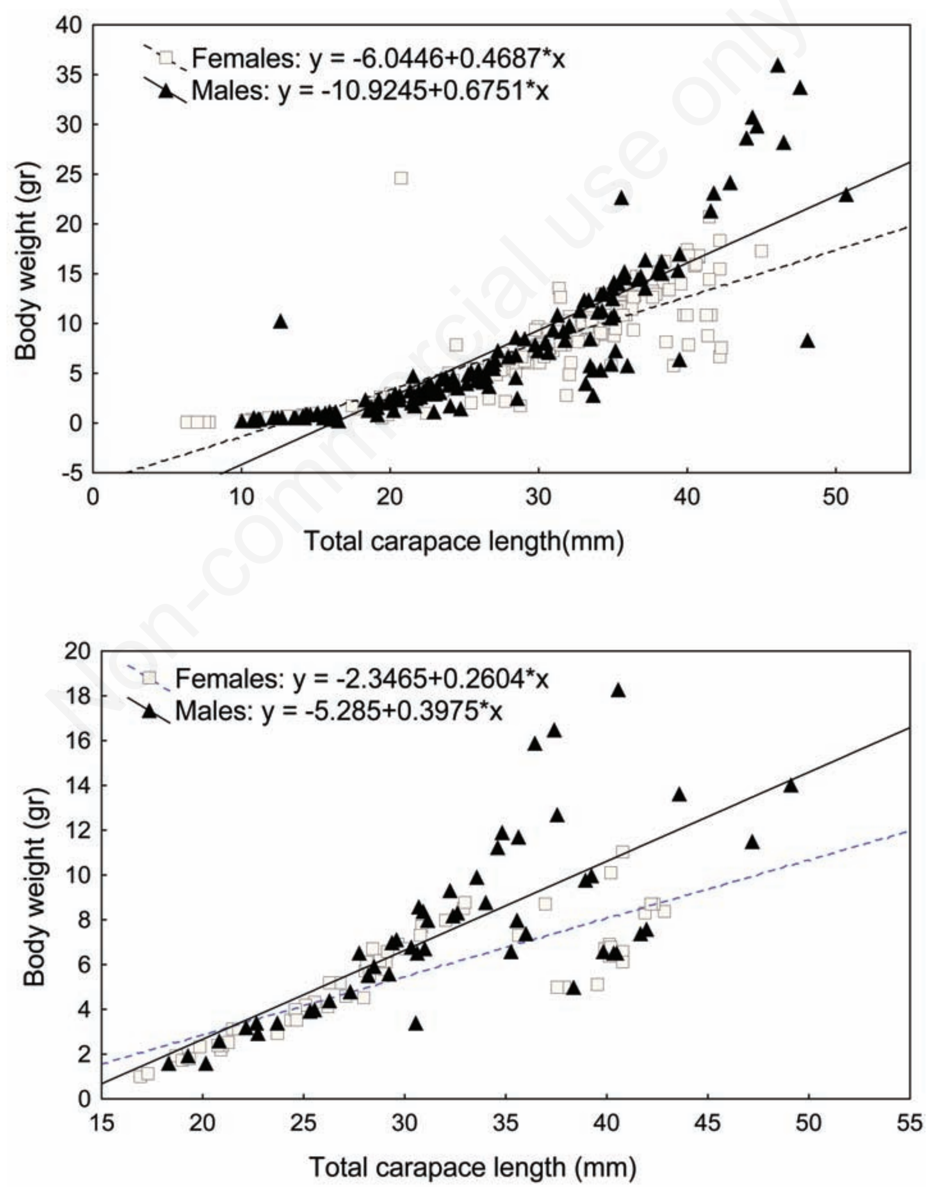

Fig. 3. Regression analysis of weight *vs* total carapace length for *Austropotamobius pallipes*. Creeks are given in the top panel, while lakes in the bottom panel.

regression analysis of weight *vs* carapace length showed significantly different growth rates for the two sexes in lakes and creeks (ANCOVA, P for predictive variable *sex*<0.001) with good correlation values for both sexes (Fig. 3). In creeks, males gained 0.68 g mm^{-1}, and females 0.47 g mm^{-1} (r=0.85 r^2=0.72 with 145 d.f. for males, and r=0.86 r^2=0.73 with 220 d.f. for females). (r=0.85 and 0.86, and r^2=0.72 and 0.73, for males and females, respectively); they grew less in lakes, where males gained 0.40 g mm^{-1}, and females 0.26 g mm^{-1} (r=0.74 and 0.84, and r^2=0.55 and 0.70, for males and females, respectively) (r=0.74 r^2=0.55 with 49 d.f. for males, and r=0.84 r^2=0.70 with 53 d.f. for females).

In *O. limosus*, the regression analysis of weight *vs* carapace length (Fig. 4) did not show significant different growth rates for the two sexes (ANCOVA, P for predictive variable *sex*>0.05) although the correlation values of both sexes were high (Fig. 4); females gained 0.94 g mm^{-1} while males increased by 0.79 g mm^{-1} (r=0.93 and 0.96, r^2=0.87 and 0.93, with 75 and 74 d.f. for males and females, respectively).

Regression analysis of chela length *vs* carapace length

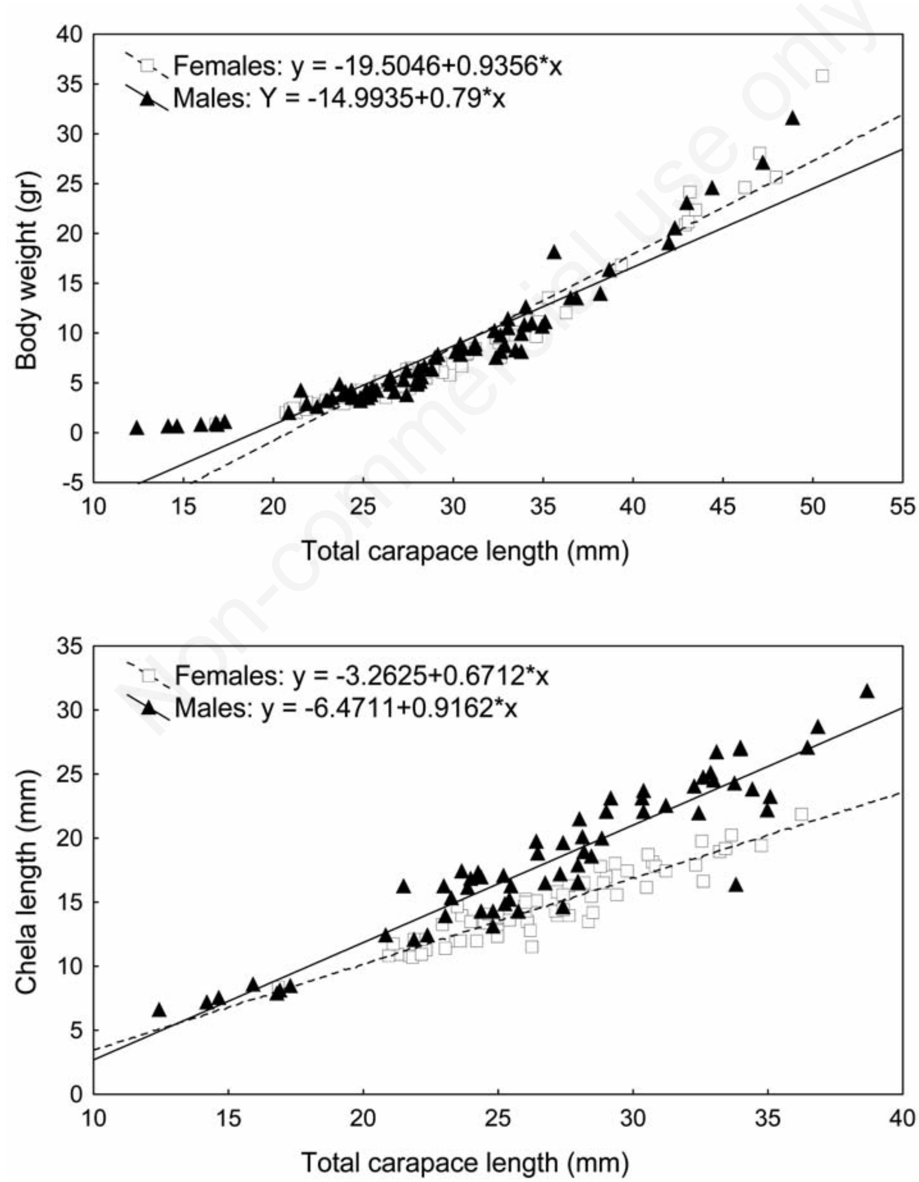

Fig. 4. Regression analysis for *Orconectes limosus*. Top panel provides weight *vs* total carapace length, while bottom panel provides chela length *vs* total carapace length.

was significant for *A. pallipes* (ANCOVA, P for predictive variable *sex*<0.001), with high correlation values in creeks (r=0.95 and 0.96, r^2=0.90 and 0.92, with 122 and 197 d.f. for males and females, respectively) and lakes (r=0.98 and 0.98, r^2=0.96 and 0.97, with 26 and 35 d.f. for males and females, respectively), and with larger chelae in males (Fig. 5). The regression was significant also for *O. limosus* (ANCOVA P<0.001), with high correlations (r=0.94 and 0.94, r^2=0.88 and 0.87, with 61 and 63 d.f. for males and females, respectively) (Fig. 4). Over the entire sampling period (2010-2012), we recorded 167 specimens with lost or regenerated chelae in *A. pallipes* (69.46% females and 30.54% males) and 35 specimens in *O. limosus*: 65.70% females and 34.30% males (Tab. 1).

DISCUSSION

Distribution

This study confirmed the presence of two species of freshwater crayfish in Trentino: the native *A. pallipes* and the invasive alien *O. limosus*. We recorded a drastic reduction in the natural distribution range of the native

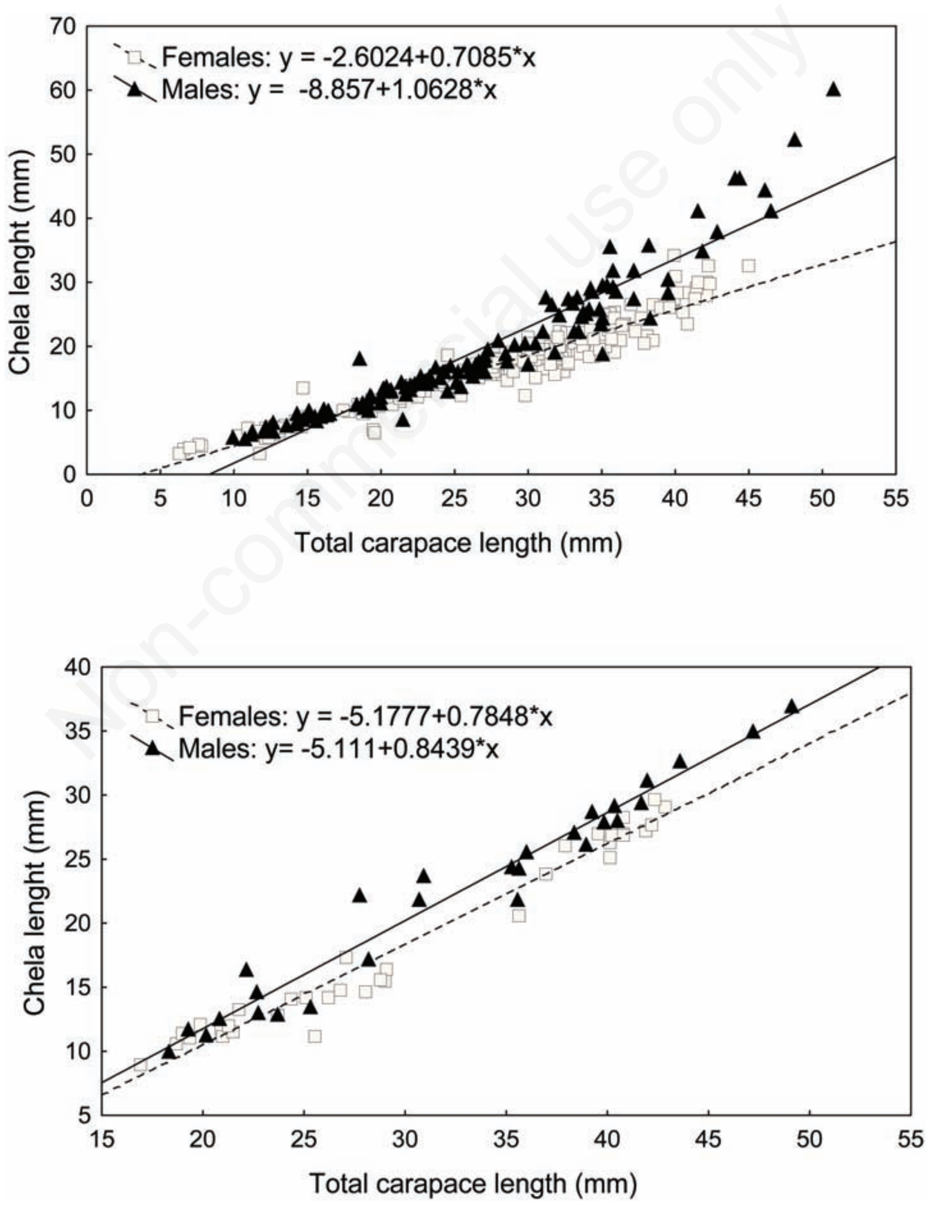

Fig. 5. Regression analysis of chela length *vs* total carapace length for *Austropotamobius pallipes.* Creeks are given in the top panel, while lakes in the bottom panel.

species in the investigated catchments: remnant populations were found in small mountain brooks and lakes, isolated from the great water bodies of the piedmont areas. Although our choice to sample lakes at night and creeks during the day introduced a bias in our results (since crayfish are active at night, if the population density is low, it is possible not to detect the crayfish, or underestimate their densities during the day), repeated surveys over three years in most of the creeks added robustness to our distribution data of the two crayfish species. The comparison of historical data form Albrecht (1982), Pagotto (1995), Provincia Autonoma di Trento (2004), Fratini *et al.* (2005), Maiolini *et al.*, 2007; Paoli (2007) and from our interviews with local residents (unpublished material), with the distribution recorded in our study, shows a progressive extinction of the native species through the last fifty years. Native populations were lost in ten running water habitats (Roncone creek and lake emissary, Gamberi, Squero, Pravert, Poia, Banale, Terlago, Scorzai, creeks, and Avisio stream), one pond (Terlago) and nine lakes (Caldonazzo, Levico, Piazze, S. Colomba, Serraia, Lases, Lamar, Roncone, Costa), and probably in Madrano and Canzolino lakes. Only in four lakes (Levico, Caldonazzo, Madrano, and Canzolino) the extinction of *A. pallipes* was followed by the invasion of *O. limosus*. So far, *O. limosus* was not found in running water sites in Trentino.

The main causes of decline or extinction of *A. pallipes* populations are the reduction of its habitat (*e.g.* physical and hydrological alterations of the waterbodies, chemical pollution, reduction of riparian vegetation), the introduction of alien species which are better competitors for resources and/or transmit diseases and parasites and, in a few cases, exploitation through fishing (Jay and Holdich, 1981; Holdich and Lowery, 1988; Souty-Grosset *et al.*, 2006; Brusconi *et al.*, 2008). Among these factors, physical and hydrological alterations of the waterbodies are reported as one of the main causes of crayfish extinction. For instance, overexploitation coupled with loss of pristine riverine landscape was responsible for the local extinction of populations of *A. pallipes* in Central Italy (Brusconi *et al.,* 2008). In the Adige watershed of South Tyrol, habitat alteration and degradation have contributed to the high degree of population decline and loss of *A. pallipes* (Füreder *et al.*, 2003; Sint *et al.*, 2007). The reduction or destruction of riparian vegetation due to the channelisation of the riverbed is one of the causes of crayfish extinction (Aquiloni *et al.*, 2010); the importance of riparian vegetation for the survival of *A. pallipes* has recently been demonstrated by Brusconi *et al.* (2008) in Tuscany and its presence is a main factor in Trentino too. In this region, populations of *A. pallipes* were mostly restricted to small creeks in wooded hillslopes, and disappeared in the downstream lowland areas, where agricultural practices and altered habitat conditions (loss of riparian habitat) were dominant. Possibly, the absence of riparian wooded vegetation was the main cause of the observed sudden reduction or disappearance of crayfish populations, which occurred also in creeks flowing in pasture areas, with little or no chemical impact but without riparian vegetation. The loss of wooded riparian vegetation implies the lack of temperature and light regulation, input of coarse particulate organic matter (CPOM) and woody debris and submerged roots on the banks for refuge. In our study, crayfish were not present in the reaches impacted by intensive agriculture, suggesting sensitivity to pollution, albeit mixed with the habitat alteration associated with agricultural practices (mainly channelisation and absence or periodic cut of riparian vegetation). *A. pallipes* has been reported to be vulnerable to organic pollution (Trouilhé *et al.*, 2007), but its susceptibility to pollution has been debated (Füreder and Reynolds, 2003), and this species has been able to survive in poor quality waters, being found in acid, peaty areas, in moorlands and in eutrophic angling lakes (Demers and Reynolds, 2002). In Italy, local extinctions of *A. pallipes* were ascribed to sporadic events of point chemical pollution (Renai *et al.*, 2006). Illegal fishing is a further cause of reduction in the integrity of crayfish populations (Aquiloni *et al*., 2010), and is reported as one of the main causes of local extinctions of *A. pallipes* in Tuscany (Renai *et al*., 2006); in Trentino, fishing was probably the cause of recent extinctions in the Sarca catchment.

In general, studies of competitive interaction between native and alien species of crayfish in Europe showed that the native species is usually negatively affected, with resulting reduction of its distribution and population density (Vorburger and Ribi, 1999; Pöckl and Pekny, 2002; Füreder *et al.*, 2006; Brusconi *et al.*, 2008). A further cause of extinction which is often associated with the introduction of alien invasive species, is the spread of parasites and diseases which alien species are resistant to, and which can be lethal for the native populations (Aquiloni *et al*., 2010). Among the most common parasites, the protozoan *Thelohania* sp. causes a chronic disease which results in death, even leading to mass kills (Diéguez-Uribeondo, 2006). A very low percentage of specimens of *A. pallipes* in the population investigated in Trentino were infested, suggesting that this parasite does not represent a threat to their conservation (Alderman and Polglase, 1988). Similar results (infestation rate ranging from 0.17 to 3.7%) were recorded in other studies in Italy and Spain (Mori and Salvidio, 2000; Diéguez-Uribeondo *et al.*, 1997a). As reported by Vogt (1999), diseases may play an important role in maintaining the ecosystem balance through the control of population density. Parasites can negatively affect populations in disturbed habitats (Vogt, 1999) and their spread rate should be monitored, as recommended by Diéguez-Uribeondo *et al.* (1997a). The oomycota *A. astaci* is the etiological agent for the crayfish plague, the principal cause of mass mortalities in native crayfish populations in Europe (Holdich, 1999), and the cause of 100% mortality in the affected populations (Diéguez-Uribeondo, 2006). The crayfish plague exterminated the *A. pallipes* populations in Lombardia region (Northern Italy) (Alderman, 1996). According to Aquiloni *et al.* (2010), by 2010 there were no populations of native crayfish infected with *A. astaci* in Italy, with the exception of one episode of mass mortality of *A. pallipes* recorded in Southern Italy and assigned to *A. astaci* infection (Cammà *et al.*, 2010). However, a screening for the assessment of infestation by *Aphanomyces astaci* in populations of *A. pallipes* and *O. limosus* from Trentino including specimens from Caldonazzo, Levico, Madrano, Canzolino lakes for *O. limosus*, was recently conducted with a histological and biomolecular approach, and the preliminary results indicate a high infestation rate for Caldonazzo, Levico and Canzolino populations (Minghetti *et al.*, 2012). The same populations were not affected by ectosimbiotic branchiobdellids, which are common in autoctonous populations of *A. pallipes* (Souty-Grosset *et al.*, 2006). The presence of infested populations of the invasive species is very likely related to the disappearance of populations of *A. pallipes* in the same sites.

The recorded distribution of the two crayfish species in Trentino for each investigated catchment is the following: for the Trentino section of the Brenta catchment, the last evidence of *A. pallipes* in the two main lakes dates back to 2004 (Provincia Autonoma di Trento, 2004). The extinction of the native populations could have been determined by the competition with the invasive species which was introduced in Levico lake before 2006 when it was first recorded by Maiolini *et al.* (2007), and by the consequent spread of *Aphanomyces astaci*. Today, the native species has completely disappeared from this lake, and the alien species is widespread and with very high population density. The oomycosis, diffused through fishing equipment or aquatic animals, was possibly the only cause of the disappearance of *A. pallipes* in Caldonazzo lake. In fact, recent data (Minghetti *et al*., 2012) confirm the presence of infested specimens of *O. limosus* in both lakes. The present distribution of the invasive species in Caldonazzo lake, where we recorded only one small population in a restricted area, suggests that the colonisation is very recent and the species still has to expand its distribution. Only one population of *A. pallipes* was recorded in running waters in the Brenta catchment, *i.e.* at the Grigno creek site; this population is isolated in a short reach with semi-natural conditions. In the Sarca catchment, the distribution of *A. pallipes* is nowadays greatly reduced: in fact, we found only three populations in three minor tributaries (Rango, Folon, and Andogno creeks) out of the twenty creeks and one site on the Sarca river we surveyed. Historically, as deducted from interviews with locals, the native crayfish were present in some minor tributaries: Banale and Travert creeks (last record around 1950), Poia creek (last record dating 2008), and Squero creek (last record at the end of the 1980s). *O. limosus* has not been recorded for this catchment. The causes of disappearance of several populations of the native species might be related to the strong capture pressure occurred in the last century in the area, where crayfish was traditionally an important food source. Recently, specimens of *A. pallipes* infested with *A. astaci* were collected in a fish farm at a short distance from a tributary of the Sarca river (Minghetti *et al*., 2012). In the Chiese catchment we surveyed only Roncone lake and its emissary and tributary where crayfish were observed from the late 1980s (Maiolini, unpublished material) to a few years ago (local interviews). However, the lake and tributary populations are today extinct, and the emissary was dry at the time of our survey. The cause of extinction for the Roncone populations was, together with habitat reduction, the possible spread of the parasite *A. astaci*, which was recorded in a population of *A. pallipes* on the Chiese river, 11 km downstream of Roncone lake, in 2010-2011 (Pretto and Manfrin, 2011). In the Fersina catchment, high density populations of *O. limosus* were observed in Madrano and Canzolino lakes. Despite the absence of historical data, the presence of the native species in Costa lake (downstream, and hydrologically connected to the above-listed ones) in 2010 implies a past presence of the native species also in these lakes. *A. pallipes* possibly disappeared there, due to the high organic pollution occurred in Madrano and Canzolino during the 1950s. The native populations were probably replaced by the more tolerant *O. limosus*. The Costa lake native population of *A. pallipes* still survived downstream from these water bodies colonised by *O. limosus*, but were extinct the second survey year (*i.e.* 2011), probably due to the transmission of *A. astaci* through the water flow from the *O. limosus* infested Canzolino lake (Minghetti *et al*., 2012). Four more populations from lakes in the catchment (Piazze, Serraia, Lases and S. Colomba) went extinct during the last six years, having been previously recorded there (Provincia Autonoma di Trento, 2004; Paoli, 2007; Maiolini, unpublished material). The strong decrease in *A. pallipes* density recorded from the first to the second year of survey in Nero creek was probably caused by the exceptional flood and related high load of fine sediment, occurred at the end of the summer 2010, which caused stranding of crayfish and massive mortality (as reported by local residents). Moreover, the population is periodically affected by the reduction of the minimum vital flow released from an upstream small dam, due to recurring partial obstruction of the outlet conduit (reported by local residents and anglers), resulting in a decrease of available microhabitats. We found four populations of *A. pallipes* in the Adige left tributaries flowing through the wooded slopes of Mount Calisio: S. Colomba, Farinella, Gardolo and Carpine creeks. Despite the good environmental conditions, the population of Gamberi creek, reported to be very abundant in the past century by local residents, was extinct when we surveyed the site in 2011. The habitat quality of these creeks decreases significantly moving downstream, especially on the valley bottom where they are often channelised and flow in urban areas. Relict isolated populations in the piedmont areas were in fact found only near to few natural reaches. For the Adige right tributaries, an unusually high crayfish density was recorded in a restricted and isolated upstream reach of Terlago creek, which is not affected by agricultural pollution and has an extended cover by riparian woods. The last evidence of *A. pallipes* in Lamar lake dates back to 2007. Local residents mentioned that illegal fishing of crayfish occurred in Lamar lake, and this could be one of the reasons of the extinction. A population of the native species still survives in Santo Lamar lake, at less than two hundred meters distance from Lamar lake. In the Noce stream, the main right tributary of the Adige, we surveyed only one site, an artificial pond in the backwater, where we recorded *A. pallipes*. More sites will possibly be added in the future. In fact, there are records of the presence of this species in the mainstream of the Noce, adjacent to the same pond, dating to the mid-90s (Maiolini, unpublished material).

Morphometry

The sexual dimorphism in growth rate, observed for *A. pallipes* in both habitats, is probably due to the different moult cycle in the two sexes. Males and females have different growth rates once they reach sexual maturity (Pratten, 1980): ovigerous females moult only once per year after they release their young, whereas males usually moult twice during the warm season (Pratten, 1980). The relationships between length and weight, and chela length and total body length obtained for the two sexes showed that males of *A. pallipes* gain weight faster than females and have proportionally larger chelae; similar results are reported in other studies which attribute the greater weight of males to the higher number of moults and to the larger size of their chelae (Brewis and Bowler, 1982; Rhodes and Holdich, 1984; Streissl and Hödl, 2002; Milka *et al*., 2006; Muzaffer and Utku, 2006). On the other hand, females of *O. limosus* increased in weight faster than males in our study, as reported by Chiesa *et al.* (2006) who ascribed the faster growth of females to the higher energy needed to develop the eggs. Results of other studies are controversial, *e.g.* differences in weight growth rate among sexes of this species were not significant (van den Brink *et al*., 1988), or males gained weight faster than females, due to the larger chela size (Ďuris *et al.*, 2006). We recorded a faster increase in chelae size in males than in females in both species, a competitively advantageous and allometric trait for males (Streissl and Hödl, 2002). Weight regulation in crayfish in our study sites is probably influenced by multiple factors, especially for *A. pallipes* which showed a higher growth rate in creeks. In fact, crayfish growth is influenced by environmental conditions: decrease in growth range was observed in crayfish as a consequence of low temperatures, short growing season, limited food availability, high competition, spread of parasites and body damages (Thomas and Ingle, 1971; Bowler and Brown, 1977; Pratten, 1980). The existence of genetic influences on the variability of weight and body length in different crayfish species is also recognised (Lutz and Wolters, 1989; Henryon *et al.*, 1999; Jones *et al.*, 2000). The morphometric differences observed in this study among crayfish populations are probably due, in most cases, to the environmental conditions of the sampling sites. The largest weight and body size recorded in Caldonazzo population can be attributed to the presence of only mature individuals which are probably recent colonisers with little or no competition for food resources. Genetic factors, due to the distribution pattern of the investigated populations, could be the reason of the phenotypic similarity observed between populations of *A. pallipes* from creeks of western Trentino (Terlago, Andogno and Rango creeks, Santo Lamar lake populations) (*i.e.* geographical proximity), and of higher phenotypic dissimilarity among lake populations (*i.e.* geographical isolation).

The incidence of chela loss in crayfish populations has been related to competition for natural resources (Figiel and Miller, 1995), mating (Ameyaw Akumfi and Hazlett, 1975; Ameyaw Akumfi, 1981) and moult processes (Powell *et al.*, 1998), and the energy required to regenerate the appendages could affect fecundity in terms of numbers and quality of eggs produced by females (Powell *et al.*, 1998). The high percentage of females with missing or regenerated chelae recorded in this study suggests the influence of reproductive behaviour in chela loss, such as aggressive interaction related to copulation (Kawai *et al.*, 1994). The populations we investigated, in fact, always had food and shelters available for the recorded densities, probably resulting in low competition for resources.

Crayfish abundance varied seasonally, reflecting a major activity during the summer period for both species. The sex ratio was unbalanced in creek populations of *A. pallipes* with a greater number of females, which may indicate high reproductive efficiency, as suggested by laboratory studies (Carral *et al.*, 1994, 2000). Moreover, each male of *A. pallipes* can mate with at least eight females (Reynolds *et al.*, 1992). These reproductively-efficient traits, however, are counterbalanced by a slow growth rate, late sexual maturity and low fertility (Brusconi *et al.* 2008; Aquiloni *et al.*, 2010). On the contrary, in our study the sex ratio of *O. limosus* populations was unbalanced towards males, as reported in other studies on this species (Ďuris *et al.*, 2006; Pilotto *et al.*, 2008). The male-biased sex ratio notwithstanding, *O. limosus* abundance appeared to increase quickly in all the monitored populations, probably due to the fast growth rate, high fertility, and early sexual maturity reported for this species (Kozák *et al.*, 2007; Souty-Grosset *et al.*, 2006).

Seasonal variations in sex ratio were observed in both species. The strong decrease of female abundance in autumn probably reflects their elusive behaviour after mating, which could have resulted in undersampling. Similarly, the total absence of soft specimens in *O. limosus* might be attributed to nocturnal sampling which involves the capture of only active individuals, moving outside their shelters.

Conservation

The environmental survey indicates that small creeks in mountain slopes, with well-developed riparian vegetation, can represent potential refuge and recruitment areas which can be reasonably expected to sustain populations of *A. pallipes* in favourable conditions for the foreseeable future, without significant management intervention. Nonetheless, a sustainable management of piedmont water bodies would allow preserving or increasing the number and density of the relict populations. Observations carried out in Grigno, Gardolo (piedmont stretch) and Terlago creeks, where *A. pallipes* is present but only in those few reaches with submerged and riparian vegetation, and disappears in the reaches where vegetation is periodically cut, suggest that crayfish are negatively affected by the practices and alterations associated with agriculture, and not necessarily by urbanisation. In these cases, stream restoration could recreate suitable habitats for crayfish, allowing their conservation also in urban environments. The removal of unnecessary barriers could improve the connections between the mountain areas and the valley bottom, thus avoiding the fragmentation of populations and increasing gene flow. Observations carried out in the Fersina catchment suggested that crayfish distribution could be limited by altitude. With increasing altitude, water velocity increases as well, temperature decreases, and the lentic habitats favoured by crayfish disappear. In fact, we collected *A. pallipes* at a maximum elevation of 1199 m asl for lentic habitats, and 805 m asl for running water habitats.

Studies conducted in mountain areas have shown a diffusion pattern of *O. limosus* limited to large rivers and standing waters (Hefti and Stucki, 2006; Pöckl and Pekny, 2002). Petrusek *et al.* (2006) observed that the colonisation of small streams was restricted to confluence areas with major rivers. *O. limosus* is a dangerous invasive crayfish moving rapidly upstream, as well as downstream (Hudina *et al.*, 2009), with a high possibility of penetration into native crayfish habitats. So far, populations of *O. limosus* observed in this study were limited to the great lakes of the piedmont areas. *O. limosus* was recorded in the Grigno ditches connected to the Brenta river one decade ago (Maiolini, unpublished material). The high risk of the alien species spreading throughout the Brenta river is a threat to the populations of *A. pallipes* living in the piedmont areas of the Brenta basin, as for instance in the Grigno population. The diffusion of *O. limosus* from the lakes through the Fersina stream could affect the native populations living in the connected streams (*e.g.* Nero, Santa Colomba, Farinella streams), especially in the confluence areas.

CONCLUSIONS

The results presented here contribute to widening the knowledge of the distribution and morphometry of crayfish in Italian Alps. More research, based on a molecular and genetic approach, is needed to propose and sustain successful conservations plans for the native species *Austropotamobius pallipes* based also on an effective reintroduction in target areas.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Andreas Meraner (Research and Innovation Centre, Fondazione E. Mach), Francesco Mezzo, Alessio Miorandi, Sara Luchetta, Michele Scala, Sara Cortellazzi, Ilaria Parisi for helping with field activities; Giovanna Minghetti (Centro Trasferimento Tecnologico, Fondazione E. Mach) for providing information on crayfish infestation; Mauro Carolli (Department of Civil, Environmental and Mechanical Engineering, University of Trento) for preparing the distribution map; and Timothy Schmidt for the linguistic revision of the last version of the manuscript.

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