

LIFE HISTORY TRAITS OF THE AMERICAN SPINY-CHEEK CRAYFISH IN TWO SUBALPINE LAKES

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DECAPODA
CAMBARIDAE
NORTHERN ITALY
INVASIVENESS
REPRODUCTION

ABSTRACT. – The ecology of *Faxonius limosus* in non-native habitats is poorly understood and needs to be broadened to prevent and control its spread and protect native riverine decapod populations. The aim of this study was to describe some autecological traits of a spiny-cheek crayfish population established in two subalpine Italian lakes. We sampled the littoral zones in May and October 2014 by using hand-nets and baited traps: captured individuals were then sexed, weighed and measured. A population analysis was also performed using the modal progression analysis of length compositions. Our findings underlined the existence of four age classes (with a life expectancy of 3.5/4 years), a lower growth rate compared to other crayfish of the same family, and shift of the reproductive period respect to other studies on *F. limosus*. Our results suggested that eradication activities ought to be developed based on the situationality, and mitigating projects should be integrated with a continuous and practical monitoring programme to assess eventual changes in the ecological requirements of the investigated species. This becomes increasingly important as reintroduction or restocking of indigenous species could also be considered as a management strategy for *F. limosus* populations in the study area.

INTRODUCTION

Preventing the arrival of invasive species remains to date the only environmentally sound approach to avoid biological pollution and detrimental environmental modifications (Gollasch & Leppäkoski 1999). Anyway, once introduced species established, attempts to eradicate aliens or mitigate their impact are often considered a priority within any restoration project (Holdich *et al.* 1999). Eradication at an early stage of invasion may be effective with little or superficial knowledge of the population biology of the invasive species (Simberloff 2003). On the contrary, when the opportunity for rapid eradication has been lost, knowledge of the species biology is however recommended to avoid useless attempts in developing management strategies (Bubb *et al.* 2006). In this case, the ecology of non-invasive species needs to be investigated to manage any released taxa for conservation or containment purposes. Among introduced taxa, crayfish are a group of species whose spread is of particular concern to conservationists, since dramatic losses of native crayfish populations coming into contact with invasive species are reported (see Lodge *et al.* 2012).

Crayfish (Astacoidea) are the largest mobile freshwater invertebrates and are considered key members of littoral communities. Their activity can be influenced by temperature (Lozan 2000, Barbaresi & Gherardi 2001, Bubb *et al.* 2004), light intensity (Bojsen *et al.* 1998, Gherardi 2002), shelter availability (Barbaresi & Gherardi 2001, Martin & Moore 2007), food availability (Statzner *et al.* 2000,

Gherardi 2002), the presence of predators (Jordan *et al.* 1996, Nyström 2002), and various interspecific interactions (Gherardi 2002, Bubb *et al.* 2006). They frequently attain high biomass and interact with multiple trophic levels due to their omnivorous feeding habits and behavioral activities, *e.g.* burrowing and bioturbation (Momot 1995, Nyström 2002).

All European indigenous crayfish are endangered throughout their central European range and the presence of non-indigenous crayfish species is among the biggest threats to remaining stocks (Souty-Grosset *et al.* 2006, Holdich *et al.* 2009). Non-indigenous crayfish species may also have strong detrimental effects on the integrity of the receiving community, including a reduction in α -diversity and species abundance (Nyström 2002, Rodriguez *et al.* 2005), since they are superior competitors or predators of native species and can profoundly affect native ecosystems (reviewed in Holdich *et al.* 2009). Furthermore, invasive crayfish can be vectors for crayfish plague, a disease caused by the fungal-like pathogen *Aphanomyces astaci* (Schikora, 1903) (Holdich *et al.* 2009).

Although not included among the ‘100 of the worst’ invasive aliens in Europe (DAISIE 2017), the spiny-cheek crayfish *Faxonius limosus* (Rafinesque, 1817) (for the taxonomic review see Crandall & De Grave 2017) is inserted in the list of Unional species of concern (EU Regulation 1143/2014). Indeed this invasive alien species receives particular attention due to its capability to carry and spread the crayfish plague (see Kozubíková *et*

al. 2011). Unfortunately, its population ecology in non-native habitats is only partially understood and needs to be broadened (see Jimenez & Faulkes 2011).

The aim of this study was to describe, for the first time, some autecological traits of a spiny-cheek crayfish population established in northern Italy, and discuss our findings implementing required management actions aimed at limiting its spread to potentially suitable nearby habitats. This study, therefore, represents a contribution to improve the knowledge on its ecological plasticity by comparing our findings to other studies, in order to (1) observe eventual differences affected by geographic areas, and (2) understand if managing activities needs some kinds of regionalization.

METHODS

Study area: The study was carried out in two subalpine lakes (Trentino, northern Italy). Lake Levico (surface area = 1.16 km²; volume = 12.942 × 10⁶m³; mean depth = 11 m; maximum depth = 38 m, Brenta Basin) is a mesotrophic lake, with banks continuously covered by abundant riparian vegetation, although aquatic plants may be missing in some areas used as bathing beaches. This lake is a Site of Community Importance (SCI) “Canneto di Levico” (IT 3120039). Lake Canzolino (surface area = 0.07 km²; volume = 0.52 × 10⁶m³; mean depth = 7 m; maximum depth = 15 m, Fersina Basin) is a small, shallow and eutrophic lake (Hansen & Flaim 2007), with forest and agricultural land cover in its catchment; the main inlet of Canzolino is a channel flowing from eutrophic lake Madrano. In rainy years, it has a hydrological connection to Lake Costa, another SCI site (SCI IT3120041) (Flaim *et al.* 2013).

Sampling design and data collection: We randomly sampled the littoral zone of the two lakes in May and October 2014 by using hand-nets during a visual census and by 18 baited traps (10 in Lake Levico and 8 in Canzolino) released *in situ* all night long.

After their capture, crayfish were sexed, measured, weighed and temporarily put in tanks. Measurements, taken with a vernier caliper to the nearest 0.05 mm, were length of right chela (RChL), finger (FL), cephalothorax (CTL), and total body (TL), and width of right chela (RChW) and width of abdomen (AW). Only the right claw was measured because of high claw symmetry (see Chiesa *et al.* 2010) and several specimens showing regeneration of the left chela. Specimens were weighed (W) by using a field balance (to the nearest 0.1g). Health status was noted along with berried females with eggs or juveniles on the abdomen and the pleopodal eggs were counted, and a correlation analysis between the pleopodal egg number and CTL was executed. The external egg diameter was also measured by vernier caliper. The range of CTL value at which 50 % of females were berried (CTL₅₀) was interpreted as the size at maturity in females.

Finally, all captured specimens were removed and subsequently sacrificed by a gradual freezing.

Data analyses: First, the balanced sex ratio was tested by the χ^2 .

Then, the relationship between CTL and all remaining measurements was first assessed by Pearson’s correlation test to evaluate the allometry degree and possible morphometric differences between populations and sexes. Allometry was described according to the following linearization:

$$\log y = \log a + b \log x$$

where y and x are the dependent variables (that is all surveyed biometrics) and reference dimension (CTL), respectively, a is the proportional factor, and b the slope, that is the coefficient of allometry. In size/size relationships, allometry is positive when $b > 1$, negative when $b < 1$, absent (= isometry) when $b = 1$. As for the weight, in the size/weight relationship, the isometry is shown when $b = 3$, while the allometry when b significantly differs from 3 (positive when $b > 3$, negative when $b < 3$). We executed the log transformation to satisfy the assumptions of regression analysis (see Sokal & Rohlf 1981).

The slope of each allometric function was tested against an isometric standard slope by a t-test. Moreover, slopes were compared (using t tests) between sexes within each lake and between the two lakes within each sex. Then, the 20 smallest and 20 biggest individuals per sex were used to generate two different regression lines (1 for smallest specimens and 1 for biggest ones), by using CTL as independent variable, and remaining surveyed biometrics as dependent ones. By drawing both lines in a diagram, the size at maturity is then given as a range where the lines cross, indicating the point when the growth rate changes.

Additionally, population analysis was carried out separately for the two sampling months in order to perform a modal progression analysis of length compositions, widely adopted for different purposes in indigenous (Brusconi *et al.* 2008, Scalici *et al.* 2008a,c) and introduced (Chiesa *et al.* 2006, Scalici & Gherardi 2007, Scalici *et al.* 2010, Dörr & Scalici 2013) freshwater decapods. Specifically, we evaluated some population feature to obtain indirect information on the health status of the American spiny-cheek crayfish in north-eastern Italy. Body size data (specifically CTL) were used to generate polymodal frequency distributions, then analyzed by the Bhattacharya (1967) method (BM) that decomposes size frequency distributions into diverse normal components (each component being identified as a cohort), up to the total decomposition of the overall diagram. The process converts normal distributions into lines that simplify the procedure. For each Gaussian component, mean (ME), standard deviation (SD), individual number per size class (N), regression line (and the respective R²), and separation index values for each adjacent group (SI) were provided. In particular, SI ≥ 2 denotes when two adjacent Gaussians can be separated (Clark 1981). At the end of the separation process, a χ^2 -test value for testing the significance of the decomposition process was provided. Depending on the month in which the major number of eggs occurred and on the recruitment period (when hatched young become ‘catchable’), we defined the first life year for the

studied population. Specifically, we attributed the age 0+ (individuals with less than 12 months) to the first class obtained by BM. Then, outputs of the BM analysis were used to evaluate the growth rate according to Von Bertalanffy (1938), by the following equation (Pauly *et al.* 1992):

$$L(t) = L_{inf} \{1 - \exp[-k(t - t_0)]\}$$

where $L(t)$ is the length at age t , L_{inf} is the asymptotic length (computed as $L_{max}/0.95$, where L_{max} is the maximum recorded length, according to Pauly 1981), k the curvature parameter, and t_0 the initial condition parameter (see Sparre & Venema 1996). The previous Von Bertalanffy parameters (VBP), that is k , L_{inf} , and t_0 , were calculated by a nonlinear regression analysis (NRA). Growth parameters obtained for males and females were then compared using the multivariate Hotelling's T^2 -test. Since crustaceans moult during their life, their growth rate may appear not conform to the Von Bertalanffy model due to some 'stepwise curve', where each step represent a moult event, overall when the sample size is not so high.

To confirm and reinforce results from NRA, VBP were also described by other two approaches: the electronic length frequency analysis (ELE), and the Gulland & Holt (1959) plot (GHP). ELE allows the estimation of growth parameters using sequential length-frequency data imported in the FiSAT (FAO ICLARM Stock Assessment Tools) software (Gayaniilo *et al.* 1996), and is based on the computation of two elements: 'available sum of peaks' (ASP) and 'explained sum of peaks' (ESP) (for more details, see Gayaniilo & Pauly 1997). In particular, here we used the scan of k -values, which allows to estimate k by plotting the fit index (*i.e.* $R_n = 10ESP/ASP/10$) versus k . The highest R_n value observed in the diagram corresponds to the estimated curvature parameter of the population. Instead, GHP is based on length-at-age data and on the concept that growth rate declines linearly with length. This method plots the difference between the means of two adjacent (not necessarily consecutive) cohorts (dL/dt) versus the mean of the same adjacent cohorts [$L'(t)$] to obtain the following linearized function:

$$dL/dt = a + b \times L'(t)$$

where the slope b with the changed sign is the curvature parameter ($k = -b$) and the ratio between the intercept (a) and the slope is the asymptotic length ($L_{inf} = -a/b$). Additionally, the coefficient of determination was calculated to determine the significance of the regression function.

Once VBP were calculated, other population properties were evaluated. In particular, the total mortality index Z (the sum of natural and fishing mortality) was calculated using the Powell-Wetherall Plot equation (Powell 1979, Wetherall 1986) based on the well-known equation of Beverton & Holt (1964), which computed the asymptotic length and the ratio between the mortality coefficient and the curvature parameter (Z/k) using length-frequency data imported in the FiSAT program. Natural mortality (M) is correlated with size (L_{inf}), curvature parameter (k), and mean environmental temperature (T – Francesca Ciutti, pers. obs.) by the following formula (Pauly 1980):

$$\log M = -0.0066 - 0.279 \log L_{inf} + 0.6543 \log k + 0.463 \log T$$

Mortality due to fishing (F) was then obtained subtracting M from Z .

Finally, to analyze demographic aspects, the following formulae were applied to assess the mean life time and the expected longevity (Gayaniilo & Pauly 1997), respectively:

$$t_{1/2} = \Sigma\{[n(t) \times t]\}/N$$

$$t_{max} = (3/k) + t_0$$

where $n(t)$ is the number of individuals at time t , and N is the total number of individuals (both obtained by BM).

To evaluate sexual dimorphism in the growth pattern, a comparison was executed for the curvature parameter values only (obtained from the non-linear regression analysis – NRA), since the close relationship between the curvature parameter k and the asymptotic length L_{inf} , observed after the run of a series of Spearman regression tests for randomized data subsets (including about 30 individuals per set) of each population, according to the (Scalici *et al.* 2008b) protocol. Specifically, we performed a total of 30 "k vs. L_{inf} " regressions for both sexes, showing significant relationships (mean values of $R_s = 0.77$, and 0.71 with $P < 0.05$ in both cases, for females and males, respectively). Then we applied the analysis of covariance (ANCOVA), using CTL as covariate, to assess an eventual sexual dimorphism in the growth rate.

All data were imported into the Statistica Statsoft program version 6.0 to carry out statistical analyses on *sex ratio*, morphometry and reproduction after having pooled data from March and October, while population structure and dynamics were executed using the FiSAT software. In the last case we pooled females and males of the two lakes in order to perform sexual comparison only and not between lake (see below).

RESULTS

Number of collected specimens

We collected 211 individuals in Lake Levico, divided in 86 females (67 in May and 19 in October) + 125 males (98 and 27), while 179 in Lake Canzolino, divided in 42 females (25 and 17) + 137 males (116 and 21). In both lakes, the *sex ratio* was unbalanced in favour of males (χ^2 , $P < 0.05$ in both cases). The numbers of individuals and associated length frequency diagrams of each sampled lake are shown in Fig. 1. In Lake Levico, females ranged from 18 to 44 mm CTL (with the exception of one specimen of 14) while males from 20 to 44. In Lake Canzolino, females ranged from 18 to 38 while males from 20 to 40 mm.

Morphometry

Allometric analyses of surveyed biometrics were executed by joining all data of the two sampling months (May and October) and highlighted significant relationships between cephalothorax length (CTL) vs. all other biometrics. They are reported in Table I per sex and sampling site, separately, together to the allometry degrees for each variable (positive when $b > 1$, and isometry

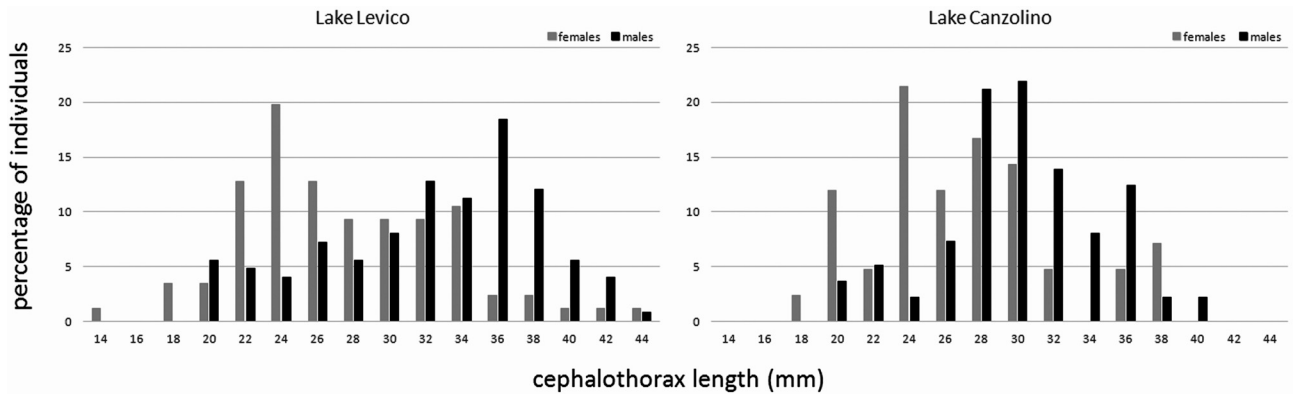


Fig. 1. – Length frequency histograms of the spiny-cheek crayfish divided per sex and sampling site, obtained using 2 mm cephalothorax length (CTL) intervals.

Table I. – Positive (+) allometry, and isometry (0) in *Faxonius limosus* individuals encompassing females (F) and males (M) divided per sampling site (R^2 significance * = $P < 0.05$; ** = $P < 0.01$). In alphabetical order: abdomen width (AW); cephalothorax length (CTL); finger length (FL); right chela length (RChL); right chela width (RChW); total length (TL); weight (W).

Sex	LogCTL vs.	Lake Levico	Lake Canzolino
Females	LogTL	0	0
	LogRChL	+	+
	LogFL	+	+
	LogRChW	+	+
	LogAW	+	+
	LogW	+	+
Males	LogTL	0	0
	LogRChL	+	+
	LogFL	+	+
	LogRChW	+	+
	LogAW	0	0
	LogW	+	+

when $b = 1$; negative allometry was not observed). While significant sexual differences in the regression slopes emerged within both lakes for right chela length (RChL), right chela width (RChW) (both bigger in males), abdomen width (AW) and weight (W) (both bigger in females) (t-test, P was always < 0.05), no divergences in all regression slopes occurred between females and between males in the two lakes (t-test, P was always > 0.05).

Executing the regressions between CTL and all the surveyed biometrics exploiting smallest and biggest specimens, and cross them in diagram, the intersection point indicated a CTL between 22-24 and 20-22 mm as size at maturity for females and males, respectively, in both lakes.

Reproduction

Berried females were observed only during the first

sampling session (May). No BF were found within the sampling session of October. Taking the size of the smallest crayfish in October into account, probably eggs hatch before June. Then it is plausible to consider that coupling, fecundation and egg carrying occur between October and June.

The number of pleopodal eggs (from near 50 in smaller individuals to near 300 in bigger ones, diameter mean 1.8 ± 0.2 mm) is significantly correlated to the cephalothorax length in both lakes ($r = 0.52$, $p = 0.012$, $N = 32$ for Lake Levico; $r = 0.48$, $p = 0.033$, $N = 15$ for Lake Canzolino) (Fig. 2). Additionally the percentage of BF per 2 mm TL intervals is shown in Fig. 3, where it is possible to observe that all females with CTL > 20 mm berried eggs, while the berried females of Lake Canzolino seemed to show a random distribution within the length-frequency diagram. As for the size at maturity, it seemed reasonable to indicate 20 and 22 mm of CTL for Lake Levico and Lake Canzolino, respectively.

Population structure and dynamics

As for the population analysis, we pooled females and males of the two lakes since no morphometric and reproductive differences occurred between them. Anyway, we analysed the sexes separately.

The results of the BM application for the two studied months are summarized in Table II, where it is possible to observe that females are composed of 3 size classes, while males of 4. Since berried females were observed only in May and the smaller individuals were collected in October, we interpreted the first size class as the cohort at the first life year. Attributing an age to the size classes obtained by the application of the BM method is important to execute the following analyses on the growth rate. As a consequence, after the BM analysis, we observed 3 age classes in females and 4 in males for Lake Levico and Lake Canzolino. The separation index values, calculated during the BM routine, were always > 2 , and all the

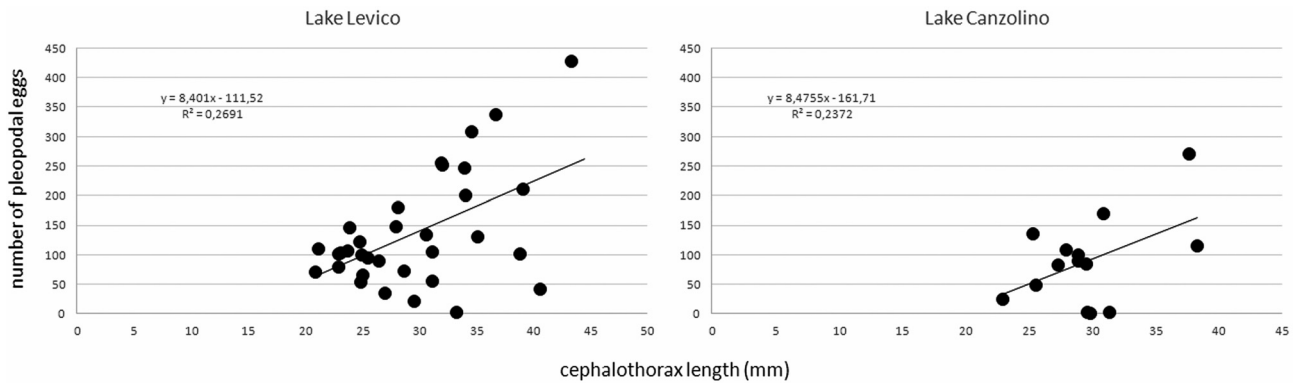


Fig. 2. – Regressions between number of pleopodal eggs and cephalothorax length in females divided per sampling site.

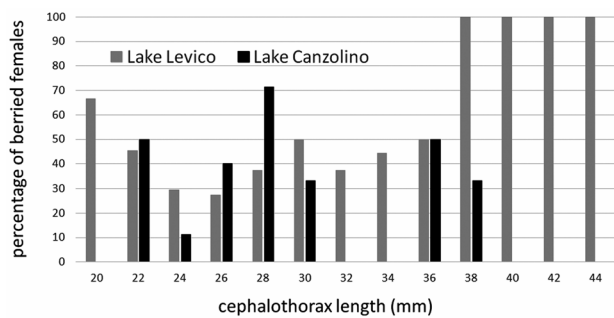


Fig. 3. – Percentage of berried females per 2 mm of cephalothorax length (CTL) recorded during the study period.

Table II. – Mean (ME) and standard deviation (SD) of the cephalothorax length, and coefficient of determination (R^2) for each age class for the two study months, obtained by the application of the Bhattacharya method.

Cohort	Females			Males		
	ME	SD	R^2	ME	SD	R^2
May 2014						
First	19.87	2.01	0.64	19.53	1.45	0.67
Second	30.71	1.36	0.81	34.01	1.59	0.78
Third	36.53	1.88	0.56	40.89	2.11	0.71
Fourth				44.19	1.82	0.49
October 2014						
First	21.12	1.13	0.77	22.49	2.29	0.71
Second	32.84	1.51	0.73	33.69	1.54	0.68
Third	38.37	2.09	0.58	42.23	2.03	0.73
Fourth				44.81	1.77	0.51

decomposition processes were significant (after χ^2 test, with P always < 0.05).

The age class mean values of both sexes (Table II) were used to estimate the VBP parameters (Table III) by NRA, with evident higher values of k and L_{inf} in males of the present study. Indeed significant sexual dimorphic differences emerged in the k values ($F_{1,369} = 8.74, P < 0.05$). On the other hand, the expected life time is higher in females. All VBP obtained by NRA are synthesized in Table III, together with those obtained by ELE and GHP, and other

Table III. – Growth and dynamic parameters of *Faxonius limosus* obtained by different approaches, for the two study months. In alphabetical order: ELE, ELEFAN; F, fishing mortality; F%, fishing mortality percentage; GHM, Gulland & Holt's (1959) method based on the plot of differences between the means of two adjacent cohorts versus the mean of the same ones; L_{inf} , asymptotic length; M, natural mortality; NRA, non-linear regression analysis; t_0 , initial condition parameter; $t_{1/2}$, expected mean lifetime; t_{max} , expected longevity; Z, total mortality.

	Females			Males		
	NRA	ELE	GHM	NRA	ELE	GHM
May 2014						
k	0.66	0.63	0.66	0.71	0.69	0.76
L_{inf}	45.17	–	–	47.14	–	–
t_0	–0.22	–0.13	–	–0.19	–0.28	–
t_{max}	4.32	4.63	–	4.03	4.07	–
$t_{1/2}$	2.23	–	–	1.97	–	–
Z	2.63	–	–	2.52	–	–
M	1.43	–	–	1.69	–	–
F	1.20	–	–	0.85	–	–
F%	45.63	–	–	33.73	–	–
October 2014						
k	0.64	0.61	0.70	0.78	0.77	0.72
L_{inf}	45.68	–	–	48.01	–	–
t_0	–0.17	–0.03	–	–0.15	–0.08	–
t_{max}	4.52	4.89	–	3.70	3.82	–
$t_{1/2}$	2.26	–	–	1.89	–	–
Z	2.33	–	–	2.17	–	–
M	1.32	–	–	1.03	–	–
F	1.01	–	–	1.14	–	–
F%	45.62	–	–	52.53	–	–

dynamic descriptors. Comparing VBP obtained with different methods, no differences seemed to emerge.

DISCUSSION

The present study described some traits of the population biology of the American spiny-cheek crayfish *Faxonius limosus* in northern Italy, mainly highlighting

the presence of an established and reproductively active population in the study area in lakes Levico and Canzolino (Trento, northern Italy), which presents suitable conditions for *F. limosus* to proliferate.

The values of the von Bertalanffy growth parameters in *F. limosus* have shown how this species has a lower growth rate than other crayfish, see the red swamp crayfish *P. clarkii* (see Scalici *et al.* 2010). Our growth rate was comparable with other studies on *F. limosus* (see Chiesa *et al.* 2006). Indeed our results underlined the existence of 4 age classes with an expected life time of 3.5/4 years, corroborating the observation of Smith (1981), Van Den Brink *et al.* (1988), Momot (1988) and Hamr (2002), although Shultz & Smietana (2001) described up to 6 age classes for females.

In contrast to previous studies, the sex ratio showed an unbalanced value with males of the present study prevailing on females; Chiesa *et al.* (2006) described almost an equal relationship between the two sexes in central Italy, confirming what Bott (1950) and Van Den Brink *et al.* (1988) also observed.

In this study, size at maturity was evaluated between 22 and 24 mm in females and 20 and 22 in males of CTL. This value was lower than the 27.75 mm CTL reported for introduced populations of *F. limosus* (Chiesa *et al.* 2006), although Huner (2002) described this value as very variable, depending on several ecological and environmental factors.

The reproductive period also seemed to be shifted respect to other studies, although we cannot be sure on the breeding range basing our observation only on berried females. This period was also inferred by other observations, such as the occurrence of mature females in October, the coupling specimens in March and October, and the females with embryoned eggs in May and June. Hamr (2002) reported the occurrence in July and August of sexually mature specimens of *F. limosus* within the original distribution area, where breeding takes place in spring (March-April) and in autumn (September-October). The occurrence in June of a newly independent juvenile confirms what was reported by the same author for Europe, where breeding takes place in spring and eggs are carried by the female until June-July, period in which they hatch. Also Van Den Brink *et al.* (1988) in the Netherlands observe the occurrence of ovigerous females from March until June, with the highest occurrence in April, and the occurrence in May of females with juveniles attached to the abdomen. This may suggest the existence of a wide reproductive interval that allows the species to breed more times during the year. However, even in this case, further research is necessary to clearly identify the breeding period in the population examined. In males observed by us, sexual maturity is reached at 24.85 mm CTL. Hamr (2002) quotes a unitary value of 25-35 mm CTL for European populations, and 45 mm CTL for those of Quebec. Even in this case, the frequency of breeding periods may

explain the result of the gonadic index with respect to CTL, which is difficult to interpret.

The most conservative characteristics of our populations compared to other studies were morphometric surveys. Indeed, although with diverse allometric trajectories, the relationships among different body measurements corroborated observations by Chiesa *et al.* (2010) and Endrizzi *et al.* (2013, and references therein).

There is a general agreement in considering eradication or reduction of alien species (when introductions cannot be prevented) as one of the most important issues in conserving indigenous ones, overall whether the native taxa occur with fragmented populations as a result of a recent local extinctions. This is the case of the indigenous crayfish *Austropotamobius pallipes* (Lereboullet, 1858) within the province including the study area, with particular reference to sites in the Natura 2000 network (Ciutti *et al.* 2013). Indeed, the presence of the non-indigenous *F. limosus* in five lakes (Canzolino; Madrano; Levico with SCI IT 3120039, Caldonazzo with SCI IT3120042 and Garda), and of *Procambarus clarkii* (Girard, 1852) in Lake Lagolo may have contributed to the *A. pallipes* reduction in northeastern Italy (Cappelletti & Ciutti 2016). Additionally, the crayfish disease caused by *A. astaci* was also detected and observed in Canzolino and Levico where the *F. limosus* populations were observed for more than a decade (Minghetti *et al.* 2012). As result, in the EU LIFE project TEN (Trentino Ecological Network, LIFE11NAT/IT/000187), the management plan for the indigenous *A. pallipes* preservation foresaw the *P. clarkii* eradication from Lake Lagolo as a priority action. Likewise, containment actions (although expansive) on *F. limosus* should be considered within the lake system of Canzolino and Madrano (connected to Lake Costa IT3120041), and Caldonazzo and Levico Lakes (both outflowing into the Brenta River). These actions should concern the modification of existing legislation as well, in order to prohibit catching indigenous species (to protect them) and non-indigenous ones (to prevent their spread), as just proposed in the EU project "Rarity" for *P. clarkii*.

Additional actions may concern: (i) the manual removal of non-indigenous crayfish (overall larger females due to their greater reproductive output – Gutiérrez-Yurrita & Montes 1999, Alcorlo *et al.* 2008) since the results of our study confirmed that baited net traps were an efficient way to easily capture and remove large amounts of crayfish individuals from a huge target population (see also Scalici *et al.* 2010); (ii) the incentive in using native predator (see Aquiloni *et al.* 2010); (iii) a continuous and practical monitoring and mitigating programmes; (iv) citizens involvement by starting a multi-level programme of environmental education and utilizing apps such as iNaturalist (<https://www.inaturalist.org/>) (Chandler *et al.* 2017).

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