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# **Control planning for invasive crayfish: the case of *Pacifastacus leniusculus* (Decapoda, Astacidae) in the Clitunno River (Central Italy).**

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1 **Control planning for invasive crayfish: the case of *Pacifastacus leniusculus* (Decapoda,**  
2 **Astacidae) in the Clitunno River (Central Italy).**

3

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9

10 **Short title:** Alien crayfish removal activities in a Mediterranean chalk stream.

11

12 **Key words:** invasive crayfish; inland waters; chalk streams; removal activities; biodiversity  
13 conservation.

14 **Abstract (Max 300 words)**

15 The signal crayfish *Pacifastacus leniusculus* (Dana, 1852) is one of the most ecologically  
16 impactful decapod crustaceans introduced in Europe. As a species of Union concern, early  
17 detection and rapid eradication measures are required to prevent its establishment and spread. We  
18 aimed to: i) test the effectiveness of the control plan prepared within the LIFE IMAGINE  
19 IPE/IT/000015 project to contrast the diffusion and contain demographic growth of *P. leniusculus*  
20 in the Clitunno River basin (Central Italy), where the species has been detected in 2020; ii) assess  
21 distribution, age structure and growth of *P. leniusculus* in this invaded area. The removal actions  
22 were conducted biweekly, from June 2022 to December 2024, using both traps and electrofishing.  
23 Biometric parameters were individually recorded, and the demographic features and growth of *P.*  
24 *leniusculus* population were assessed. In total, 259 removal actions were carried out and a biomass  
25 of 39.74 Kg has been removed. Maximum size (Total Length = 14.8 cm) was consistent with what  
26 has been reported for another Italian population, but greater than some native Californian ones.  
27 The presence of six cohorts (from 0+ to 5+), including the young-of-the-year (0+), attested the  
28 quick acclimatization of *P. leniusculus*, which gave rise to a self-sustaining population in a short  
29 time. The greatest removal efforts have been concentrated on a small tributary, named Fosso  
30 Vecchio, where the average values of Catch Per Unit Effort (CPUE) showed a significant  
31 decreasing trend over time, arguing in favour of the removal actions' effectiveness. Our findings  
32 provided some evidence that early detection and control measures conducted at a small scale,  
33 applying strong catching efforts, represent effective management tools to limit population  
34 abundance and prevent invasive crayfish from spreading further.

35

## 36 **1 Introduction**

37 Biological invasions represent one of the major causes of biodiversity loss in freshwaters  
38 (Ricciardi and MacIsaac 2011; Reid et al. 2019). Acting as powerful generalist omnivores,  
39 decapods are included among the most successful invaders in these environments (Gherardi 2012;  
40 Twardochleb et al. 2013; Wacker and Harzsch 2021; Soto et al. 2023; Carvalho et al. 2025).  
41 Crayfish have strong opportunities for invading aquatic systems due to their capacity to enter the  
42 food chain at various trophic levels, and to exploit the considerable energy reserves of the detrital  
43 pool (Gherardi 2007). In the invaded areas, significant management efforts are required to prevent  
44 damage from alien crayfish, since they have the potential to directly affect native species (through  
45 predation, competition for food and shelters, disease transmission, etc.) as well as indirectly affect  
46 ecosystems (through food webs and habitat alterations) (Ficetola et al. 2012; Twardochleb et al.  
47 2013). North American crayfish are of particular concern, considering their aptitude to assume a  
48 preponderant role in aquatic communities when introduced outside their original range (Soto et al.  
49 2023); their detrimental effects have been linked to life history traits (early sexual maturity, high  
50 fecundity), wide tolerance to various environmental conditions, scarcity of natural predators, and  
51 competition with native crayfish (Soto et al. 2023). Because of the widespread diffusion of North  
52 American crayfish in Europe, many local cases of native species extinction have been observed  
53 (Kouba et al. 2014); the combination of biodiversity loss and ecosystem processes alteration often  
54 resulted in ecological and economic disruptions (Lodge et al. 2000).

55 The signal crayfish *Pacifastacus leniusculus* (Dana, 1852), native to the northwestern coast of the  
56 United States, is considered one of the most widespread and ecologically impactful decapod  
57 crustaceans introduced in Europe (Rebrina et al. 2015; Chucholl and Chucholl 2021). The species  
58 is a vector of the crayfish plague, that is lethal for the most widespread native European crayfish  
59 (Holdich et al. 2009). Moreover, its ecological niche largely overlaps that of native crayfish; other  
60 detrimental effects are related to predatory activity and environmental alterations in the invaded  
61 sites. With its feeding habits, *P. leniusculus* modifies the food web and alters the composition of  
62 aquatic communities, consuming macrophytes, organic detritus, and preying on  
63 macroinvertebrates, amphibians and small benthic fish (Souty-Grosset et al. 2006; Procopio 2020).

64 Furthermore, the digging activity carried out by the species to create shelters, increases the water  
65 turbidity, reducing the light penetration with a consequent drop in primary productivity. Its  
66 digging activity can also damage riverbanks, facilitating their collapse (Harvey et al. 2011). The  
67 species has led to the local extinction of many native crayfish populations in Scandinavia, England,  
68 and northern Europe (Lodge et al. 2000; Green et al. 2018). Even in Italy, as in other European  
69 countries, local extinction of the native crayfish *Austropotamobius pallipes* complex (Lereboullet,  
70 1858) has been reported immediately after the introduction of the signal crayfish (Ghia et al. 2017);  
71 there are few cases in which a native population of *A. pallipes* complex was observed in syntopy  
72 with *P. leniusculus* (Ghia et al. 2019).

73 The distribution range of *P. leniusculus* in Italy, in the period 1981-2015, was limited to some  
74 watercourses in the northern part of the country (Capurro et al. 2007; Morpurgo et al. 2010; Bo et  
75 al. 2016; Ghia et al. 2017); the first report of the species in Central Italy date back to 2020, when  
76 some individuals were found in the Clitunno River (Umbrian portion of the Tiber River basin)  
77 (Della Bella et al. 2021). The further spread of *P. leniusculus* in the Apennine watercourses raises  
78 serious concern, because among all the North American crayfish, its ecological preferences largely  
79 overlap with that of *A. pallipes* complex (Souty-Grosset et al. 2006; Chucholl 2013), which being  
80 a cold-water species preferentially colonize the upper river stretches (Bo et al., 2016).

81 According to the European Invasive Alien Species Regulation (EU 1143/2014), due to the  
82 detrimental multi-level impacts posed by the species on native biodiversity and ecosystem services,  
83 *P. leniusculus* is deemed as species of Union Concern. Therefore, population monitoring and  
84 quick removal actions shortly after its detection are required, to prevent a further spread of the  
85 species. Based on these regulatory requirements, the Umbria Region prepared, within the LIFE  
86 IMAGINE IPE/IT/000015 project, a control plan started in 2022, with the purpose to contrast the  
87 diffusion and contain demographic growth of *P. leniusculus* in the Clitunno River basin.

88 Two crucial traits expressing species fitness, population persistence and habitat adaptation are:  
89 population growth and demographic characteristics (Hoffmann et al. 2017). Thus, a deepen  
90 knowledge of these aspects play a major role in crayfish invasions management (Guan and Wiles

91 1999), and it is essential for predicting potential range expansion of invasive species (Chuang and  
92 Peterson 2016).

93 Within this context, the present study aimed to i) test the effectiveness of the control plan, in terms  
94 of limiting *P. leniusculus* spread and population abundance in the Clitunno River basin; and ii)  
95 assess distribution, age structure and growth of *P. leniusculus* population in the invaded area.

96

## 97 **2 Material and methods**

### 98 **2.1 Study area**

99 The Clitunno River (Tiber River basin, central Italy) is a chalk stream that flows for 18 km, in  
100 agricultural context, with an almost constant slope (0.18%), and stable thermal and hydrological  
101 regime (water temperature range: 11.8-20°C; average flow rate: 1.5 m<sup>3</sup>/s) (Lorenzoni et al. 2010;  
102 2023). The stream originates from a rheo-limnocratic spring system, called “Fonti del Clitunno”.  
103 Despite the high cultural, naturalistic, and landscape value of the Clitunno River, which has  
104 inspired artists and poets since Roman times (Pascual et al. 2024), its riverbed is characterised by  
105 the presence of significant silt deposits, that have built up over the years because of civil and  
106 productive discharges (Lorenzoni et al. 2010). The Clitunno River has been classified in "moderate  
107 ecological status", in line with the assessments carried out by the regional Environmental  
108 Protection Agency (ARPA Umbria) in the years 2016–2017 (Della Bella et al. 2021). The  
109 hydrographic network of the Clitunno River basin is very complex and articulated, due to the  
110 numerous human-made hydraulic system modifications carried out over the centuries (Lorenzoni  
111 et al. 2023). The Clitunno River hosts an abundant macrophyte community (Cingolani et al. 2008),  
112 while among the most widespread fish species, several are currently facing the threat of extinction  
113 according to the IUCN Red List (IUCN 2024). These include *Anguilla anguilla* (Linnaeus, 1758)  
114 (CR), *Sarmarutilus rubilio* (Bonaparte, 1837) (VU), and *Barbus tyberinus* (EN). Additionally,  
115 according to the IUCN Red List of Italian Vertebrates (Rondinini et al. 2022), the cyclostome  
116 *Lampetra planeri* (Bloch, 1784) is listed as vulnerable. The native crayfish *A. pallipes* complex is  
117 locally extinct, while the invasive *Procambarus clarkii* Girard, 1852 is widely distributed  
118 throughout the basin.

119 Figure 1 schematically shows the selected area for *P. leniusculus* removal activities, located in the  
120 upper part of the Clitunno river basin. The removal plan was based on the results of preliminary  
121 monitoring carried out in 2021 (Lorenzoni et al. 2023). The entire area has been divided into 13  
122 stretches, homogeneous in terms of environmental features, situated both upstream and  
123 downstream from the first detection point of *P. leniusculus*; table 1 shows the main environmental  
124 characteristics for each river stretch.

125

## 126 **2.2 Removal activities**

### 127 **2.2.1 Baited traps and artificial refuge traps**

128 The removal actions were conducted biweekly, from June 2022 to December 2024, using two  
129 types of traps: baited and artificial refuge traps. Baited traps consisting of double entry cylindrical  
130 (50 x Ø 30 cm) plastic cages, with a mesh size of 0.5 cm. They were baited with dry food for cats  
131 or fish. The traps were placed, approximately 25 m apart from each other, in a semi-submerged  
132 position along the banks, to avoid causing disturbance to non-target species that could be attracted  
133 by the bait, following the indications reported in the California crayfish National Management  
134 Plan (Tricarico et al. 2021).

135 Following Green et al. (2018), to collect more individual representative of little size ranges, the  
136 use of baited traps has been integrated by artificial refuge trap (ART). ARTs were made of a set of  
137 PVC tubes of 10-, 20-, 32- and 50-mm diameter and 400 mm long, joined by a clamp, and closed  
138 by a fine mesh net at one end. Trend in catches over time was assessed by Catch per Unit Effort  
139 (CPUE), calculated as a daily average of catches for trap (baited and ART). Being *P. leniusculus* a  
140 species of Union concern, all individuals were frozen immediately after capture, and all specimens  
141 were brought to the laboratory for sex determination and biometric measurements. To test the  
142 differences between the CPUE mean values among sites, among years and the crossing effect  
143 between the two categorical variables (site x year), a two-way factorial ANOVA has been  
144 performed.

145

### 146 **2.2.2 Electrofishing**

147 Limited to wadable areas, in all the 13 river stretches, the removal activities were carried out also  
148 by electrofishing, using a 4.5 kW electro shocker. The average length of the river stretches was  
149 equal to 40 m (range: 15 - 120 m); they were sampled by at least 3 operators, proceeding from  
150 downstream to upstream. In the Clitunno River, when it wasn't possible to carry out electrofishing  
151 by wading because of the high-water depth, the catches were made along the banks.

152

### 153 **2.2.3 Environmental characterization**

154 To investigate the environmental conditions that could most influence the presence and activity of  
155 the signal crayfish, the following physico-chemical parameters were detected: water temperature  
156 (°C), pH (units), electric conductivity ( $\mu\text{S cm}^{-1}$ ), dissolved oxygen ( $\text{mg L}^{-1}$ ). The measurements  
157 were conducted simultaneously with the electrofishing removal activities, using a multiparametric  
158 probe (Hanna Instruments, Padova, Italy). A one-way ANOVA has been performed to compare  
159 mean values among sites.

160

### 161 **2.3 Laboratory activities**

162 For each specimen identified as *P. leniusculus* based on some morphological characters (i.  
163 e. white oval patch at the joint of the claw fingers), sex was determined, the total weight was  
164 measured using a digital scale (accuracy  $\pm 0.1$  g), and the following biometric parameters were  
165 measured using a caliper (accuracy  $\pm 1$  mm): cephalothorax length (CL) from the tip of the  
166 rostrum to the posterior edge of the carapace, and the total length (TL) from the tip of the rostrum  
167 to the telson. Cheliped damage (absent or regenerating) or the presence of other macroscopic  
168 lesions were also recorded.

169

### 170 **2.4 Demographic features and growth of *P. leniusculus* population**

171 For the F. Vecchio3P population, the total length-weight relationship (LWR) was estimated by the  
172 least-squares method (Ricker, 1975), based on the logarithmic equation:  $\log_{10} W \text{ (g)} = a + b \log_{10}$   
173  $CL \text{ (cm)}$ , where  $a$  is the intercept on the Y-axis and  $b$  is the regression coefficient. The standard

174 error was calculated for the slope (b) of LWR. Isometric growth was tested through *t*-test, using  
175 the equation:

$$176 \quad t_s = b-3/S_b,$$

177 where  $S_b$  is the standard error of the slope (b), for  $\alpha=0.05$  (Sokal and Rohlf, 1987). The LWR was  
178 fitted both to the total sample and separately to males and females, and differences between sexes  
179 were assessed by ANCOVA. All statistical tests were conducted using Dell STATISTICA 13  
180 software for Windows.

181 Age classes were estimated by Bhattacharya's method based on the CL frequency distribution data  
182 (Bhattacharya, 1967), using the software FiSAT II (FAO-ICLARM Stock Assessment Tools,  
183 version 1.2.2).

184 Theoretical growth was estimated by the von Bertalanffy growth curve model (VBGE) (von  
185 Bertalanffy, 1938):  $TL_t = L_\infty (1 - e^{-k(t-t_0)})$  where  $TL_t$  is the total length of the crayfish at time *t*,  
186  $L_\infty$  the theoretical maximum length (cm), *k* the rate of approach to  $L_\infty$ , and  $t_0$  the theoretical age (in  
187 years) at which  $TL_t = 0$ . Furthermore, the index of growth performance ( $\Phi'$ ) was calculated by the  
188 equation of Pauly and Munro (1984):  $\Phi' = \log_{10} k + 2 \log_{10} L_\infty$  where *k* and  $L_\infty$  are the growth  
189 parameters of the von Bertalanffy model.

190

## 191 **3 Results**

### 192 **3.1 Removal activities and *P. leniusculus* distribution**

#### 193 **3.1.1 Baited traps and artificial refuge traps**

194 A total of 180 removal actions were conducted. The overall catching effort applied through setting  
195 traps was equal to 2057 trap · day in 2022, 5583 trap · day in 2023, and 8708 trap · day in 2024.

196 Overall, 1075 *P. leniusculus* individuals were removed for a total biomass of 37.9 kg. The species  
197 was only caught upstream from the first detection site (Fig. 1), where the largest sample (91.63%  
198 of total individuals) has been removed from Fosso Vecchio, a small tributary of the Clitunno upper  
199 stretch. In F. Vecchio3P we observed a progressive decreasing trend over time of the mean annual  
200 CPUE values ( $\pm$  SE), which dropped from  $0.28 \pm 0.07$  ind · trap<sup>-1</sup> · day<sup>-1</sup> recorded in 2022 to  $0.07 \pm$   
201  $0.01$  in 2024 (Fig. 2). A drastic reduction of CPUE was also observed in the same period for F.

202 Media3P and Clitunno3P, where the mean annual values ( $\pm$  SE) decreased from  $0.07 \pm 0.06$   
203  $0.024 \pm 0.008$  and from  $0.018 \pm 0.061$  to  $0.001 \pm 0.014$  ind· trap<sup>-1</sup>·day<sup>-1</sup>, respectively. At all other  
204 sites, no *P. leniusculus* individuals were captured in 2024 by traps. The differences between the  
205 mean values among sites ( $F = 32.19$ ;  $P = 0.004$ ), and the crossing effect between the two  
206 categorical variables site x year ( $F = 2.51$ ;  $P = 0.001$ ) were statistically significant to the two-way  
207 factorial ANOVA.

208 During the eradication interventions, a progressive significant decreasing trend over time of the  
209 average daily CPUE values for traps was observed in the F. Vecchio3P (Fig. 3). The computed  
210 equation was  $y = 13.78 - 0.0003 \cdot x$  ( $F = 27.05$ ;  $P < 0.001$ ;  $r^2 = 0.13$ ); the regression slope  
211 significantly differed from 0 ( $t = 5.2$ ;  $df = 178$ ;  $P < 0.001$ ).

212 The analysis of the monthly trend in CPUE values allowed us to identify two peaks, occurring in  
213 April and August (Fig. 4), with mean values ( $\pm$  SE) recorded at  $0.23 \pm 0.06$  and  $0.21 \pm 0.02$ ,  
214 respectively. The lowest values were observed in January (mean  $\pm$  SE =  $0.02 \pm 0.006$ ) and  
215 February (mean $\pm$ SE =  $0.02\pm 0.007$ ).

216

### 217 3.1.2 Electrofishing

218 A total of 79 removal interventions through electrofishing were carried out, during which overall  
219 28 specimens were collected. The mean total length of individuals ( $\pm$  SE) was  $10.0 \pm 2.59$  cm  
220 (range 4.3 - 13.5 cm); the mean weight ( $\pm$  SE) was  $38.66 \pm 23.77$ g (range 2.3 - 7 5.9 g). This  
221 method proved its effectiveness in ClitunnoPP and F. Vecchio3P, with captures recorded  
222 exclusively in 2022 and 2023. In 2024, two individuals were also captured from F. Media3P (Fig.  
223 5).

224

### 225 3.1.3 Selectivity of removal methods

226 The mean length ( $\pm$  SE) of individuals caught with ARTs ( $7.36 \pm 0.27$  cm) was significantly lower  
227 from those of both baited traps ( $10.04 \pm 0.04$  cm) and electrofishing ( $9.60 \pm 0.58$  cm) at the  
228 Kruskal-Wallis ANOVA ( $\chi^2 = 38.44$ ;  $P < 0.001$ ). To check the normality of length data

229 distribution, ANOVA was preceded by Shapiro–Wilk test. Sex ratio didn't differ significantly  
230 among different removal methods at the chi-square test ( $\chi^2 = 1.10$ ;  $P = 0.578$ ).

231

### 232 **3.2 Environmental characterization**

233 Water temperature ranged from 11.20 °C, recorded in F. Media3P, to 19.36 °C, recorded in F.  
234 Nuova3P (Fig. 6a). The differences between the mean values calculated for the 13 sites were  
235 statistically significant at the one-way ANOVA ( $F = 5.27$ ;  $P < 0.001$ ). The mean value ( $\pm$ SD)  
236 recorded for F. Vecchio3P ( $13.97 \pm 1.29$  °C) was statistically different from the mean values  
237 recorded for Clitunno3P ( $12.63 \pm 0.77$  °C) and F. Nuova3P ( $15.92 \pm 2.28$  °C), as revealed by the  
238 Fisher LSD post hoc test.

239 FiumicellaPI presented the lowest pH value (6.54 units), while the highest value was measured in  
240 F. NuovaSP (8.39 units) (Fig. 6b). The differences between the mean values were statistically  
241 significant at the one-way ANOVA ( $F = 2.69$ ;  $P = 0.004$ ). The average value ( $\pm$ SD) observed in F  
242 Vecchio3P ( $7.60 \pm 0.32$  °C) was significantly different from those observed in Clitunno3P ( $7.23 \pm$   
243  $0.83$  °C) and Fosso3P ( $7.98 \pm 0.22$  °C), as determined by the Fisher LSD post hoc test.

244 Conductivity values covered a wide range, from 79  $\mu$ S  $\text{cm}^{-1}$ , measured in Sportella PI, to 2254  $\mu$ S  
245  $\text{cm}^{-1}$ , measured in Clitunno PI (Fig. 6c). The mean value differences among sites were statistically  
246 significant at the one-way ANOVA ( $F = 4.08$ ;  $P < 0.001$ ). The mean value ( $\pm$  SD) observed for F.  
247 Vecchio3P ( $614.35 \pm 141.21$   $\mu$ S  $\text{cm}^{-1}$ ) exhibited a statistically significant difference when  
248 compared to the mean values calculated for ClitunnoPI ( $952.40 \pm 0731.65$   $\mu$ S  $\text{cm}^{-1}$ ) and  
249 Clitunno3P ( $817.40 \pm 114.33$   $\mu$ S  $\text{cm}^{-1}$ ), as determined by the Fisher LSD post hoc test.

250 Dissolved oxygen varied from 1.42 °C, recorded in SportellaBT, to 14.80 °C, recorded in F.  
251 Media3P (Fig. 6d). The differences between the mean values calculated for sites were not  
252 statistically significant at the one-way ANOVA ( $F = 1.56$ ;  $P = 0.115$ ).

253

### 254 **3.3 Demographic features and growth**

255 The total length of crayfish ranged from 1.1 to 14.8 cm (mean  $\pm$  SE =  $9.82 \pm 0.06$ ) and weight  
256 from 0.025 to 118.70 g (mean  $\pm$  SE =  $36.05 \pm 0.60$ ). The largest size recorded was 14.8 cm in total

257 length for females, and 13.9 cm for males. Females represented 50.4% of the total sample; sex  
258 ratio (1 : 1.1 males/females) didn't differ significantly from the theoretical 1:1 value ( $\chi^2 = 0.004$ ;  
259  $P > 0.05$ ). For both sexes of F. Vecchio3P population, a total of six cohorts, extended with  
260 continuity from 0+ to 5+, were identified, based on the carapace length frequency distribution data  
261 (Fig. 7).

262 The LWR equations for males and females were:  $\log_{10} W = -0.68 + 3.26 \log_{10} CL$  ( $R^2 = 0.98$ ;  $P <$   
263  $0.001$ ), and  $\log_{10} W = -0.61 + 3.11 \log_{10} CL$  ( $R^2 = 0.98$ ;  $P < 0.001$ ), respectively (Fig. 8). In both  
264 equations the b slopes were significantly higher than three ( $df = 387$ ;  $t = 11.95$ ;  $P < 0.001$  for  
265 males;  $df = 395$ ;  $t = 4.82$ ;  $P < 0.001$  for females), indicating positive allometric growth (Ricker,  
266 1975). The LWRs calculated for both sexes were statistically different at the ANCOVA ( $F = 71.74$ ;  
267  $P < 0.001$ ). Based on this latter result, the growth of males and females was analyzed separately.

268 The VBGEs calculated for females and males were  $TLt = 15.53 (1 - e^{-0.42(t)})$  and  $TLt = 17.54 (1 -$   
269  $e^{-0.27(t+0.23)})$ , respectively. The parameters obtained for the theoretical growth highlighted a better  
270 performance of females ( $\Phi' = 2.00$ ), showing a lower theoretical length ( $L_{\infty} = 15.53$  cm) than  
271 males ( $L_{\infty} = 17.54$  cm), but a faster growth rate ( $K = 0.42$ ) (Fig. 9).

272

#### 273 **4 Discussion**

274 Our research provided evidence that, within a short period from its first detection, *P. leniusculus*  
275 has naturalized in the Clitunno river basin, quickly resulting in a self-sustaining population mainly  
276 confined in a small tributary (F. Vecchio3P). Through intensive removal efforts, a drastic  
277 reduction in the population abundance was achieved over nearly three years of eradication  
278 activities. Additionally, concurrent monitoring conducted at control sites proved that the species  
279 has not further extended its invasive range.

280 The distribution analysis revealed that the current invasive range of the species covers 3.5 km of  
281 the Clitunno hydrographic network, extending from the springs downstream. The established and  
282 most abundant population in F. Vecchio 3P could be identified as the "invasion core" (i. e. where  
283 the species was probably initially introduced and established a stable population, *sensu* Carvalho  
284 et al. 2025), while the Clitunno 3P distribution point could be considered the downstream

285 “invasion front” (i. e. invasion edge), with much lower abundance than the core area. This is not a  
286 surprising result, considering the high dispersal rate of the species, which can reach 6.4-24 km/yr  
287 (Hudina et al. 2009; Bernardo et al. 2011; Anastácio et al. 2015). Various authors have reported  
288 both downstream and upstream *P. leniusculus* invasion range expansions, through active and  
289 passive movements, including secondary introductions (Anastácio et al. 2015; Rebrina et al. 2015;  
290 Carvalho et al. 2024). Such movements could be driven both by environmental conditions (thermal  
291 conditions, high flows, food availability) and biological factors (high population density,  
292 occurrence of other crayfish) (Rebrina et al. 2015). In the Clitunno river basin, where the  
293 environmental characteristics of watercourses are quite stable over time, but more heterogeneous  
294 in space, water temperature probably played an important role in *P. leniusculus* naturalization  
295 process and dispersal pattern. The physico-chemical characterization revealed the presence of  
296 significant differences between the mean temperatures of the F. Vecchio3P and the adjacent sites,  
297 even if the thermal conditions were always still within the tolerance range (4-25°C) reported for  
298 the species (Shimizu and Goldman 1983). Another key element in the invasion process could be  
299 represented by the co-occurrence with *P. clarkii*, which can potentially exert competitive pressure,  
300 even if the two species have shown diversifications in terms of thermal preferences and habitat use  
301 (Henttonen and Huner 1999; Bernardo et al. 2011; Anastácio et al. 2015). Even the dissolved  
302 oxygen concentration, and in general the human impact level and water pollution that  
303 characterizes the different sites, could justify the different distribution of *P. leniusculus* within the  
304 investigated area. Further analyses are required to inform these hypotheses.

305 Despite a fairly widespread distribution and a well-structured population, the data collected during  
306 the eradication activities indicated that the relative abundance that characterized the population of  
307 *P. leniusculus* in the Clitunno River basin was not particularly high, except for a few localized  
308 areas, in comparison with what reported in the literature for other Italian invaded areas which  
309 reported maximum CPUE values  $> 4 \text{ ind-trap}^{-1}\text{-day}^{-1}$  (Capurro et al. 2009; Ghia et al. 2017). This  
310 condition probably played a crucial role in the success of the eradication efforts.

311 The greatest removal efforts have been concentrated on the invasion core (F. Vecchio3P), where  
312 the average CPUE values and the number of crayfish removed by traps were much higher than

313 elsewhere. Here, the average CPUE values of traps showed a significant decreasing trend over  
314 time, arguing in favour of the removal actions' effectiveness. The trend of catches over time  
315 highlighted the presence of peaks concentrated in the spring and summer periods, when *P.*  
316 *leniusculus* is most active, due to the strong positive relationship between water temperature and  
317 crayfish movements (Bubb et al. 2002). This result could also reflect moulting activities (Shimizu  
318 and Goldman 1983). In winter catches were low, but crayfish never completely disappear. The  
319 peak in catches occurring in late summer was also probably due to the intensification of crayfish  
320 movements close to reproductive period.

321 The outcomes of electrofishing confirmed the effectiveness of this method in the capture of  
322 crayfish; the limited number of specimens obtained may be linked to the high abundance of  
323 shelters that provided opportunities for crayfish to escape capture. One additional limitation  
324 pertains to the selectivity of the method, which, like baited traps, tends to favour the capture of  
325 larger individuals. In contrast, the application of ARTs has demonstrated effectiveness in  
326 capturing juvenile crayfish (i.e. TL < 6 cm), confirming the findings of Green et al. (2018).

327 The overall sample of *P. leniusculus* caught during the removal actions was quite large, well  
328 distributed in size classes and balanced in the sex ratio. Maximum size (TL = 14.8 cm) was  
329 consistent with what has been reported for another Italian population (Capurro et al. 2007), but  
330 greater than some native Californian ones, for which a maximum CL of 4.28 cm was found  
331 (Shimizu and Goldman 1983). The analysis of the population structure highlighted the prevalence,  
332 in the overall sample, of sexually mature individuals, with TL > 6 cm (Souty-Grosset et al. 2006).

333 The overall effectiveness of removal actions for young individuals seems to be limited, even with  
334 the implementation of ARTs. This factor should be considered in the ongoing efforts related to  
335 removal initiatives. The presence of six cohorts (from 0+ to 5+), including the young-of-the-year  
336 (0+), suggested that the species had already been present in the Clitunno River basin for several  
337 years. The number of age classes and the maximum size, and positive allometric growth (with  
338 significant difference between sexes), are consistent with the findings of Guan and Wiles (1999)  
339 for British invaded lowland rivers. The fast growth rate, observed especially for females, and the  
340 theoretical maximum length achievable, which approaches the maximum size reported for the

341 species (i.e. 20 cm, according to Tucker 2019), could be related to the availability of resources.  
342 Population growth rate is often fast when species initially colonize new environments with  
343 adequate resources; however, this growth tends to decelerate as population density rises and  
344 resources become constrained (Guan and Wiles 1996). For instance, significantly high growth  
345 rates were observed for *P. leniusculus* during the initial four years following its release into a lake  
346 in southern Britain (Richards 1983; Hogger 1986). Furthermore, it should be considered that  
347 eradication activities have progressively reduced the size of the population, making intraspecific  
348 competition less likely.

349 It is possible to hypothesize, based on population structure and the already quite widespread  
350 diffusion, that the species has been present in the Clitunno for some time, and that its introduction  
351 preceded its first detection. This hypothesis is consistent with the existence of a “lag phase” during  
352 which the species was present but given the low population abundance it escaped detection.

353 Reconstructing the dispersal patterns of invasive species and the dynamics of the invasion process  
354 is crucial for the effectiveness of management strategies aimed at mitigating their further spread  
355 (Hudina et al. 2017; Carvalho et al. 2025). This analysis facilitates: i) the containment of ongoing  
356 invasions by alleviating the propagule pressure (Simberloff 2009), ii) the reduction of the  
357 likelihood that existing populations could serve as sources for secondary introductions  
358 (Bertelsmeier and Keller 2018), and iii) the minimization of recolonization chances following  
359 successful eradication efforts (Britton et al. 2011). It is crucial to implement prolonged monitoring  
360 across all regions affected by the species, particularly in areas where native crayfish exist  
361 (Kirjavainen and Westman 1999). This is necessary due to the current lack of comprehensive  
362 information regarding the population dynamics of *P. leniusculus* within its invaded range  
363 (Kirjavainen and Westman 1999).

364

## 365 **5 Conclusions**

366 Even though it is unlikely that invasive crayfish could be completely eradicated within a system  
367 where acclimatization has taken place, our findings show how effective control measures are at  
368 limiting population abundance and preventing the alien species from spreading further. Early

369 detection and removal actions conducted at small scale, applying strong catching efforts, could  
370 represent effective management tools to counteract the spread of invasive crayfish. Our results  
371 allowed us to improve the removal effectiveness by concentrating the capture efforts more on the  
372 most favourable periods (spring and late summer) and areas (F. Vecchio3P). Additional research is  
373 required to clarify the environmental and biological factors that most significantly affect the  
374 distribution of the species within the study area, such as the water chemistry and the co-occurrence  
375 with *P. clarkii*. This will help to elucidate if the conditions are conducive to further expansion or if  
376 the species has already reached its maximum capacity to invade all potentially suitable locations.

377

### 378 **References**

- 379 Anastácio PM, Banha F, Capinha C, Bernardo JM, Costa AM, Teixeira A, Bruxelas S (2015)  
380 Indicators of movement and space use for two co-occurring invasive crayfish species. *Ecological*  
381 *Indicators* 53: 171-181. <https://doi.org/10.1016/j.ecolind.2015.01.019>
- 382 Bernardo JM, Costa AM, Bruxelas S, Teixeira A (2011) Dispersal and coexistence of two non-  
383 native crayfish species (*Pacifastacus leniusculus* and *Procambarus clarkii*) in NE Portugal over a  
384 10-year period. *Knowledge and Management of aquatic Ecosystems* (401): 28.  
385 <https://doi.org/10.1051/kmae/2011047>
- 386 Bertelsmeier C, Keller L (2018) Bridgehead effects and role of adaptive evolution in invasive  
387 populations. *Trends in Ecology & Evolution* 33(7): 527-534.
- 388 Bhattacharya CG (1967) A simple method of resolution of a distribution into Gaussian  
389 components. *Biometrics* 23: 115–135. <https://doi.org/10.2307/2528285>
- 390 Bo T, Candiotta A, Delmastro GB, Fea G, Fenoglio S, Ghia D, Gruppuso L, (2016) Prima  
391 segnalazione del gambero alloctono *Pacifastacus leniusculus* (Decapoda, Astacidae) in Provincia  
392 di Savona, Italia. *Natural History Sciences. Atti della Società Italiana di Scienze Naturali e del*  
393 *Museo Civico di Storia Naturale di Milano* 3(1): 63-65. <https://doi.org/10.30463/es211.002>
- 394 Britton JR, Gozlan RE, Copp GH (2011) Managing non-native fish in the environment. *Fish and*  
395 *fisheries* 12(3): 256-274 <https://doi.org/10.1111/j.1467-2979.2010.00390.x>

- 396 Bubb DH, Lucas MC, Thom TJ (2002) Winter movements and activity of signal crayfish  
397 *Pacifastacus leniusculus* in an upland river, determined by radio telemetry. *Hydrobiologia* 483:  
398 111–119. <https://doi.org/10.1023/A:1021363109155>
- 399 Capurro M, Galli L, Mori M, Salvidio S, Arillo A (2007) The signal crayfish, *Pacifastacus*  
400 *leniusculus* (Dana, 1852) [Crustacea: Decapoda: Astacidae], in the Brugnato Lake (Liguria, NW  
401 Italy). The beginning of the invasion of the River Po watershed. *Aquatic Invasions* 2: 17-24.  
402 <http://dx.doi.org/10.3391/ai.2007.2.1.2>
- 403 Chuang A, Peterson CR (2016) Expanding population edges: theories, traits, and trade-  
404 offs. *Global change biology* 22(2): 494-512. <https://doi.org/10.1111/gcb.13107>
- 405 Carvalho F, Alves H, Pascoal C, Castro P, Miranda F, Teixeira A, Cássio F, Sousa R (2025)  
406 Invasive dynamics of the signal crayfish *Pacifastacus leniusculus* in a protected area.  
407 *Hydrobiologia* 852: 705–720. <https://doi.org/10.1007/s10750-024-05717-w>
- 408 Chucholl C (2013) Feeding ecology and ecological impact of an alien ‘warm-water’ omnivore in  
409 cold lakes. *Limnologica* 43(4): 219-229. <https://doi.org/10.1016/j.limno.2012.10.001>
- 410 Chucholl F, Chucholl C (2021) Differences in the functional responses of four invasive and one  
411 native crayfish species suggest invader-specific ecological impacts. *Freshwater Biology* 66: 2051-  
412 2063. <https://doi.org/10.1111/fwb.13813>
- 413 Cingolani L, Lazzerini G, Padula R (2008) Metodologia per l’individuazione e valutazione dei  
414 possibili impatti su un ecosistema fiume, derivanti da interventi di ripristino ambientale e di  
415 restauro della continuità fluviale. Arpa Umbria 1-90.  
416 <https://www.arpa.umbria.it/au/clitunno/1%20FASE.pdf>
- 417 Della Bella V, Mauro N, Tricarico E (2021) Prima segnalazione in Umbria del gambero della  
418 California *Pacifastacus leniusculus* (Dana, 1852), specie esotica invasiva di interesse unionale.  
419 *Biologia ambientale* 35: 12-17. <https://doi.org/10.30463/es211.002>.
- 420 Ficetola GF, Siesa ME, Padoa-Schioppa E, De Bernardi F (2012) Wetland features, amphibian  
421 communities and distribution of the alien crayfish, *Procambarus clarkii*. *Alytes* 29(1-4): 75-87.

- 422 Gherardi, F. (2007). Understanding the impact of invasive crayfish. In: Gherardi, F. (eds)  
423 Biological invaders in inland waters: Profiles, distribution, and threats. Springer (Dordrecht): 507-  
424 542. [https://doi.org/10.1007/978-1-4020-6029-8\\_28](https://doi.org/10.1007/978-1-4020-6029-8_28)
- 425 Gherardi F (2012) Control and management of non-indigenous crayfish. In: Reynolds J, Souty-  
426 Grosset C (Eds) Management of freshwater biodiversity: crayfish as bioindicators. Cambridge  
427 University Press (Cambridge): 195-197. <https://doi.org/10.1017/CBO9781139031790>
- 428 Ghia D, Fea G, Gruppuso L, Bo T, Candiotta A, Fenoglio S, Sacchi R (2017) Distribution and  
429 establishment of the invasive signal crayfish *Pacifastacus leniusculus* in the Valla Stream (N-W  
430 Italy). Italian Journal of Freshwater Ichthyology 4: 101-108.
- 431 Ghia D, Fea G, Ventimiglia M, Capurro M, Oneto F, Ottonello D, Bo T, Candiotta A, Sacchi R  
432 (2019) Il gambero autoctono italiano e il gambero della California coesistono in un tratto del  
433 torrente Valla (Italia nord-occidentale). Italian Journal of Freshwater Ichthyology 5 (1): 120-131.
- 434 Green N, Bentley M, Stebbing P, Andreou D, Britton R (2018) Trapping for invasive crayfish:  
435 comparisons of efficacy and selectivity of baited traps versus novel artificial refuge traps.  
436 Knowledge and Management of Aquatic Ecosystems 419: 15.  
437 <https://doi.org/10.1051/kmae/2018007>
- 438 Guan R, Wiles PR (1996) Growth, density and biomass of crayfish, *Pacifastacus leniusculus*, in a  
439 British lowland river. Aquatic Living Resources 9(3): 265-272. <https://doi.org/10.1051/alr:1996030>
- 440 Guan RZ, Wiles PR (1999) Growth and reproduction of the introduced crayfish *Pacifastacus*  
441 *leniusculus* in a British lowland river. Fisheries Research 42(3): 245-259.  
442 [https://doi.org/10.1016/S0165-7836\(99\)00044-2](https://doi.org/10.1016/S0165-7836(99)00044-2)
- 443 Harvey GL, Moorhouse TP, Clifford NJ, Henshaw AJ, Johnson MF, Macdonald DW, Reid I, Rice  
444 SP (2011) Evaluating the role of invasive aquatic species as drivers of fine sediment-related river  
445 management problems: the case of the signal crayfish (*Pacifastacus leniusculus*). Progress in  
446 Physical Geography 35(4): 517-533. <https://doi.org/10.1177/0309133311409092>
- 447 Henttonen P, Huner JV (1999) The introduction of alien species of crayfish in Europe: A historical  
448 introduction. In: Gherardi F, Holdich DM (Eds) Crayfish in Europe as alien species. Routledge  
449 (Oxfordshire): 13-22.

- 450 Hoffmann AA, Sgrò CM, Kristensen TN (2017) Revisiting adaptive potential, population size, and  
451 conservation. *Trends in Ecology & Evolution* 32(7): 506-517.  
452 <https://doi.org/10.1016/j.tree.2017.03.012>
- 453 Hogger JB (1986) Aspects of the introduction of “signal crayfish”, *Pacifastacus leniusculus*  
454 (Dana), into the southern United Kingdom. 1. Growth and survival. *Aquaculture* 58(1-2): 27-44.  
455 [https://doi.org/10.1016/0044-8486\(86\)90154-7](https://doi.org/10.1016/0044-8486(86)90154-7)
- 456 Holdich DM, Reynolds JD, Souty-Grosset C, Sibley PJ (2009) A review of the ever-increasing  
457 threat to European crayfish from non- indigenous crayfish species. *Knowledge and Management of*  
458 *Aquatic Ecosystems* 11: 394–395. <https://doi.org/10.1051/kmae/2009025>
- 459 Hudina S, Faller M, Lucić A, Klobučar G, Maguire I (2009) Distribution and dispersal of two  
460 invasive crayfish species in the Drava River basin, Croatia. *Knowledge and Management of*  
461 *Aquatic Ecosystems* 9: 394–395. <http://dx.doi.org/10.1051/kmae/2009023>
- 462 Hudina S, Kutleša P, Trgovčić K, Duplić A (2017) Dynamics of range expansion of the signal  
463 crayfish (*Pacifastacus leniusculus*) in a recently invaded region in Croatia. *Aquatic Invasions*  
464 12(1): 67-75. <https://doi.org/10.3391/AI.2017.12.1.07>
- 465 IUCN. 2024. The IUCN Red List of Threatened Species. Version 2024-2.  
466 <https://www.iucnredlist.org>. Accessed on 14 January 2025.
- 467 Kirjavainen J, Westman K (1999) Natural history and development of the introduced signal  
468 crayfish, *Pacifastacus leniusculus*, in a small, isolated Finnish lake, from 1968 to 1993. *Aquatic*  
469 *Living Resources* 12(6): 387-401. [https://doi.org/10.1016/S0990-7440\(99\)00110-2](https://doi.org/10.1016/S0990-7440(99)00110-2)
- 470 Kouba A, Petrusek A, Kozák P (2014) Continental-wide distribution of crayfish species in Europe:  
471 update and maps. *Knowledge and Management of Aquatic Ecosystems* 413: 05.  
472 <https://doi.org/10.1051/kmae/2014007>
- 473 Lodge DM, Taylor CA, Holdich DM, Skurdal J (2000) Nonindigenous crayfishes threaten North  
474 American freshwater biodiversity: lessons from Europe. *Fisheries* 25(8): 7-20.  
475 [https://doi.org/10.1577/1548-8446\(2000\)025<0007:NCTNAF>2.0.CO;2](https://doi.org/10.1577/1548-8446(2000)025<0007:NCTNAF>2.0.CO;2)
- 476 Lorenzoni M, Ghetti L, Carosi A, Dolciami R (2010) La fauna ittica e i corsi d'acqua dell'Umbria.  
477 Sintesi delle Carte Ittiche regionali dal 1986 al 2009. Petrucci Editore (Perugia, Italy): 1-288.

- 478 Lorenzoni M, Croce M, Lorenzoni F, Tagliaferri G, Joubert A, Ricci S, Carosi A (2023) Il  
479 gambero della California *Pacifastacus leniusculus* (Dana, 1852) nel Fiume Clitunno (Italia  
480 centrale): monitoraggio e piano di eradicazione. Italian Journal of Freshwater Ichthyology 9(1):  
481 107-124.
- 482 Morpurgo M, Aquiloni L, Bertocchi S, Brusconi S, Tricarico E, Gherardi F (2010) Distribuzione  
483 dei gamberi d'acqua dolce in Italia. Studi Trentini di Scienze Naturali 87: 125-132.
- 484 Pascual R, Piana L, Bhat SU, Castro PF, Corbera J, Cummings D et al. (2024) The Cultural  
485 Ecohydrogeology of Mediterranean-Climate Springs: A Global Review with Case  
486 Studies. Environments 11(6): 110. <https://doi.org/10.3390/environments11060110>
- 487 Pauly D, Munro JL (1984) Once more on comparison of growth in fish and invertebrates.  
488 ICLARM Fishbyte 2(1): 21–22.
- 489 Procopio J (2020) *Pacifastacus leniusculus* (Dana, 1852): U.S. Geological Survey, Nonindigenous  
490 Aquatic Species Database. <https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=200>
- 491 Rebrina F, Skejo J, Lucić A, Hudina S (2015) Trait variability of the signal crayfish (*Pacifastacus*  
492 *leniusculus*) in a recently invaded region reflects potential benefits and trade-offs during  
493 dispersal. Aquatic Invasions, 10(1): 41-50. <http://dx.doi.org/10.3391/ai.2015.10.1.04>
- 494 Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PT et al. (2019) Emerging threats  
495 and persistent conservation challenges for freshwater biodiversity. Biological reviews 94(3): 849-  
496 873. <https://doi.org/10.1111/brv.12480>
- 497 Ricciardi A, MacIsaac HJ (2011) Impacts of biological invasions on freshwater ecosystems. In:  
498 Richardson DM (Eds) Fifty years of invasion ecology: the legacy of Charles Elton. Blackwell  
499 Publishing Ltd (Oxford) 1: 211-224. <https://doi.org/10.1002/9781444329988.ch16>
- 500 Richards KJ (1983) The introduction of the signal crayfish into the United Kingdom and its  
501 development as a farm crop. Freshwater Crayfish 5: 557-563.  
502 <https://doi.org/10.5869/fc.1983.v5.557>
- 503 Ricker WE (1975) Computation and interpretation of biological statistics of fish populations. J.  
504 Bulletin of Fisheries Research Board of Canada 191: 1–382.

- 505 Rondinini C, Battistoni A, Teofili C (2022) Lista Rossa IUCN dei vertebrati italiani 2022  
506 Comitato Italiano IUCN e Ministero dell'Ambiente e della Sicurezza Energetica (Roma): 1-57
- 507 Shimizu SJ, Goldman CR (1983) *Pacifastacus leniusculus* (Dana) production in the Sacramento  
508 River. *Freshwater Crayfish* 5: 210-228.
- 509 Simberloff D (2009) The role of propagule pressure in biological invasions. *Annual review of*  
510 *ecology, evolution, and systematics* 40(1): 81-102.  
511 <https://doi.org/10.1146/annurev.ecolsys.110308.120304>
- 512 Sokal RR, Rohlf FL (1987) *Introduction to biostatistics*. Freeman (New York).
- 513 Soto I, Ahmed DA, Beidas A, Oficialdegui FJ, Tricarico E, Angeler DG et al. (2023) Long-term  
514 trends in crayfish invasions across European rivers. *Science of the Total Environment* 867: 161537.  
515 <https://doi.org/10.1016/j.scitotenv.2023.161537>
- 516 Souty-Grosset C, Holdic DM, Noë PY (2006) *Atlas of crayfish in Europe*. Muséum National  
517 d'Histoire Naturelle (Paris): 1-187.
- 518 Twardochleb LA, Olden JD, Larson ER (2013) A global meta-analysis of the ecological impacts  
519 of nonnative crayfish. *Freshwater Science* 32(4): 1367-1382. <https://doi.org/10.1899/12-203.1>
- 520 Tricarico E, Ghia D, Fea G (2021) *Piano di gestione nazionale del gambero della California*  
521 *(Pacifastacus leniusculus)*. Ministero della Transizione Ecologica (Roma): 1-25.
- 522 von Bertalanffy L (1938) A quantitative theory of organic growth. *Human Biology* 10: 181-243.
- 523 Wacker A, Harzsch S (2021) Crustaceans in a changing world. *Zoology* 146: 125921.  
524 <https://doi.org/10.1016/j.zool.2021.125921>.

525 Table 1 – List of sampling sites along geographical coordinates, and main environmental features.  
526

River stretches code and geographic coordinates	Average depth (m)	Average width (m)	Canopy cover (%)	Prevalent substrate	Shaded area (%)	Anthropization level	Type of disturbance
<b>1)</b> Clitunno Borgo Trevi 33 T 4749177.40 m N; 314823.23 m E	0.8	12	90	Silt	90	High	Channelization, Diffuse organic pollution, Presence of obstacles
<b>2)</b> Fosso Sportella Pietrarossa 33 T 4750596.97 m N; 313932.25 m E	0.4	1	20	Silt	50	Very high	Channelization, Point-source organic pollution
<b>3)</b> Irrigation channel Borgo Trevi 33 T 4750793.29 m N; 314195.95 m E	0.5	2	10	Silt	10	Molto elevato	Channelization, Diffuse organic pollution
<b>4)</b> Clitunno Piatto Bridge 33 T 4747402.16 m N; 315656.63 m E	0.9	6	75	Silt	30	Very high	Channelization, Diffuse organic pollution
<b>5)</b> Clitunno Pigge 33 T 4746511.93 m N; 316183.45 m E	0.7	12	70	Silt - Fine Gravel	10	Moderate	Channelization
<b>6)</b> Fiumicella Pigge 33 T 4746497.39 m N; 316178.65 m E	0.7	2.5	30	Silt	20	Moderate	Channelization, Diffuse organic pollution
<b>7)</b> Clitunno Treponti 33 T 4745128.83 m N; 316837.82 m E	1	10	100	Silt -Sand	30	Moderate	Channelization, Diffuse organic pollution
<b>8)</b> Forma media Treponti 33 T 4745058.25 m N; 316802.64 m E	0.4	4	20	Silt -Fine Gravel	80	High	Channelization, Diffuse organic pollution
<b>9)</b> Fosso vecchio Treponti 33 T 4745008.15 m N; 316869.76 m E	0.5	3	80	Silt	80	High	Channelization, Diffuse organic pollution
<b>10)</b> Forma nuova Treponti 33 T 4744929.48 m N; 316845.18 m E	0.5	2.5	70	Silt	50	Very high	Channelization, Point-source organic pollution
<b>11)</b> Fosso vecchio Fonti 33 T 4744664.53 m N; 317446.05 m E	0.3	2.5	20	Silt	100	Very high	Channelization, Diffuse organic pollution, Presence of obstacles
<b>12)</b> Clitunno Fonti 33 T 4744708.80 m N; 317445.71 m E	1	8	90	Silt - Sand	70	Moderate	Channelization
<b>13)</b> Fosso Treponti 33 T 4745034.65 m N; 316789.89 m E	1	2.5	20	Silt	50	High	Channelization, Diffuse organic pollution

527 **Figure legends**

528

529 **Fig. 1** Location of the sites selected for *P. leniusculus* removal activities. A) Tiber River  
530 watershed; b) Topino River sub-watershed; c) Hydrographic network of the upper Clitunno River  
531 basin and location of the eradicated stretches. An asterisk indicates the first detection site. The  
532 shaded area includes the species occurrence stretches.

533 **Fig. 2** Baited traps and artificial refuge traps: mean annual CPUE values for *P. leniusculus* are  
534 broken down by year and removal sites falling within the invaded area. Vertical bars denote  
535 standard errors.

536 **Fig. 3** Trend over time in mean daily CPUE values for *P. leniusculus* during eradication  
537 interventions conducted with Baited traps and artificial refuge traps in the Fosso Vecchio river.  
538 The dotted lines represent confidence limits (95%).

539 **Fig. 4** Monthly mean CPUE values for *P. leniusculus* during eradication interventions conducted  
540 with Baited traps and artificial refuge traps in the Fosso Vecchio river. Vertical bars denote  
541 confidence limits.

542 **Fig. 5** Electrofishing: mean annual CPUE values for *P. leniusculus* are broken down by year and  
543 removal sites falling within the invaded area. Vertical bars denote standard errors.

544 **Fig. 6** Box-plots of physico-chemical parameters collected during eradication activities carried out  
545 by electrofishing.

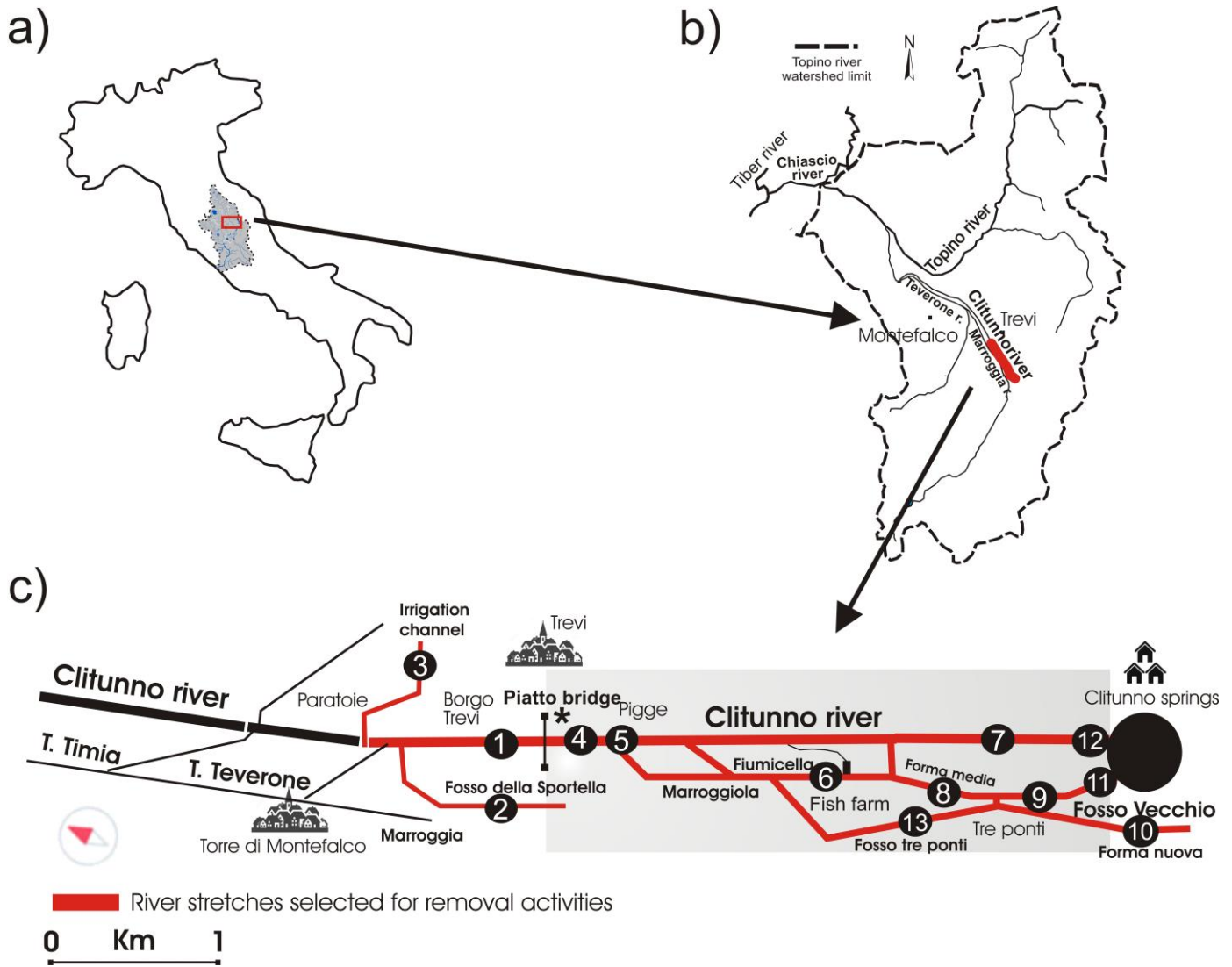
546 **Fig. 7** Frequency chart of carapace length for the total sample of males (black bars) and females  
547 (grey bars).

548 **Fig. 8** LWRs for *P. leniusculus* population of Fosso Vecchio river. Black and grey dots represent  
549 males and females, respectively.

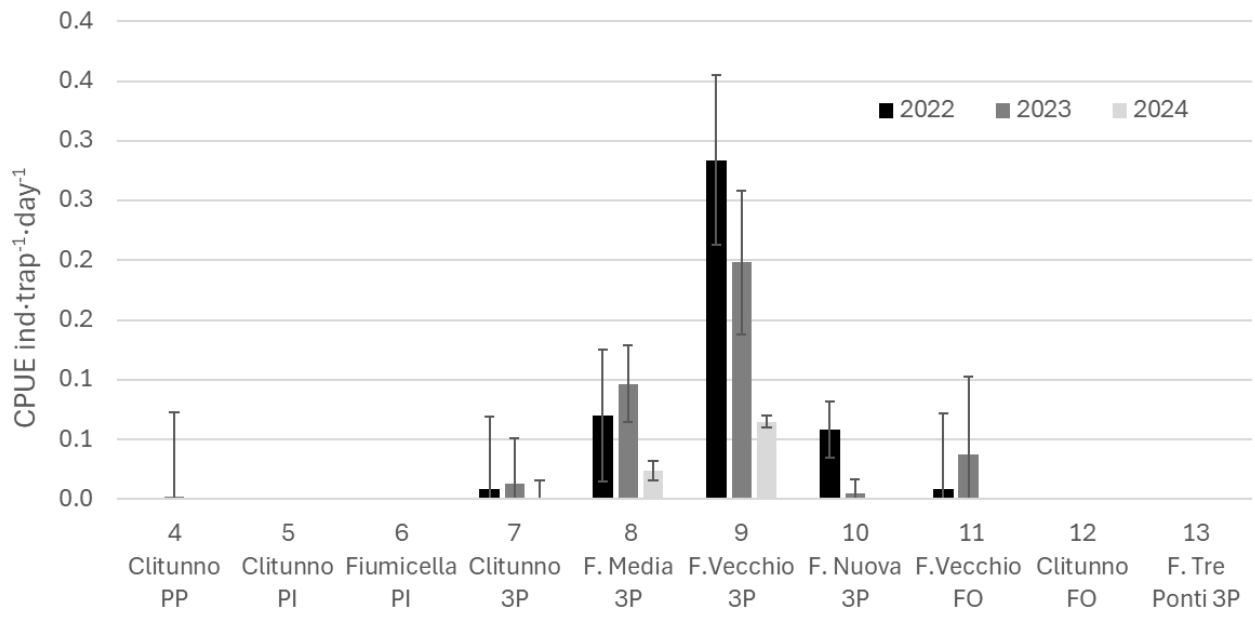
550 **Fig. 9** VBGEs for *P. leniusculus* calculated for females (grey) and males (black).

551 Fig. 1

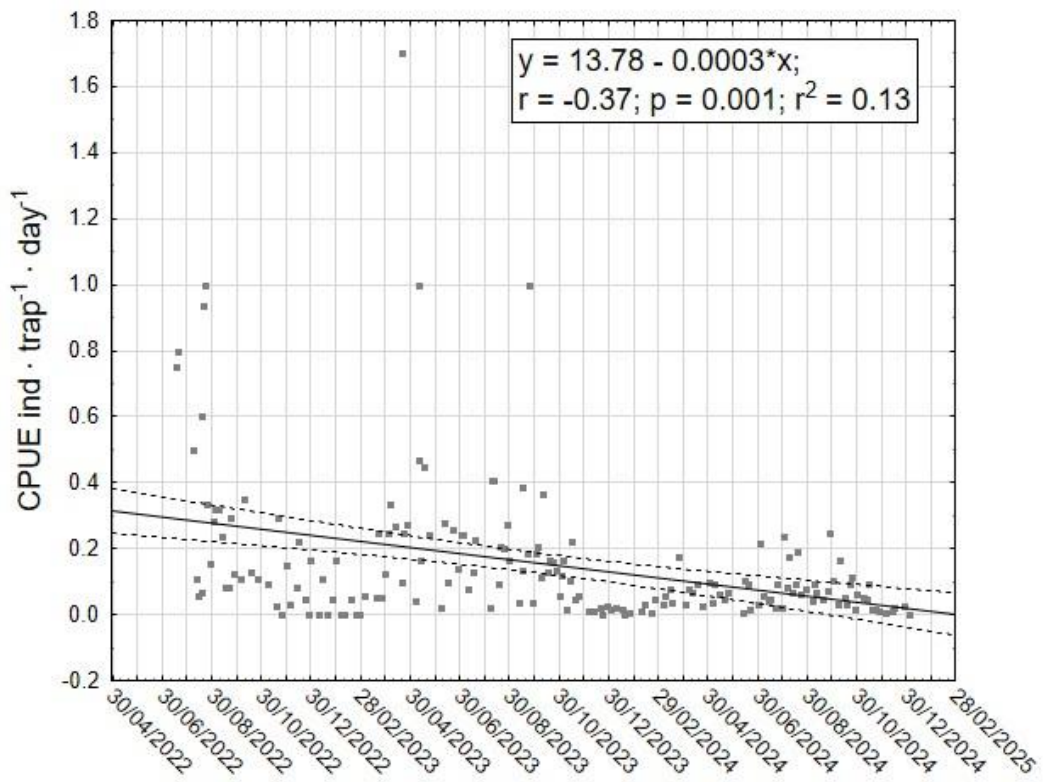
552



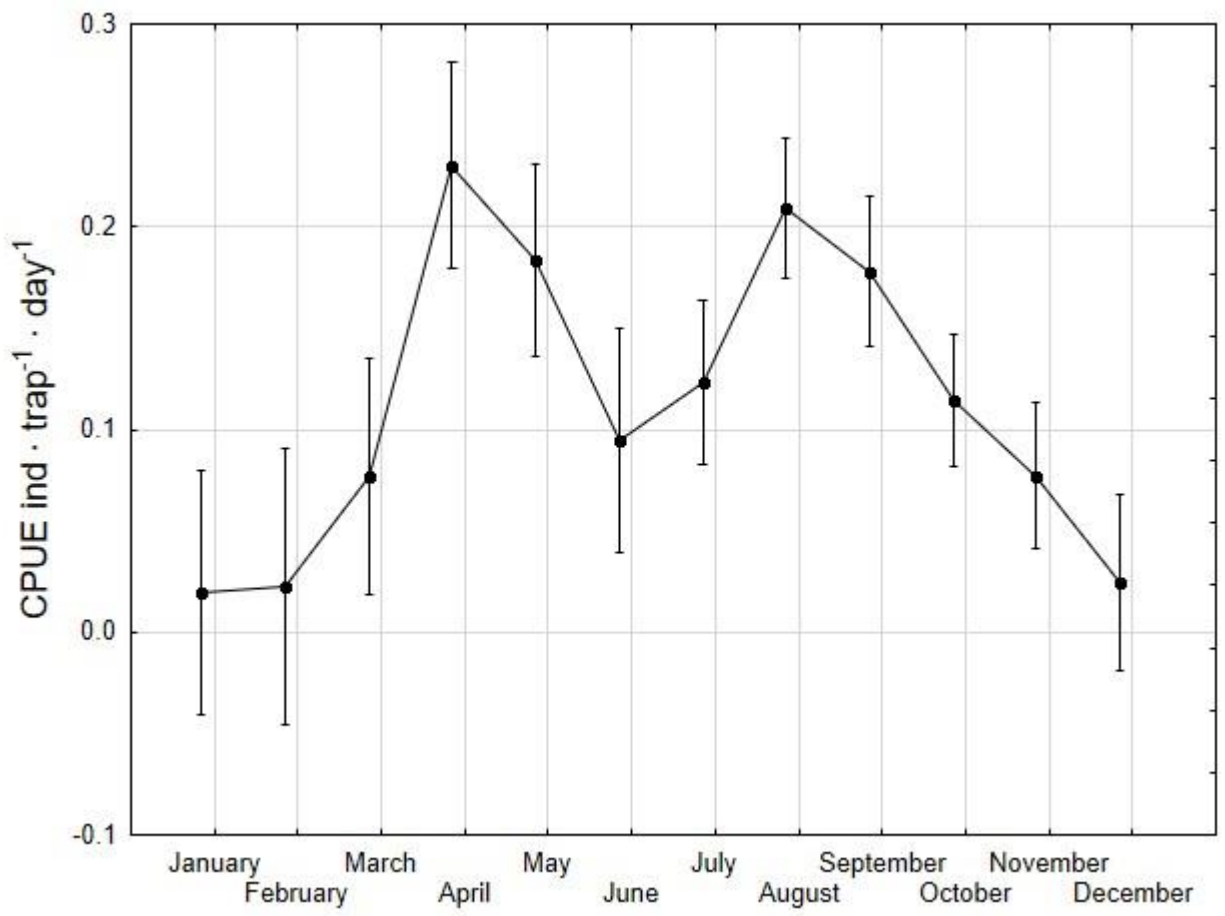
553 Fig. 2



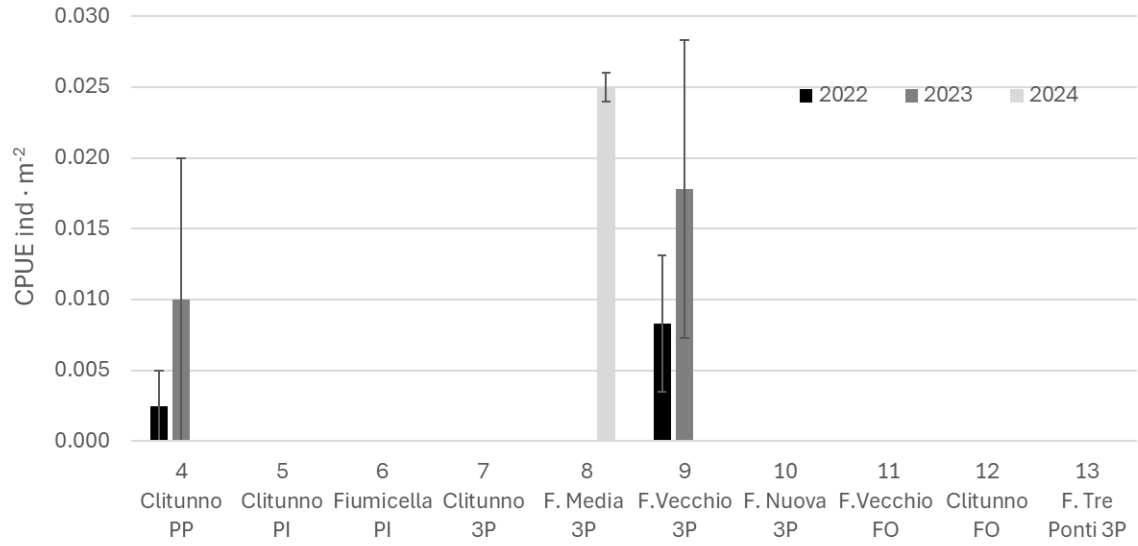
554 Fig. 3



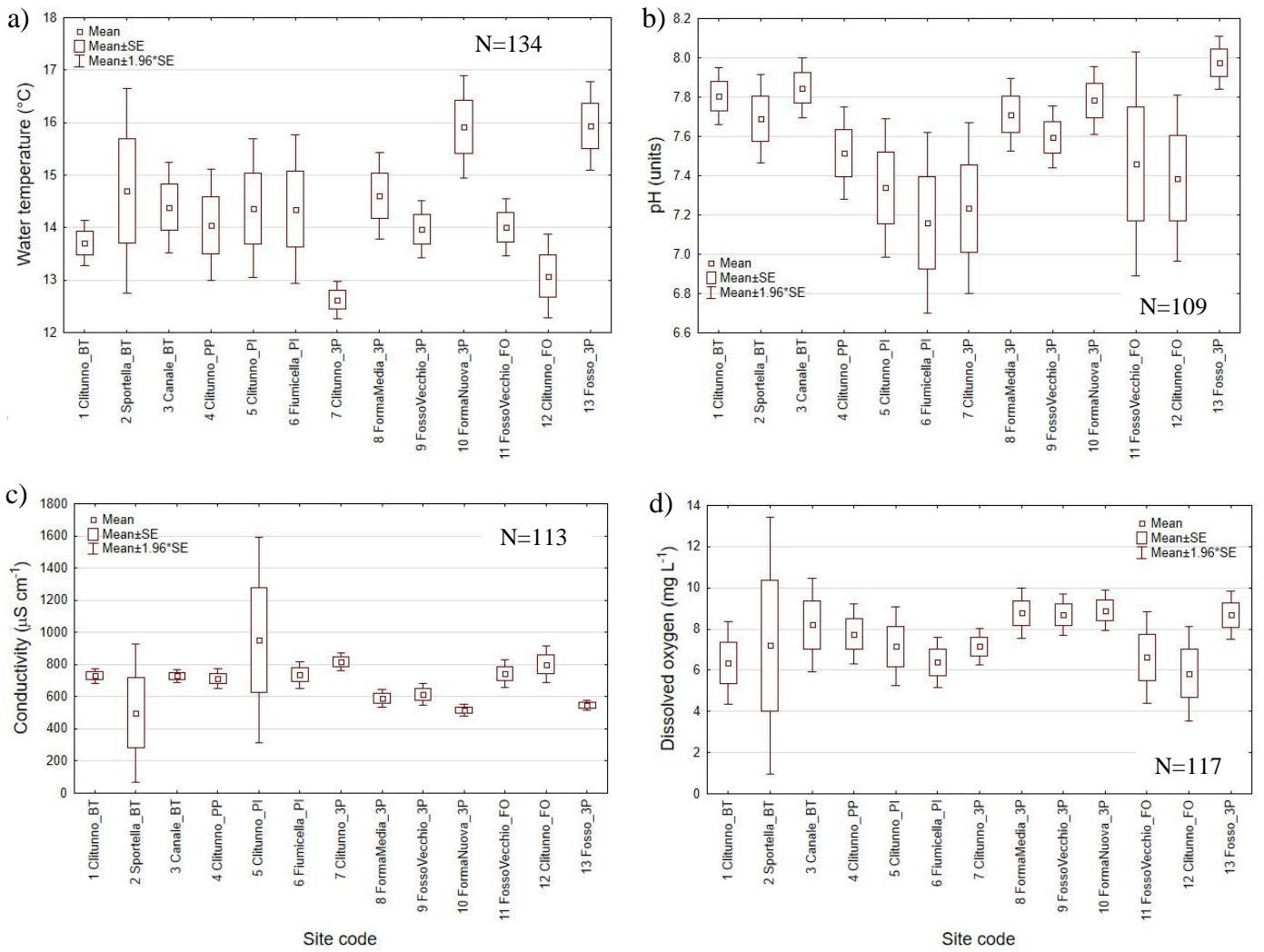
555 Fig. 4



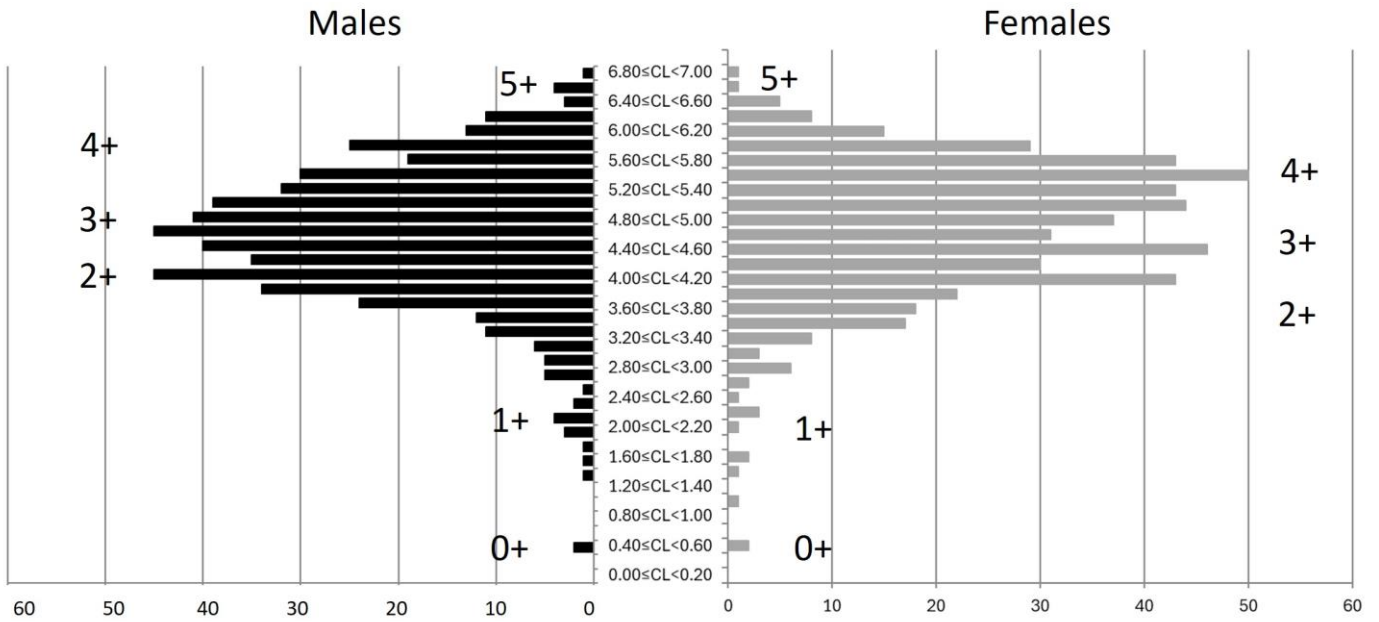
556 Fig. 5



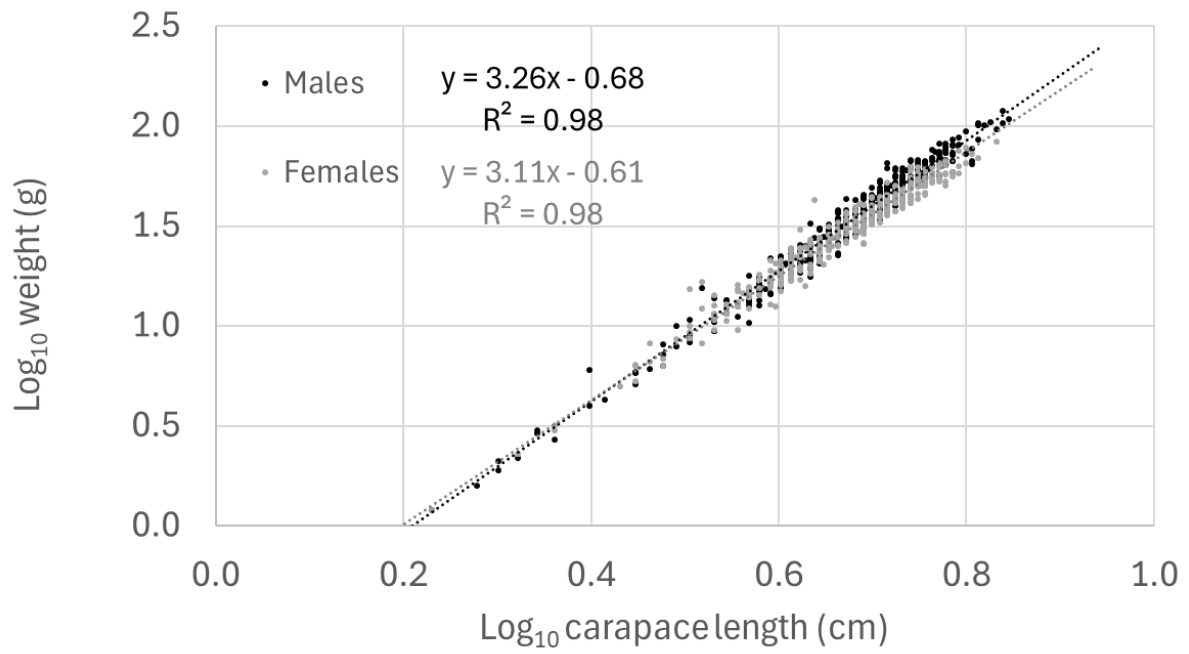
557 Fig. 6



558 Fig. 7



559 Fig. 8



560 Fig. 9

