

Article

Alien Crayfish Species in the Deep Subalpine Lake Maggiore (NW-Italy), with a Focus on the Biometry and Habitat Preferences of the Spiny-Cheek Crayfish

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Abstract: Invasive alien species are a major threat to biodiversity. Thus, it is fundamental to implement control strategies at the early stages of invasions. In the framework of the Italian-Swiss Alien Invasive Species in Lake Maggiore cooperative programme, we performed an extensive study on the occurrence and ecology of alien crayfish, one of the most significant invaders of freshwater habitats. From April 2017 to July 2018, we inspected seventy-five sites along the coastline to verify crayfish occurrence. We recorded, for the first time, the signal crayfish *Pacifastacus leniusculus*. Additionally, we found few individuals and remains of the red swamp crayfish *Procambarus clarkii*, and confirmed the presence of a consistent population of the spinycheek crayfish *Orconectes limosus*. Given the high number of *O. limosus*' individuals found, it was possible to perform in-depth biometric and ecological analyses for this abundant species only. We observed no significant differences of biometric measures between males and females of *O. limosus*. We explore its habitat preferences with a generalized linear model, detecting a significant relationship between mean annual temperatures and the presence of shelters of this species. These results, together, have direct implications for planning rapid management response actions on alien crayfish in large and deep lakes.

Keywords: lakes; invasion biology; non-indigenous species; *Procambarus Clarkii*; *Pacifastacus Leniusculus*; *Orconectes Limosus*

1. Introduction

Invasive Alien Species (IAS) are regarded as one of the major drivers of the global biodiversity decline [1–3]. They impact native species and ecosystems, but also the human society, by threatening Nature's Contribution to People (NCP) and causing economic and cultural damages [4]. In Europe, crayfish represent the most frequent freshwater invaders, responsible for local extinctions of native species, damage to freshwater resources, as well as to productive activities [5,6]. Their introduction is a serious threat to these key habitats [7], which are usually already compromised by several anthropogenic stressors including chemical pollution, climate change, and water derivations and withdrawals [8,9]. The spread of IAS is a further impeding factor that limits the effectiveness of protection, vanishing the areas of concern management, and the restoration strategies [10]. In Europe, there are at least eight species of alien crayfish, introduced intentionally or accidentally during the nineteenth and twentieth centuries [11]. Five of these species are considered of Union Concern [12]: *Orconectes limosus* (Rafinesque, 1817), *O. virilis* (Hagen, 1870), *Pacifastacus leniusculus* (Dana, 1852), *Procambarus clarkii* (Girard, 1852), and *Procambarus fallax* (Hagen, 1870) f. *virginalis*.

During the last 50 years, Italian lakes and rivers have been heavily affected by anthropogenic pressures, including the arrival of neobiota that have altered the native communities [13,14], and have

caused major impacts on the environment, as well as on socio-economic activities. Due to the complexity of their food webs structures and their socio-economic relevance, deep lakes deserve to be studied using deeper and articulated approaches than the standard routine (Water Framework Directive 2000/60/EC), in order to obtain a more nuanced understanding of their short- to long-term evolution.

Lake Maggiore is a large and deep lake of fluvio-glacial origin located on the southern side of the Western Alps, straddling the Italian-Swiss border. Over the past century, the catchment area of the lake has been interested by intense urbanization and industrial activities, which contributed to chemical and organic pollution of lake waters. In the past, eutrophication was one of the primary impacts to the lake ecosystem [15], followed by the rise in temperature of 1.4 °C in the upper 30 m depth (historical period 1965–2010). The synergistic effect of these two factors resulted in the extension of the epilimnion at lower depth and an increase in harmful cyanobacteria blooms frequency [16]. All these changes also favoured IAS arrival and acclimatization in the lake [17–20]. Two species of alien crayfish are reported to inhabit the lake: The red swamp crayfish *Procambarus clarkii* [21] and the spiny-cheek crayfish *Orconectes limosus* [22]. Since the 1970s, the lake has been included in the monitoring program of the International Commission for the Protection of Italian-Swiss Waters (CIPAI), which provides periodic monitoring of biological (phyto- and zooplankton, fish) and of physical-chemical parameters along the water column. Using this lake as a model system, in 2017 the project “Alien Invasive Species in Lake Maggiore” (SPAM) began, aiming at documenting the occurrence, the spatial distribution, and the abundance of alien macrophytes, crayfish and bivalves along the lake shores.

Within the SPAM framework, we conducted an extensive monitoring of the coastline of Lake Maggiore, aiming to identify the occurrence of alien crayfish. Once we identified the resident crayfish within the lake, we conducted an in-depth analysis focused on the most abundant species, to shed light on its natural history and autoecology. Specifically, we acquired biometric data on *O. limosus* in order to describe population size and variation in body traits, across the different life stages, and we studied the most important drivers of its spatial distribution. Building upon this evidence, our over-arching goal is to provide indications for the long-term management strategies to control alien crayfish in Lake Maggiore.

2. Materials and Methods

2.1. Study Site

Located in NW-Italy, Lake Maggiore is the second Italian lake by extension (area of 212 km², maximum depth 370 m, volume of 37.5 km³) [17]. Its surface area partly belongs to the Italian territory (80%), and partly to Switzerland (20%), while its catchment (6599 km²) is roughly equally divided between the two Countries. Due to the presence of the Alps, the local climate is characterised by high mean annual precipitation (1658 mm; reference period 1981–2018), with increasingly frequent extreme events primarily concentrated in the last 20 years [23].

2.2. Sampling Procedures

We monitored the whole coastline of Lake Maggiore for the occurrence of alien crayfish. In 2017, we carried out preliminary inspections to record the environmental features of the shorelines and to develop a standardized sampling protocol. We set the minimum distance between two different recording stations at 1.5–2 km along the coastline, to cover the entire perimeter of the lake (Figure 1), for a total of 75 sampling stations. We evaluated the following environmental features: type of substrate, dominant vegetation of the banks and of the shores, and the presence of native and alien crayfish (traces, burrows, remains, or live individuals). We classified the type of substrate basing on their granulometry as sand/silt (particle size < 1 mm), pebbles (particle size range 2–256 mm), and boulders (particle size > 256 mm) [24]. We assessed the occurrence of alien crayfish by visual encounter surveys [25] in the sites visited for preliminary inspections. We standardize research efforts

by setting visual assessment time at 20 min/site [25]. In parallel, local fishermen were interviewed to gain anecdotal data on the distribution of the species.

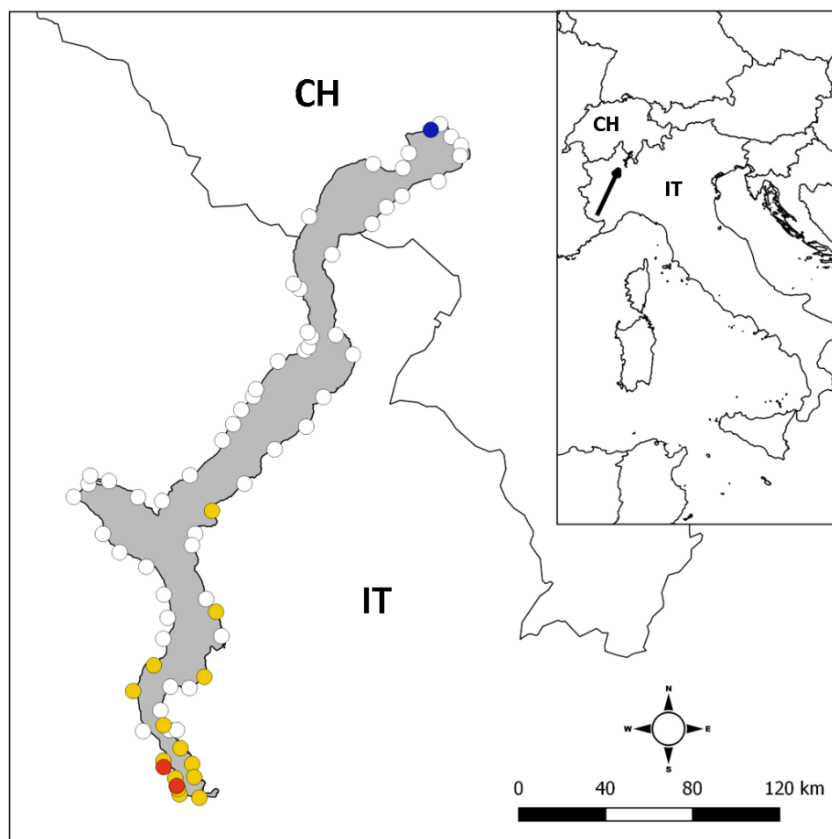


Figure 1. Sampling sites (dots) across Lake Maggiore (IT: Italy, CH: Switzerland). Red dots: sites of occurrence of *Procambarus clarkii*; Yellow dots: *Orconectes limosus*; Blue dot: *Pacifastacus leniusculus*; White dots: no alien crayfish detected.

In a second step, we selected eight sites on both eastern and western lake sides, to confirm the previous findings and for a deeper investigation through trapping, by drawing an imaginary line of representative sites along the north-south axis of the lake. We carried out sampling procedures tailoring the protocol described in Tricarico and coauthors [26] to a deep and large lake, placing 25 wire mesh double entrances cylindrical traps (30 × 60 cm) for each site, at a regular distance of ca. 10 m to one another. We used approx. 20 g cat food/trap as a bait to attract crayfish. We checked and removed traps after 24 h to: (i) Avoid wounds and cannibalism phenomena between individuals due to an excessive permanence; (ii) allow the release of by-catches; (iii) avoid the removal of traps by unauthorized personnel.

2.3. Biometric Analysis

We identified crayfish taxonomically according to Mazzoni and coauthors [27] and Souty-Grosset and coauthors [28]. For each captured specimen, we recorded: (i) sex; (ii) total body length (Lt_{tot}, from the tip of the rostrum to the tip of the telson); (iii) length of the cephalothorax (Lc_{ft}, from the tip of the rostrum to the end of the carapace); (iv) total individual weight (W_{tot}); (v) presence and number of eggs in ovigerous females. Crayfish belonging to the species *O. limosus* were divided in age/size classes [29] (Table 1):

Table 1. Age-size classes of *Orconectes limosus* according to Pieplow [29].

Age	Body Length (mm)
0+	Up to 40–65
1+	65–80
2+	80–95
3+	95–110

2.4. Statistical Analyses and Models

We performed all analyses in R software, version 2.13.2 [30]. *Orconectes limosus* was the only abundant species recorded along the lake shores, and thus, all in-depth analyses refer to this species. We tested the differences in biometry using a Student's t-test, taking into consideration the effect of size classes, and comparing present-day biometric data with historical data available in Bazzoni [22]. We graphically compared data using density plots in ggplot2 [31].

To understand environmental preferences of the species, we obtained present-day climatic data on average annual temperatures, maximum annual temperatures, and minimum annual temperatures from WorldClim 2 [32], all variables at a resolution of 30 arc-sec (ca. 1 km at the equator). We derived precipitation data from daily surveys of Lake Maggiore catchment areas used for the preparation of reports for CIP AIS (<http://www.cipais.org>, accessed on 13 May 2020) and related to the seasonality preceding the sampling period (December 2016–December 2017). We then stacked these environmental variables in a single raster and extracted the climatic conditions for each sampling location.

We defined the habitat preferences of *O. limosus* using regression-type analysis (generalized linear model; GLM) [33]. In contrast to univariate analyses, the use of GLM allowed us to account for the combined effect of explanatory variables as well as potential interactions [33–35]. Given the low abundance of individuals detected in each sampling site (ranging 1–3 individuals), we expressed counts as presence/absence (i.e., Bernoulli distribution 0–1). Thus, we modeled the probability of occurrence rather than abundance values.

Prior to model fitting, we explored the dataset following the protocol for data exploration by Zuur and coauthors [34]. We checked for the presence of outliers using Cleveland's dotplots and we investigated multi-collinearity among continuous covariates using pairwise Pearson correlation tests (r), setting the threshold for collinearity at $|r| > 0.7$. We inferred the associations between categorical and continuous covariates graphically, with boxplots. Finally, we used coplots to explore possible interaction among covariates.

We developed Bernoulli GLMs in R [30] using a complementary log-log link function (clog-log) as recommended in Zuur and coauthors [35] for datasets with unbalanced presence/absences data (in our case, ~70% observations were absences). Once we fitted the initial model, including all covariates and interactions of interest, we applied model selection [36,37]. We carried out model reduction (backward elimination) on the full model by sequentially deleting terms according to corrected Akaike criterion for finite sample size (AICc) values [38]. We reiterated the reduction process until a minimum adequate model remained, namely the best model supported by observations that avoided overfitting [39].

3. Results

3.1. Occurrence of Alien Crayfish

We confirmed the occurrence of three alien crayfish in Lake Maggiore. The map of distribution is presented in Figure 1. We recorded the occurrence of the signal crayfish *Pacifastacus leniusculus* for the first time in Lake Maggiore (Canton Tessin, Switzerland) based on three specimens collected in Tenero (Mappo and Rivapiana Minusio) by a professional fisherman [27]. The species was not found in the Italian side of the lake.

We detected a live individual of the red swamp crayfish *Procambarus clarkii* (Palude Bruschera Nature Reserve SCI IT2010015-Lombardy, Italy), while we found only remains in two Dormelletto sites (Piedmont, Italy).

As anticipated in the methods, *O. limosus* was the most abundant invasive crayfish (Figure 1). We found alive specimens in 13 sites along the central-southern Italian shoreline (17.3% of investigated sites), while in 7 additional sites we confirmed the species presence through remains (9.3% of sites).

3.2. Biometric Analyses and Population Size Structure of *O. Limosus*

We collected a total of 238 specimens of *O. limosus*, 72% males and 28% females. A total of 157 crayfish were collected through trapping in 3 out of 8 selected sites (66% of total individuals). In the other five sites there were no stable populations. A summary of biometrics is available in Table 2.

Table 2. Body size measures. Distribution range (minimum, maximum, mean, and standard deviation values) for the main biometric features for *Orconectes limosus*. Wtot: $n = 227$, Ltot and Lcft: $n = 238$.

Sex	Total Body Length			Total Weight			Cephalo-Thorax Length		
	L min	L max	L mean	W min	W max	W mean	CL min	CL max	CL mean
M	3.94	10.57	6.66 ± 1.19	1.75	27.20	10.07 ± 5.37	1.3	4.54	3.15 ± 0.73
F	3.04	9.88	6.86 ± 1.71	0.80	30.43	11.30 ± 7.47	1.1	4.89	3.24 ± 0.98
Total M+F	3.04	10.57	6.71 ± 1.35	0.80	30.43	10.41 ± 6.03	1.1	4.89	3.18 ± 0.80

We observed no significant differences between males and females for either cephalothorax length ($t = -0.72$, $p > 0.05$), total length ($t = -1.01$, $p > 0.05$), or weight ($t = -1.39$, $p > 0.05$), even within size classes (Figure 2).

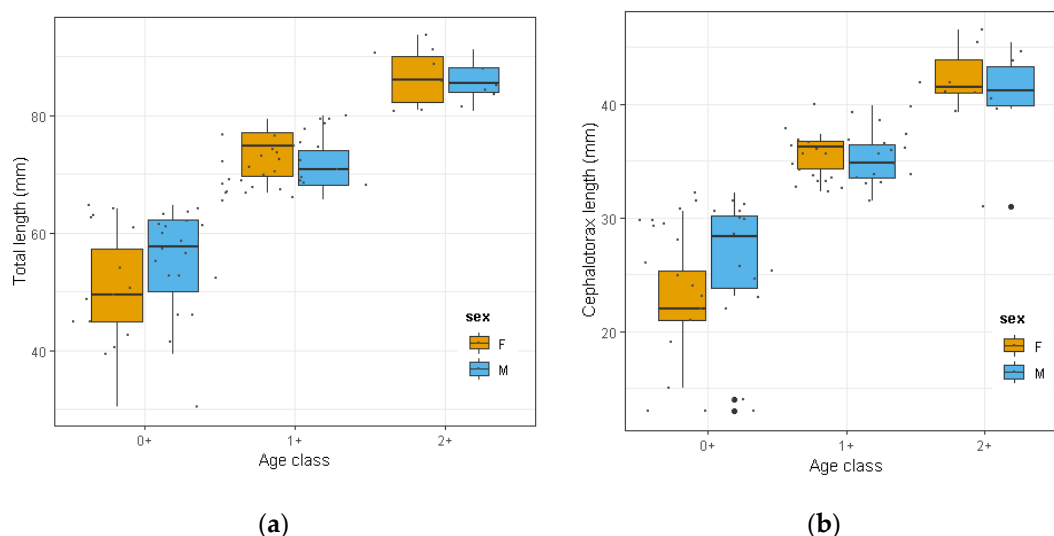


Figure 2. Boxplots showing variations in total body length (a) and cephalothorax length; (b) among age classes of *Orconectes limosus* in Lake Maggiore. Grey dots are observed values used to calculate each boxplot. A random noise (jitter) is applied to aid visualization of otherwise superimposed dots.

The comparisons of data acquired in this study with the ones collected in 2006 by Bazzoni [22], highlighted a significant difference both in total body length ($t = 14.66$, $p < 0.001$) and total weight ($t = 13.36$, $p < 0.001$), and, in the former case, the observed difference is ascribable also to sex ($t = -2.19$, $p = 0.03$) (Figure 3).

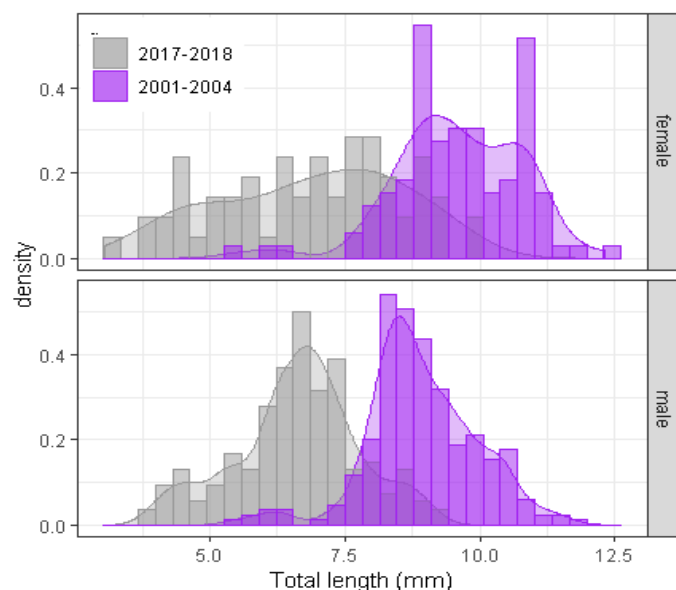


Figure 3. Comparison between 2017–2018 (grey) and 2001–2004 (purple) data [22] on body length of females (top) and males (bottom) of *Orconectes limosus* in Lake Maggiore. Histograms represent the observed values, whereas the smoothed line is the kernel density estimate of the distribution.

In Spring 2018 (between 27th April and 30th May), ten ovigerous females were captured in two sites (Dormelletto and Lisanza) in four different sampling sessions. Females measured between 50.4 mm and 78.6 mm (mean number of eggs/female: 72)

3.3. Influence of Environmental Factors on *O. limosus* Distribution

Following data exploration [34], we dropped from the regression analysis the variable average precipitation, warmer temperature, and colder temperature, being mutually collinear ($|r| > 0.7$) and also associated with the categorical variables of shelter presence. No outliers were present in the dataset. Coplot revealed a potential interaction between mean annual temperature and the presence of shelters, which we incorporated in the initial regression structure.

The initial model included substrate type (Substrate), presence of algae (Algae), presence of macrophytes (Macrophytes), and presence of shelters in interaction with mean annual temperature (Shelters * Tmean).

According to model selection (Table 3), the most appropriate model structure supported by the observations explaining the habitat preference of the alien crayfish had the following structure:

$$y \sim \text{Tmean} * \text{Shelters} \quad (1)$$

Table 3. Model selection according to the corrected Akaike information criterion for finite sample size (AICc [35]). Model are ordered from the least to the most appropriate. df: degrees of freedom, ΔAICc : difference of AICc, wi (AIC): Rounded Akaike weights *sensu* Burnham & Anderson [33].

Model Structure	Df	AICc	ΔAICc	wi(AIC)
$y \sim \text{Tmean} * \text{Shelters} + \text{Substrate} + \text{Algae} + \text{Macrophytes}$	8	74.00	5.27	0.02
$y \sim \text{Tmean} * \text{Shelters} + \text{Algae} + \text{Macrophytes}$	6	70.27	1.54	0.18
$y \sim \text{Tmean} * \text{Shelters} + \text{Macrophytes}$	5	68.81	0.08	0.38
$y \sim \text{Tmean} * \text{Shelters}$	4	68.73	0.00	0.40

The significant interaction (Tmean * Shelters, estimated $\beta \pm \text{s.e.} = -6.66 \pm 3.02$, $p = 0.02$) reflects a differential response to mean annual temperature (Tmean = 4.89 ± 2.23 , $p = 0.02$) depending on the

presence or absence of shelters (Shelters = 80.97 ± 36.09 , $p = 0.02$). The probability of presence of the species increased positively with increasing mean annual temperature in habitats lacking shelters, whereas the trend was flat to slightly negative in areas with shelters (Figure 4).

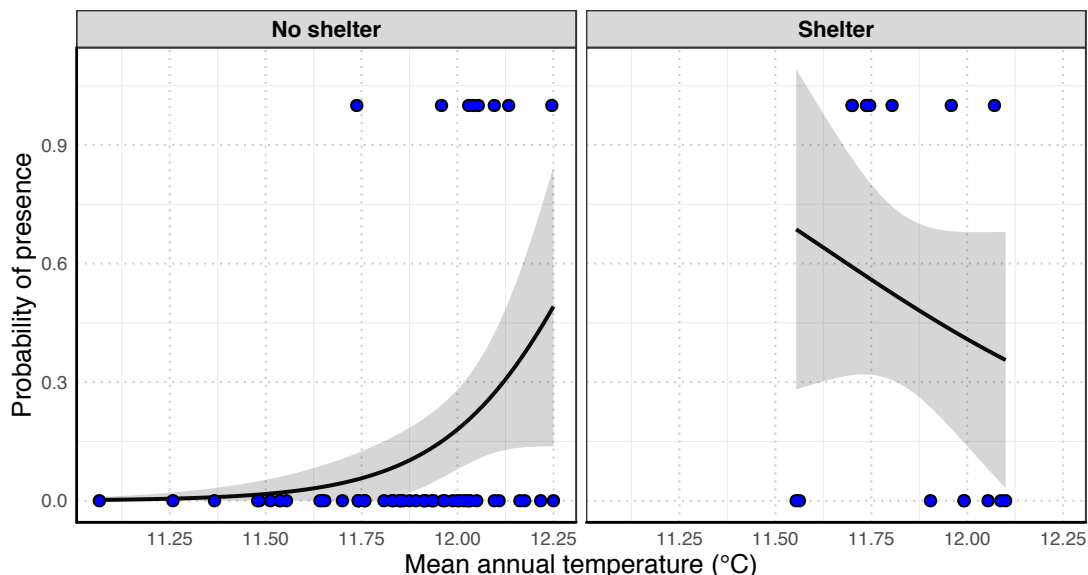


Figure 4. Predicted relationships between the presence-absence of *Orconectes limosus* and the mean annual temperature in interaction with the presence of shelters. Shaded grey surfaces are 95% confidence intervals. Blue dots are observed values.

4. Discussion

We documented the occurrence of three alien crayfish species in the deep subalpine Lake Maggiore: *Pacifastacus leniusculus*, *Procambarus clarkii*, and *Orconectes limosus*. Concerning the first species, this is the first record in Lake Maggiore. In Switzerland, *P. leniusculus* was introduced around 1980, although it showed a scattered distribution [40]. In 2007, one specimen was also found in Canton Tessin, in a tributary of the lake near Minusio [41]. These authors hypothesized that the finding was linked to an occasional introduction, and argued that the species was still not of concern. The specimens from our study were found in 2015 (one individual) and in 2017 (two individuals), and were reported by a local fisherman in the lake zone beyond Tenero/Mappo and Rivapiana of Minusio. This is suggestive of an on-going process of acclimatization of the species on the littoral shores of the lake standing Minusio's river tributary. However, as low water temperature seems to be the major predictor that determines the abundance of the signal crayfish (12.7 °C) [42], the cold waters of the lake recorded during winter seasons (average winter temperature 3.9 °C, data available at www.cipais.org) could prevent, or slow down, its spread. Further analyses are needed to clarify this aspect.

The species *P. clarkii* has been regularly found in Lake Maggiore since 2016, although records are only anecdotal [21]. This species is recognized as the most successful IAS in Italy [43]. *Procambarus clarkii* is considered a typical inhabitant of warm waters, being tolerant to water eutrophication and mineralization [44]. Notwithstanding suitable environmental features for its establishment are present in the lake, and the fact that a stable population have been reported for the nearby Lake Orta [45], our data suggests that the species is not widespread in Lake Maggiore yet. Delmastro [21] reported observations from researchers and operators of the Environmental Agencies during monitoring activities in the lake, but its occurrence is seemingly linked to the presence of waterways, such as tributaries or outlet of the lake. Local fishermen, who were interviewed, confirmed this tendency. At present, no record is available for the lake side representing the Verbano-Cusio-Ossola province.

A thorough population analysis was possible only for *O. limosus*. In Italy, the species is known since 1991 [46], when it was accidentally introduced from Poland. Currently, it is widespread particularly in

the Po Plain [43]. In Lake Maggiore, it was firstly reported by Bazzoni [22], who found specimens in the Borromean Gulf on the central part of the lake. Our results demonstrate that *O. limosus* occurs mainly on the central-southern part of the lake, near the River Ticino outlet connecting the lake to the Po Plain. In a previous paper, focused on the spatial dynamic of invasion and on the establishment of the invasive bivalves *Corbicula fluminea* Müller, 1774 in the lake, Kamburska and coauthors [20] hypothesized that this mollusc initially settled in the southern basin of the lake, starting from populations established in the River Ticino outlet [47]. Quite possibly, we are observing a similar invasion dynamic, i.e., the spread of the species from the River Ticino lake-outlet to the southern and northern district of the lake. Bazzoni [22] reported that the population was changing during the monitoring period, observing a decrease in the number of individuals in the Fondotoce Nature Reserve from 2000 to 2004. In the Nature Reserve, we never detected the species, both through visual census or trapping sessions in two consecutive years. Tentatively, we suggest that its decrease in the area is linked to the presence of a large Grey Heron (*Ardea cinerea*) colony near the Reserve (Figure 5). Indeed, terrestrial predators such as herons, belonging to *Butorides* and *Ardea* genera, have been demonstrated to caused little crayfish mortality in deep areas, but can rapidly consume both small and large crayfish exposed in shallow areas [48]. Unfortunately, the heronry is outside the Nature Reserve, on the opposite side of a nearby congested road that is periodically subjected to side tree cutting. Further studies are required to understand the effectiveness of bird predation on the population of *O. limosus*. However, since these ardeids (species included in the Bird Directive 2009/147/EC) can consume a large amount of crayfish, we suggest that the heronry of the Fondotoce Nature Reserve to be included in under the umbrella of protection of the Site of Community Importance (SCI).

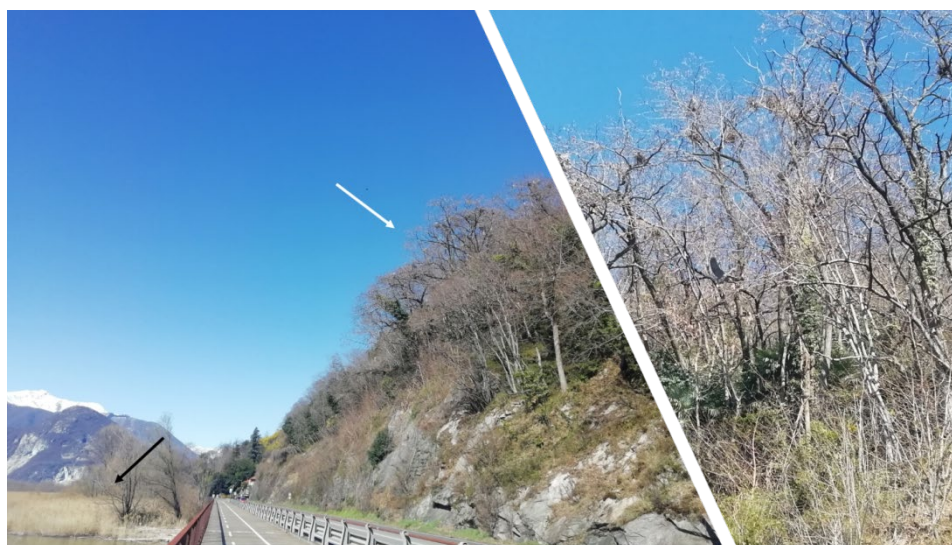


Figure 5. The heronry (**white arrow**) near the Fondotoce Nature Reserve (**black arrow**) in Lake Maggiore.

Concerning biometrics, we did not find a significant difference between males and females for total body length and total weight. Although sexual dimorphism is widely diffused in Decapods [49], this has not been fully demonstrated for *O. limosus*. Pieplow [29] did not distinguished between males and females in total body length. Důriš and coauthors [50] found a difference in the total body length between sexes, but males were larger than females in brooks and isolated waters, and smaller in large rivers. Pilotto and coauthors [51], who observed a similar biometry in *O. limosus* populations in the nearby Lake Varese, did not found significant differences in cephalotorax length between sexes, observing differences only within age classes. These differences in findings are probably related to a unique feature of the females of this species: similarly to the conspecific males, they undergo significant

cyclic changes in size, producing larger chelae, abdomen, and body dimensions, especially during the moult to form I (i.e., when an adult crayfish changes from a sexual inactive to a sexual active form) [52].

Differences in biometry and size classes, between present and past results available for the Lake Maggiore [22], can be linked to the different trapping method employed in the present time respect to the past (2001–2004). We employed traps with a mesh size of 1 cm², allowing us to catch also young-of-the-year individuals, while Bazzoni [22] employed traps with meshes of 4–5 cm, unsuitable for catching small-sized individuals (Figure 3).

Concerning the environmental factors affecting crayfish distribution, we found that, in the absence of shelters, the probability of occurrence of *O. limosus* increases with the raise of mean annual temperatures. This result suggests that the species succeeds in warmer areas, while in presence of refugees, there is not a specific selection of areas to be colonized. Thus, the lack of suitable refuges may be a strong limiting factor for the species spread in absence of optimal climatic conditions, as confirmed for the species *P. leniusculus* [53]. This result is in accordance with general studies on the ecological drivers of invasive crustaceans, such as crayfish [53–55] and crabs [56], pointing out the crucial role of climate in determining the successful outcome of biological invasions.

It is interesting to note that we did not found any specimen in the northern part of the lake. The substrate along the coasts changes along the south-north axes from sandy beaches, mixed with gravel and algae formations in the southern part, into rocky cliffs in the central part of the lake, to beaches with boulders in the northern part. The boulders were settled in by humans to create narrow beaches for tourists and as docking sites for small boats. However, the substrate was not a significant factor in our regression model, and was dropped through model selection. This result contrasts with previous observations on other crayfish species, where significant relationships between granulometry and species abundance were observed [42,57].

The finding of few ovigerous females in April and May 2018 indicate the Lake Maggiore as a potentially suitable reproductive site for the species. Furthermore, according to the structure of age/size classes based on the classic work of Pieplow [29], females were between the first and the second year of life. This is contrasting to previous observations [58,59], which suggested that females reach sexual maturity from the second year of life.

From a methodological point of view, we support the efficiency of visual inspection for the detection of crayfish [25,60] to be used as a complementary tool of traps. By setting a standard time for preliminary inspection through visual survey, we were able to verify 75 sampling points for a total of 170 km of inspected shoreline in a reasonable time. Moreover, only through inspections we were able to assess the occurrence of *P. clarkii*, as this species never fell into traps. Although the method is not without bias [25], visual encounter survey is an effective and inexpensive approach to screen for the presence of alien crayfish from large lakes.

5. Conclusions

The Invasive Alien Species in Lake Maggiore monitoring project was a pilot study that allowed us to detect the occurrence of three alien species in this large subalpine lake, and to shed some light on the autoecology of the most abundant species, *O. limosus*. The recovery of these species of Union Concern raises important conservation issues that need to be addressed. The finding of *P. leniusculus* in the Swiss part of the lake is worrisome because of the proximity of the Bolle di Magadino Nature Reserve, and of several source population of the native *Austropotamobius pallipes* in the lake catchment. Moreover, the alien species, observed in this study, not only compete with the native fauna, but are also the primary vectors of the oomycetes *Aphanomyces astaci* Schikora 1906, the causative agent of the crayfish plague [61]. We suggest that Italy and Switzerland should agree on concrete actions to prevent the spread of crayfish by intensifying trapping sessions in the spring reproductive period. Further investigations are needed to understand the ecology and the behavior of invasive crayfish, in particular, concerning their vertical distribution in relation to depth and the possible interaction

with natural predators such as fish and ardeids. Altogether, the evidence may contribute to reducing the level of uncertainty of environmental management actions to be proposed at the catchment scale.

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Conflicts of Interest: The authors declare no conflict of interest.

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