

Research Article

Environmental conditions influencing the early colonization stage of *Ludwigia hexapetala*, an aquatic plant recently invasive in Italy

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Abstract

Freshwater ecosystems are among the most susceptible to biological invasions. The South American Ludwigia hexapetala is an aquatic plant that is becoming an increasing threat in many European waterbodies, recently including Italy. This study aimed to define the main parameters influencing the early colonization stage of L. hexapetala by overlapping the percentage cover of this species with environmental parameter data collected at 24 aquatic sites from six waterbodies in north-central Italy. At each site, chemical and physical characteristics of the water (temperature, pH, dissolved oxygen, conductivity, nitrates, phosphates, ammonia, depth, transparency), grain size of the substrate and level of anthropogenic disturbance were evaluated. The results showed that although *L. hexapetala* prefers shallow, warm, alkaline, moderately rich in ions and nutrients (especially phosphates) and oxygen-poor waters, it can grow in a wide range of environmental conditions. Moreover, as a typical invasive alien species, it spreads opportunistically in disturbed, unstable sites. Thus, L. hexapetala can invade freshwater habitats with different environmental conditions and subjected to anthropogenic disturbance. However, the results suggest that water depth may be a limiting factor in the early colonization stage of this species, which does not seem to be able to colonise waters deeper than 1 m in investigated sites, while it has been observed in significantly deeper waters in other European countries with a longer invasion history. Detecting the environmental parameters that most influence the growth of *L. hexapetala* becomes crucial both to identify the sites most at-risk of invasion in which to initiate timely monitoring actions for the species, and to be able to develop better management and control actions for this alien species in sites that have already been invaded.

Key words: aquatic invasion, autoecology, biological pollution, freshwater ecosystem, invasive alien species, non-native macrophyte, water primrose

Introduction

Biological invasions, meaning colonization of alien species to areas outside of their home range, are a major cause of biodiversity loss and functional and structural alteration of ecosystems worldwide (Seebens et al. 2017). Among the environments

most susceptible to biological invasions, freshwater ecosystems occupy a critical place for their intrinsic vulnerability and because they are often subject to anthropogenic disturbance, which generally favors the spread of alien plants (Shea 2002; Anufriieva and Shadrin 2018; Dimitrakopoulos et al. 2022); indeed, freshwater ecosystems exhibit the highest number of invasive alien plant species (Lazzaro et al. 2020).

Invasive alien plants are adaptable species, with high reproductive capacity, that often outcompete native species, causing deterioration of local biodiversity and alteration of plant communities in colonized habitats (Pyšek et al. 2012; Gigante et al. 2018; Viciani et al. 2020). Although awareness of the impacts exerted by these species on invaded habitats has increased in the last decade (e.g., Ceschin et al. 2020; Lazzaro et al. 2020; Kerns et al. 2021), studies regarding the ecological traits that make some alien aquatic plants highly competitive and invasive in Europe are still quite scarce (Lazzaro et al. 2020; Viciani et al. 2020). Therefore, filling this knowledge gap should be one of the priorities in scientific research to fully understand the ecological traits of invasive species, including both their strengths and possible limits, comprehension of which becomes crucial for their better management and control.

A highly invasive aquatic plant in several European countries, recently including Italy, is Ludwigia hexapetala (Hook. & Arn.) Zardini, H.Y. Gu and P.H. Raven (Onagraceae), which is native to South America (Wagner et al. 2007; Liu et al. 2017). Ludwigia hexapetala was first reported in Italy in 1934 in the northern regions (Di Pietro et al. 2007), where it probably arrived as a consequence of its expansion throughout Europe due to its use as an ornamental plant (Dandelot et al. 2008). It has since spread in more recent times to the central parts of the Italian peninsula, invading major waterbodies, such as the volcanic Lake Bracciano (Azzella and Iberite 2010; Buono et al. 2019). Ludwigia hexapetala can colonize still or slow-flowing waters, marshy wetlands and banks of lakes, rivers and canals (Dandelot et al. 2005; Rolon et al. 2008; EPPO 2011; Thouvenot et al. 2013b). It can grow in both aquatic and terrestrial habitats, showing remarkable morphological plasticity, evidenced by possessing two different morphotypes (Billet et al. 2018; Thiébaut et al. 2018): an aquatic one and a terrestrial one. The aquatic morphotype is characterized by round leaves grouped in rosettes at the stem apex, and leaves of varying shape, ranging from elliptic to oblanceolate, along the stems below the water surface. The terrestrial morphotype is characterized by elongated vertical flowering stems and mostly lanceolate leaves, although leaf shape in both forms is highly plastic in response to life stage and local conditions (Thouvenot et al. 2013b; Hussner et al. 2016). Ludwigia hexapetala also produces three types of roots: traditional branched roots, which grow downward, anchoring the plant to the sediment; spongy pneumatophores, which grow upwards, that act as specialized structures for tissue aeration (Ellmore 1981); adventitious roots produced at floating stem nodes that acquire nutrients from the water column and facilitate propagules dispersal and establishment (Gérard et al. 2014; Hussner et al. 2016; Billet et al. 2018; Skaer Thomason et al. 2018b). These morphological adaptations, combined with high growth rate, photosynthetic efficiency (Thouvenot et al. 2013b), and the ability to produce allelopathic substances that hinder other plant species (Dandelot et al. 2008; Thiébaut et al. 2018), denote the clear competitive nature of this alien species (Billet et al. 2018; Genitoni et al. 2020). The phenotypic plasticity of *L. hexapetala* would also highlight the broad ecology of the species, another key determinant factor in the success of many alien species in invaded areas (Richards et al. 2006; Davidson et al. 2011; El-Barougy et al. 2021). However, it is still unclear whether such ecological breadth of many invasive alien species may concern all ecological parameters or only some (Palacio-López and Gianoli

2011; Zhang et al. 2022). Therefore, a detailed investigation of the ecological requirements of invasive alien species, such as *L. hexapetala*, becomes essential to identify both the optimal environmental conditions that favour their colonization and expansion, and any limiting conditions that might control its growth.

The aim of the present study has been to define the main environmental parameters that influence the colonization and abundance of *L. hexapetala* by collecting field data in aquatic habitats in north-central Italy recently invaded by this alien species. Particular attention was paid to the environmental characteristics of aquatic habitats, which represent the first "front" of invasion of this species, in the early colonization stage of a new area. Detecting the environmental parameters that most influence the growth of *L. hexapetala* becomes crucial, both to identify the sites most at-risk of invasion in which to initiate timely monitoring actions for the alien species in sites that have already been invaded.

Materials and methods

Study area

A total of 24 relevés were performed (one in each sampling site) in six waterbodies in north-central Italy (Fig. 1), where the species was previously reported (17 relevés in invaded areas) (Lucchese 2017) or where it could potentially grow (7 relevés in uninvaded areas). The sampled waterbodies included the Middle and Superior Lakes in Mantova, Lake Bracciano, a canal in Torvaianica, and two canals in Latina province (see Suppl. material 1). The Mantova Lakes are a system of three shallow, fluvial lakes (Superior, Middle, Inferior), located along the Mincio River and adjacent to the city of Mantova, where L. hexapetala has spread in the last few years (Tóth et al. 2019). Bracciano Lake is an oligo-mesotrophic hard water volcanic lake where *L. hexapetala* was originally reported in 2010, although initially mistaken for the congeneric L. peploides (Kunth) P.H. Raven (Azzella and Iberite 2010). After the drastic decrease of water levels in 2017 (Giuliani et al. 2019), the spread of this alien species in the area has increased, becoming a real threat to the conservation of local populations of native and vulnerable plant species (Buono et al. 2019), such as the endemic Isoëtes sabatina Troia, Azzella (Troia and Azzella 2013) and the aquatic carnivorous *Utricularia australis* R. Br. (Ceschin et al. 2022; Pelella et al. 2023a,b). The canal in Torvaianica is a drainage ditch surrounded by urban areas, extending for about 600 meters before flowing into the Tyrrhenian Sea. It is highly disturbed, especially in summer, since it is frequently visited by citizens walking the path alongside it to get to the beach. Here, the invasion of L. hexapetala seems to be very recent, as it was observed for the first time in the summer of 2021 (F. Mariani, personal obs.). The canals in Latina province constitute a large network of waterbodies, carrying water used for irrigation and agriculture. The invasion of these canals by L. hexapetala would appear to be recent, having first been reported here in 2017, specifically in the Schiazza Canal (Lucchese 2017). This currently represents the southernmost population of this alien species in Italy.

Sampling procedures

The study was performed between late June and early September 2022, the most favourable months for the growth of *L. hexapetala* (Vernay 2022); all populations were sampled during the flowering period. Data collection for evaluation



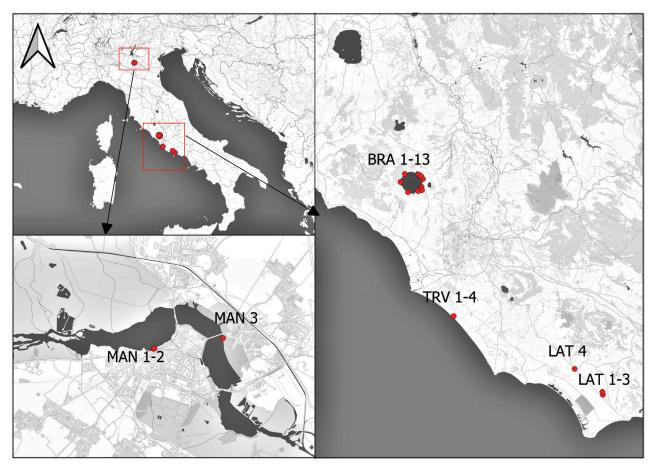


Figure 1. Map of study area in north-central Italy. A detailed view of sampling sites in the Lombardy and Latium regions is provided. Sites are pictured with red dots, with each site corresponding to one relevé. The arrow in the top left points to the north.

of L. hexapetala abundance (% cover) and associated environmental variables was carried out in aquatic habitat at sites where L. hexapetala occurred with different percentage covers. In each waterbody, one or more sampling sites were randomly selected; in each site, one relevé was performed in a standard rectangular area (10 \times 3 m), with the longest side parallel to the bank. The number of relevés for each waterbody is proportional to the invaded area and reflects the diversification and in-site variability of each waterbody. To quantify the abundance of L. hexapetala, an ocular estimate of relative % cover was conducted by the same researcher in all relevés for uniformity of data. To identify factors potentially limiting the growth of L. hexapetala, some relevés were also performed in sites without, or with low abundance of *L. hexapetala* (see Suppl. material 1: table S1). In addition to plant cover, water chemical and physical parameters were measured at each site. Specifically, temperature (°C), conductivity (µS/cm), pH and dissolved oxygen (mg/l) were measured three times using a multiparametric immersion probe (Hach-Lange HQ40d) positioned 20 cm below the water surface and a mean was calculated. A 50 ml water sample was collected and transported to the laboratory to measure ammonia, nitrate, and phosphate concentration (mg/l) using a spectrophotometer (Hach-Lange DR 3900). The ratio of nitrate to phosphate (N:P) content in the water was calculated. At each site, 3 water depth measurements were taken at different subsites using a graduated shaft and an average depth was calculated for each site. Water transparency was assessed by direct observations in field, using the following empirical 5-level qualitative scale: "null" (high turbid waters), "null/partial", "partial", "partial/total", "total" transparency (clear waters)



(see Suppl. material 2: table S2a). In addition, grain size was considered by noting the main substrate categories (silt, sand, pebbles, rock, artificial), covering more than 40% of the sampling area. The anthropogenic disturbance level of each site was assessed using an empirical 5-level scale ("low", "mid/low", "mid", "mid/high", "high"), assessing the level of anthropogenic disturbance due to activities such as bathing, boating, fishing or factors such as water pollution, wastewater discharge, in-water dredging, and the presence of litter or mowing along the banks (see Suppl. material 2: table S2b).

Statistical analyses

To identify the quantitative and qualitative parameters most influencing the distribution of *L. hexapetala*, environmental data collected in the field were overlapped with percentage of species cover. This was done by analysing quantitative parameters (temperature, pH, dissolved oxygen, conductivity, ammonia, nitrates, phosphates, nitrate/phosphate ratio, depth) separately from qualitative environmental parameters (water transparency, substrate grain size, site disturbance level), which were evaluated using nominal categories. To investigate the relationship between abundance of the alien species and environmental conditions as a whole, ordination analysis was performed on environmental data. In order to do so, assumptions of linearity were checked, and an unconstrained linear method (Principal Component Analysis, PCA) was chosen. All variables were standardized setting scale = TRUE in the "rda" function while performing the ordination. Subsequently, L. hexapetala cover was considered as a continuous response variable and it was fit over the unconstrained ordination results using a post-hoc test (envfit) to find out if there was a correlation with the PCA axes, in order to identify which of the environmental parameters most influenced the alien species distribution. The posthoc test was performed using the "envfit" function from package vegan (Oksanen et al. 2022). Ordination plots were made using ggfortify methods (Oksanen 2015; Tang et al. 2016; Horikoshi and Tang 2018). The ordination analysis includes both sites where L. hexapetala was absent and sites with varying cover of the alien species, to better characterize the environmental characteristics of the habitat it invades, and to better understand its invasion process. Two - tailed, one - way ANOVA tests were also performed to investigate the difference in L. hexapetala cover between sites with different water transparency and disturbance levels, using the alien species % cover as response variable, and the different categorical levels of the qualitative parameter (water transparency, disturbance level) as explanatory variables. Assumptions of normality and homoscedasticity were verified using the appropriate tests (Shapiro-Wilk and Levene) and a non-parametric alternative was used where the assumptions were not met (Kruskal-Wallis). In order to define the environmental conditions supporting the invasion and abundance of L. hexapetala, ranges of the water chemical and physical parameters of the sites where the alien species occurred were visualized through boxplots using ggplot2 package (Wickham 2016a, b). All statistical analyses were performed using R software (R Core Team 2021).

Results

As for the quantitative chemical and physical water data, the first two PCA axes were chosen, together explaining over 55% of the total variance (Fig. 2). The first axis (PC1) was negatively correlated with conductivity and nitrogen content (ammonia and nitrate concentration), while the second axis (PC2) was negatively



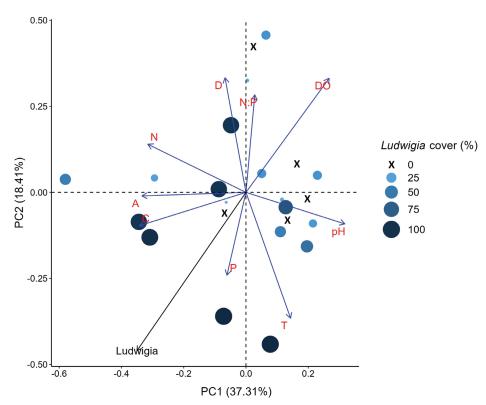


Figure 2. Ordination plot of quantitative environmental parameters. Each dot represents a sampled site. The size of the dots increases with *L. hexapetala* cover, considered as a continuous variable; reference measurements are provided in the legend. The colour of the dots also gets darker with increasing *L. hexapetala* % cover. Black crosses represent uninvaded sites where *L. hexapetala* cover = 0. The black arrow shows the post-hoc analysis result, indicating the direction in which *L. hexapetala* cover increases. The blue arrows indicate the direction of each environmental parameter. Acronyms: C = conductivity; D = depth; DO = dissolved oxygen; pH = pH value; A = ammonia; N = nitrates; P = phosphates; N:P = N:P ratio; T = temperature; N:P = N:P ratio.

correlated with temperature and phosphate concentration, and positively correlated with water depth and dissolved oxygen. The post-hoc test for *L. hexapetala* cover was significant (p < 0.01), indicating a correlation between *L. hexapetala* cover and ordination analysis results. In particular, *L. hexapetala* cover was significantly negatively correlated with both the first and second axes (see Suppl. material 3: table S3), indicating that the alien species cover increased with conductivity, ammonia, nitrates and phosphates, while it decreased with increasing values of dissolved oxygen and depth.

In ordination analysis of environmental qualitative parameters, the first two axes explained about 50% of the total variance (Fig. 3). The first axis (PC1) was mostly negatively correlated with a silty substrate and high to mid-high disturbance level, while the second axis (PC2) was correlated with partial-null water transparency and sandy substrate. The post-hoc test revealed a significant correlation between *L. hexapetala* cover and these ordination results (p < 0.05), with the species cover highly negatively correlated with the first axis (see Suppl. material 4: table S4), thus increasing significantly with high disturbance levels and silty substrate.

Water chemical and physical data collected in all sampled sites (see Suppl. material 5: table S5) were used to further explore the relationship between the cover of the alien species and each environmental parameter analysed (Fig. 2). Specifically, *L. hexapetala* was found in waters with different levels of oxygenation, although showing higher cover in oxygen-poor waters (oxygen concentration < 7 mg/l; Fig. 4a). The species showed the highest cover in waters with average temperatures



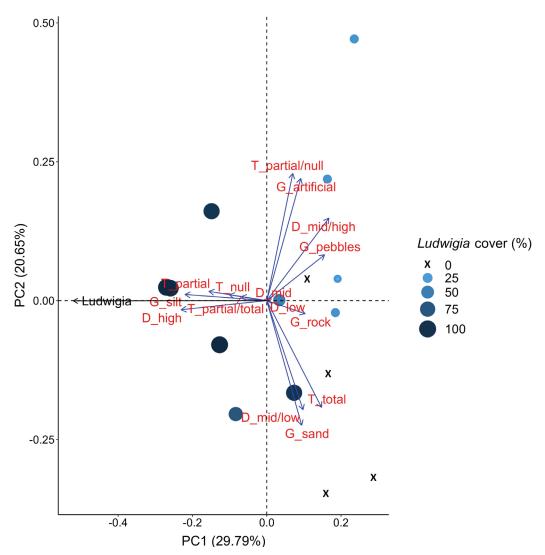
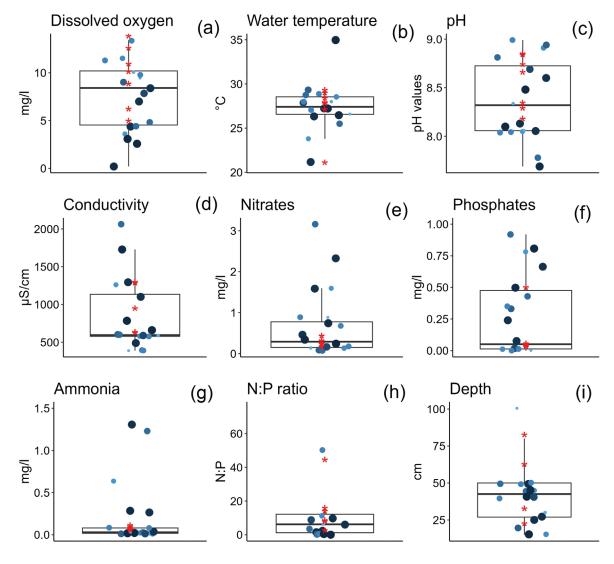


Figure 3. Ordination plot of qualitative environmental parameters. Each dot represents a sampled site. The size of the dots increases with *L. hexapetala* cover, considered as a continuous variable; reference measurements are provided in the legend. The colour of the dots also gets darker with increasing *L. hexapetala* % cover. Black crosses represent uninvaded sites where *L. hexapetala* cover = 0. The black arrow shows the post-hoc analysis result, indicating the direction in which *L. hexapetala* cover increases. The blue arrows indicate the direction of each environmental parameter. Acronyms: G = grain size; T = transparency; D = site disturbance level. For the category explanation of transparency and site disturbance level, see Suppl. material 2.

of about 27°C (Fig. 4b) and pH values ranging from 7.7 to 9.0 (Fig. 4c). It was also found in waters with a wide range of conductivity values, although the average conductivity was very high (> 800 μ S/cm; Fig. 4d). Regarding water nutrient levels, *L. hexapetala* showed high coverage in both highly nutrient-poor and moderately nutrient-rich waters (Fig. 4e–h). The species was present almost exclusively in shallow waters (< 50 cm; Fig. 4i).

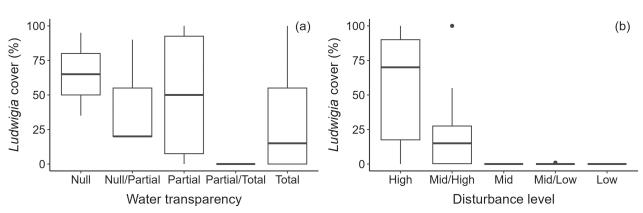
The analysis of variance (ANOVA) on environmental qualitative parameters pointed out that *L. hexapetala* cover was not significantly correlated with water transparency (p > 0.05), since the species showed the same cover values in both transparent and turbid waters (Fig. 5a). Regarding site disturbance level, the species cover was significantly different between sites with various disturbance conditions (p < 0.001). Specifically, most of the *L. hexapetala* populations were found in sites with a "high" or "mid/high" disturbance level (Fig. 5b).

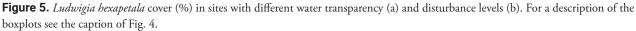




Ludwigia cover (%) * 0 • 10 • 30 • 60 • 90

Figure 4. Variations in chemical and physical water parameters in all sampled sites. Boxplots show the median (line across the box), upper and lower quartiles (the upper and lower parts of the box), and values outside the quartiles (the whiskers). The dots represent each sampled site and their size increases proportionally with the percentage cover of *L. hexapetala*; reference measurements are provided in the legend. The colour of the dots also gets darker with increasing cover of the alien species. Red asterisks show uninvaded sites where *L. hexapetala* cover = 0.







Discussion

We found that certain environmental conditions influence the growth of L. hexapetala, with shallow and poorly oxygenated waters favouring high abundance of the species. In particular, at investigated sites, *L. hexapetala* showed a marked preference for shallow waters (i.e., less than 50 cm depth) where its aquatic morphotype, characterized by short vertical stems, can better root on the bottom and emerge at the surface. However, established populations of the species have been found in its native range (Fortney et al. 2004), and in some European sites that have been invaded for longer than those investigated here (Lambert et al. 2010), even in waters up to 3 m deep. In investigated waterbodies, where the species is in an early stage of invasion, it does not seem to be able to grow where water depth exceeds 50 cm, except in one case, where it was found in water 1 m deep. The fact that the colonization of L. hexapetala in the study area appears to be constrained to the presence of shallow water, suggests that water depth may represent a limiting environmental factor for this species in an early colonization stage, while it does not in the case of longer established populations. Some experimental studies also show that L. hexapetala growth rate does not appear to depend on water level (Hussner 2010), meaning that this species behaviour could change as the invasion progresses. Nevertheless, this environmental parameter should be taken into account in the management of waterbodies potentially susceptible to its invasion. Shallow waters warm up quickly during the summer, especially in the Mediterranean area in which the sampled waterbodies fall and where in fact recorded water temperature was relatively high. However, it has been observed in literature that L. hexapetala, which is native to temperate to subtropical regions, tolerates a wide range of thermal conditions, even resisting much lower temperatures (Thouvenot et al. 2013a). This underlines the wide ecological tolerance of this species for different climatic conditions. Another environmental driving force explaining the abundance of L. hexapetala is the concentration of dissolved oxygen in the water. Our data indicate that L. hexapetala grows well in oxygenated water, although populations of L. hexapetala with the highest percentage cover have mainly been found in poorly oxygenated waters; this underlines the high tolerance of this alien species to a variety of oxygenation conditions, including those that are generally limiting for most other aquatic plants. This tolerance is related to the ability of the species, similarly to the congeneric L. peploides (Rejmánková 1992), to produce pneumatophores that increase aeration of plant tissues submerged in oxygen-poor waters (Gérard et al. 2014). Thus, it is evident that under such limiting conditions, L. hexapetala successfully outcompetes the other aquatic plants by producing extensive, often monospecific populations. It should be considered that the reduction of dissolved water oxygen could be the consequence of the presence of *L. hexapetala*; indeed, this species often forms dense floating mats on the water surface that limit oxygen exchange at the air-water interface and reduce the concentration of dissolved oxygen in water (Dandelot et al. 2005; Thouvenot et al. 2013a; Pelella et al. 2023b). Moreover, due to pneumatophores (Ellmore 1981; Armitage et al. 2013; Hoch et al. 2015), this species is also particularly efficient in absorbing large amounts of oxygen from the water (Dandelot et al. 2005; Pelella et al. 2023a,b), further limiting its availability to other plants.

Taking the other water parameters into consideration, in the study area *L. hexa-petala* showed a tendency to prefer alkaline and moderately ion-rich waters, although it has been found in waters with a wide range of conductivity. It should be noted that the positive relationship between *L. hexapetala* cover and water conductivity could also be a consequence of the presence of the alien species rather than a driving factor, since *L. hexapetala* has been found to increase water conductivity.



ty (Pelella et al. 2023a). In addition, it produces dense populations in both very nutrient-poor and moderately nutrient-rich waters, growing in different trophic water conditions. These results are consistent with Grewell et al. (2016), and with those of Matrat et al. (2006) who showed that aquatic species of the genus *Ludwigia*, particularly *L. grandiflora* (Michx.) Greuter & Burdet and *L. peploides*, grow in a wide range of conditions in terms of nutrient availability. In any case, among the various nutrients measured in this study, phosphates were found to be the most relevant in affecting the growth of *L. hexapetala*, as in most cases the densest populations were found in phosphate-rich waters. This would confirm what was reported by Skaer Thomason et al. (2018a), according to whom aqueous phosphorous is an important environmental parameter that favours the abundance of *L. hexapetala*.

According to our data, *L. hexapetala* grows mainly in sites with a substrate characterized by fine grain size (i.e., silt and sand), which allows for better rooting of the aquatic morphotype on the substrate. In addition, the alien species cover did not vary significantly in different water transparency conditions, underlining its tolerance for a wide variety of conditions. Furthermore, it is noteworthy that the percentage cover of this species increased significantly in sites with a high anthropogenic disturbance level. This supports the idea that *L. hexapetala*, as a typical invasive alien plant, can successfully and opportunistically colonise disturbed, altered, and unstable sites, which are known in literature to be more susceptible to biological invasions (Schröter et al. 2005; Kowarik 2008; Meyer et al. 2021).

Conclusions

Based on the results that emerged from this field study, pioneer L. hexapetala populations were able to grow in different environmental conditions. This wide ecological breadth is later confirmed in established populations, when L. hexapetala can produce extensive stands in both oxygenated and poorly oxygenated waters, in clear and turbid waters, in light and shaded conditions, in oligo-mesotrophic and eutrophic waters, although its growth increases with nutrient enrichment regardless of light regime (Hussner et al. 2010; Lambert et al. 2010; Thouvenot 2013a; Grewell et al. 2016). In any case, despite this large ecological breadth, some environmental conditions seem to have favoured the establishment and colonization of L. hexapetala in investigated sites. Specifically, the species showed the highest cover in anthropically disturbed sites with shallow, warm, poorly oxygenated, and alkaline waters, moderately rich in minerals and nutrients. It should be emphasized that water depth would seem to be among the few environmental parameters capable of limiting the establishment, and thus the invasion of this species, since it has been found in waters that are always shallow and, in any case, never deeper than 1 meter. To attain an even more complete picture of the ecology of *L. hexapetala*, further analyses would be needed to also assess the ecological parameters that most influence the spread of the terrestrial morphotype of the species along the banks of invaded waterbodies. It would also be interesting to come back to the investigated sites in the following years, monitoring the invasion trend, as well as analysing more environmental parameters that were not included in this study, such as water flow, water level fluctuation and other hydrological variables. Often, data regarding the early invasion stages of alien plant species are not available; therefore, the strength of this study lies in its provision of ecological data that pertains to such stages. This can provide new insights when compared to studies concerning long-established populations in other invaded countries. Consequently, the more limiting environmental conditions for the initial establishment of L. hexapetala, as documented in this one-year study, should be taken into account when formulating timely and



effective management plans for its eradication in the invaded areas, before the species can establish well developed, stabilized populations. Although *L. hexapetala* is a relatively recent invader in Italy, its rapid spread in the investigated Italian waterbodies is alarming and should draw the attention of both scientific researchers and local environmental managers for the purpose of its containment.

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The authors have no specific funding to report.

Author contributions

Conceptualization: E.P., S.C.; Y; Methodology: E.P., S.C.; Formal analysis: E.P.; Investigation: E.P., F.M., B.Q., S.C.; Resources: S.C.; Data Curation E.P., B.Q.; Writing - Original draft E.P., B.Q; Writing - Review and Editing E.P., F.M., S.C.; Visualization E.P., S.C.; Supervision: S.C.; Project administration: S.C.; Funding Acquisition: S.C.

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References

- Anufriieva EV, Shadrin NV (2018) Extreme hydrological events destabilize aquatic ecosystems and open doors for alien species. Quaternary International 475: 11–15. https://doi.org/10.1016/j. quaint.2017.12.006
- Armitage JD, Könyves K, Bailey JP, David JC, Culham A (2013) A molecular, morphological and cytological investigation of the identity of non-native *Ludwigia* (Onagraceae) populations in Britain. New Journal of Botany 3(2): 88–95. https://doi.org/10.1179/2042349713Y.000000023
- Azzella MM, Iberite M (2010) Notula: 40. In: Nepi C, Peccenini S, Peruzzi L (Eds) Notulae alla flora esotica d'Italia: 3(38–53). Informatore Botanico Italiano 42(2): 533.
- Billet K, Genitoni J, Bozec M, Renault D, Barloy D (2018) Aquatic and terrestrial morphotypes of the aquatic invasive plant, *Ludwigia grandiflora*, show distinct morphological and metabolomic responses. Ecology and Evolution 8(5): 2568–2579. https://doi.org/10.1002/ece3.3848
- Buono S, Azzella MM, Magrini S (2019) Ludwigia hexapetala (Hook. &Arn.) Zardini, H.Y. Gu & P.H. Raven (Onagraceae). In: Galasso G et al. (Eds) Notulae to the Italian alien vascular flora: 8. Italian Botanist 8: 72.
- Ceschin S, Ferrante G, Mariani F, Traversetti L, Ellwood NTW (2020) Habitat change and alteration of plant and invertebrate communities in waterbodies dominated by the invasive alien macro-phyte *Lemna minuta* Kunth. Biological Invasions 22(4): 1325–1337. https://doi.org/10.1007/ s10530-019-02185-5
- Ceschin S, Pelella E, Azzella MM, Bellini A, Ellwood NTW (2022) Unusual underwater flowering of *Utricularia australis* populations: a botanical enigma? Aquatic Botany 178: 103487. https://doi.org/10.1016/j.aquabot.2021.103487



- Dandelot S, Verlaque R, Dutartre A, Cazaubon A (2005) Ecological, dynamic and taxonomic problems due to *Ludwigia* (Onagraceae) in France. Hydrobiologia 551(1): 131–136. https://doi. org/10.1007/s10750-005-4455-0
- Dandelot S, Robles C, Pech N, Cazaubon A, Verlaque R (2008) Allelopathic potential of two invasive alien *Ludwigia* spp. Aquatic Botany 88(4): 311–316. https://doi.org/10.1016/j. aquabot.2007.12.004
- Davidson AM, Jennions M, Nicotra AB (2011) Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis: Invasive species have higher phenotypic plasticity. Ecology Letters 14(4): 419–431. https://doi.org/10.1111/j.1461-0248.2011.01596.x
- Di Pietro R, Misano G, Wagensommer R (2007) Notulae alla checklist della flora vascolare italiana 4: 1311–1419. Informatore Botanico Italiano 39(2): 401–408.
- Dimitrakopoulos PG, Koukoulas S, Michelaki C, Galanidis A (2022) Anthropogenic and environmental determinants of alien plant species spatial distribution on an island scale. Science of the Total Environment 805: 150314. https://doi.org/10.1016/j.scitotenv.2021.150314
- El-Barougy RF, Dakhil MA, Abdelaal M, El-Keblawy A, Bersier LF (2021) Trait-environment relationships reveal the success of alien plants invasiveness in an urbanized landscape. Plants 10(8): 1519. https://doi.org/10.3390/plants10081519
- Ellmore GS (1981) Root dimorphism in *Ludwigia peploides* (Onagraceae): development of two root types from similar primordia. Botanical Gazette 142(4) University of Chicago Press : 525–533. https://doi.org/10.1086/337255
- EPPO (2011) Data sheets on invasive alien plants. Fiches informatives sur les plantes exotiques envahissantes *Ludwigia grandiflora* and *L. peploides* Onagraceae – Water primroses. EPPO Bulletin 41(3): 414–418. https://doi.org/10.1111/j.1365-2338.2011.02511.x
- Fortney RH, Benedict M, Gottgens JF, Walters TL, Leady BS, Rentch J (2004) Aquatic plant community composition and distribution along an inundation gradient at two ecologically-distinct sites in the Pantanal region of Brazil. Wetlands Ecology and Management 12(6): 575–585. https://doi.org/10.1007/s11273-005-1763-0
- Genitoni J, Vassaux D, Delaunay A, Citerne S, Portillo Lemus L, Etienne M, Renault D, Stoeckel S, Barloy D, Maury S (2020) Hypomethylation of the aquatic invasive plant, *Ludwigia grandiflora* subsp. *hexapetala* mimics the adaptive transition into the terrestrial morphotype. Physiologia Plantarum 170(2): 280–298. https://doi.org/10.1111/ppl.13162
- Gérard J, Brion N, Triest L (2014) Effect of water column phosphorus reduction on competitive outcome and traits of *Ludwigia grandiflora* and *L. peploides*, invasive species in Europe. Aquatic Invasions 9(2): 157–166. https://doi.org/10.3391/ai.2014.9.2.04
- Gigante D, Acosta ATR, Agrillo E, Armiraglio S, Assini S, Attorre F, Bagella S, Buffa G, Casella L, Giancola C, Giusso del Galdo GP, Marcenò C, Pezzi G, Prisco I, Venanzoni R, Viciani D (2018) Habitat conservation in Italy: the state of the art in the light of the first European red list of terrestrial and freshwater Habitats. Rendiconti Lincei Scienze Fisiche e Naturali 29(2): 251–265. https://doi.org/10.1007/s12210-018-0688-5
- Giuliani C, Veisz AC, Piccinno M, Recanatesi F (2019) Estimating vulnerability of water body using Sentinel-2 images and environmental modelling: the study case of Bracciano Lake (Italy). European Journal of Remote Sensing 52(sup4): 64–73. https://doi.org/10.1080/22797254.2019.1689796
- Grewell BJ, Skaer Thomason MJ, Futrell CJ, Iannucci M, Drenovsky RE (2016) Trait responses of invasive aquatic macrophyte congeners: colonizing diploid outperforms polyploid. AoB PLANTS 8: plw014. https://doi.org/10.1093/aobpla/plw014
- Hoch PC, Wagner WL, Raven P (2015) The correct name for a section of *Ludwigia* L. (Onagraceae). Phytokeys 50: 31–34. https://doi.org/10.3897/phytokeys.50.4887
- Horikoshi M, Tang Y (2018) ggfortify: Data Visualization Tools for Statistical Analysis Results.
- Hussner A (2010) Growth response and root system development of the invasive *Ludwigia grandi-flora* and *Ludwigia peploides* to nutrient availability and water level. Fundamental and Applied Limnology 177:189–196. https://doi.org/10.1127/1863-9135/2010/0177-0189



- Hussner A, Windhaus M, Starfinger U (2016) From weed biology to successful control: an example of successful management of *Ludwigia grandiflora* in Germany. Weed Research 56(6): 434–441. https://doi.org/10.1111/wre.12224
- Kerns BK, Poland TM, Venette RC, Patel-Weynand T, Finch DM, Rowley A, Hayes DC, Ielmini M (2021) Future invasive species research challenges and opportunities. In: Poland TM, Patel-Weynand T, Finch DM, Miniat CF, Hayes DC, Lopez VM (Eds) Invasive species in forests and rangelands of the United States. Springer International Publishing, Cham, 329–333. https://doi. org/10.1007/978-3-030-45367-1_16
- Kowarik I (2008) On the role of alien species in urban flora and vegetation. In: Marzluff JM, Shulenberger E, Endlicher W, Alberti M, Bradley G, Ryan C, Simon U, ZumBrunnen C (Eds) Urban Ecology. Springer US, Boston, MA, 321–338. https://doi.org/10.1007/978-0-387-73412-5_20
- Lambert E, Dutartre A, Coudreuse J, Haury J (2010) Relationships between the biomass production of invasive *Ludwigia* species and physical properties of habitats in France. Hydrobiologia 656(1): 173–186. https://doi.org/10.1007/s10750-010-0440-3
- Lazzaro L, Bolpagni R, Buffa G, Gentili R, Lonati M, Stinca A, Acosta ATR, Adorni M, Aleffi M, Allegrezza M, Angiolini C, Assini S, Bagella S, Bonari G, Bovio M, Bracco F, Brundu G, Caccianiga M, Carnevali L, Di Cecco V, Ceschin S, Ciaschetti G, Cogoni A, Foggi B, Frattaroli AR, Genovesi P, Gigante D, Lucchese F, Mainetti A, Mariotti M, Minissale P, Paura B, Pellizzari M, Perrino EV, Pirone G, Poggio L, Poldini L, Poponessi S, Prisco I, Prosser F, Puglisi M, Rosati L, Selvaggi A, Sottovia L, Spampinato G, Stanisci A, Venanzoni R, Viciani D, Vidali M, Villani M, Lastrucci L (2020) Impact of invasive alien plants on native plant communities and Natura 2000 habitats: State of the art, gap analysis and perspectives in Italy. Journal of Environmental Management 274: 111140. https://doi.org/10.1016/j.jenvman.2020.111140
- Liu S-H, Hoch PC, Diazgranados M, Raven PH, Barber JC (2017) Multi-locus phylogeny of *Ludwigia* (Onagraceae): Insights on infra- generic relationships and the current classification of the genus. TAXON 66: 1112–1127. https://doi.org/10.12705/665.7
- Lucchese F (2017) Atlante della flora vascolare del Lazio: Cartografia, ecologia e biogeografia., Volume 1, 279 pp.
- Matrat R, Anras L, Vienne L, Hervochon F, Pineau C, Bastian S, Dutartre A, Haury J, Lambert E, Gilet H, Lacroix P, Maman L (2006) Gestion des plantes exotiques envahissantes – Guide Technique. (Comité des Pays de la Loire de gestion des plantes exotiques envahissantes, Agence de l'Eau Loire-Bretagne, Forum des Marais atlantiques, DIREN Pays de la Loire &: Conservatoire régional des rives de la Loire et de ses affluents) revue et augmentée, 86 pp. [In French]
- Meyer SE, Callaham MA, Stewart JE, Warren SD (2021) Invasive species response to natural and anthropogenic disturbance. In: Poland TM, Patel-Weynand T, Finch DM, Miniat CF, Hayes DC, Lopez VM (Eds) Invasive Species in Forests and Rangelands of the United States. Springer International Publishing, Cham, 85–110. https://doi.org/10.1007/978-3-030-45367-1_5
- Oksanen J (2015) natto/as.prcomp.rda.R at master jarioksa/natto GitHub. https://github.com/ jarioksa/natto/blob/master/R/as.prcomp.rda.R [Accessed 14 March 2023]
- Oksanen J, Simpson GL, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Solymos P, Stevens MHH, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, Caceres MD, Durand S, Evangelista HBA, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill MO, Lahti L, McGlinn D, Ouellette MH, Cunha ER, Smith T, Stier A, Braak CJFT, Weedon J (2022) Vegan: Community Ecology Package.
- Palacio-López K, Gianoli E (2011) Invasive plants do not display greater phenotypic plasticity than their native or non-invasive counterparts: a meta-analysis. Oikos 120(9): 1393–1401. https://doi.org/10.1111/j.1600-0706.2010.19114.x
- Pelella E, Questino B, Ceschin S (2023a) Impact of the alien aquatic plant *Ludwigia hexapetala* on the native *Utricularia australis*: evidence from an indoor experiment. Plants 12(4): 811. https://doi.org/10.3390/plants12040811



- Pelella E, Questino B, Luzi B, Mariani F, Ceschin S (2023b) Impact of the invasive alien macrophyte *Ludwigia hexapetala* on freshwater ecosystems: evidence from field data. Biology 12(6): 794. https://doi.org/10.3390/biology12060794
- Pyšek P, Jarošík V, Hulme PE, Pergl J, Hejda M, Schaffner U, Vilà M (2012) A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. Global Change Biology 18(5): 1725–1737. https://doi.org/10.1111/j.1365-2486.2011.02636.x
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rejmánková E (1992) Ecology of creeping macrophytes with special reference to *Ludwigia pep-loides* (H.B.K.) Raven. Aquatic Botany 43(3): 283–299. https://doi.org/10.1016/0304-3770(92)90073-R
- Richards CL, Bossdorf O, Muth NZ, Gurevitch J, Pigliucci M (2006) Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters 9(8): 981–993. https://doi.org/10.1111/j.1461-0248.2006.00950.x
- Rolon AS, Lacerda T, Maltchik L, Guadagnin DL (2008) Influence of area, habitat and water chemistry on richness and composition of macrophyte assemblages in southern Brazilian wetlands. Journal of Vegetation Science 19(2): 221–228. https://doi.org/10.3170/2008-8-18359
- Schröter D, Cramer W, Leemans R, Prentice IC, Araújo MB, Arnell NW, Bondeau A, Bugmann H, Carter TR, Gracia CA, Vega-Leinert AC de la, Erhard M, Ewert F, Glendining M, House JI, Kankaanpää S, Klein RJT, Lavorel S, Lindner M, Metzger MJ, Meyer J, Mitchell TD, Reginster I, Rounsevell M, Sabaté S, Sitch S, Smith B, Smith J, Smith P, Sykes MT, Thonicke K, Thuiller W, Tuck G, Zaehle S, Zierl B (2005) Ecosystem service supply and vulnerability to global change in Europe. Science 310(5752): 1333–1337. https://doi.org/10.1126/science.1115233
- Seebens H, Blackburn TM, Dyer EE, Genovesi P, Hulme PE, Jeschke JM, Pagad S, Pyšek P, Winter M, Arianoutsou M, Bacher S, Blasius B, Brundu G, Capinha C, Celesti-Grapow L, Dawson W, Dullinger S, Fuentes N, Jäger H, Kartesz J, Kenis M, Kreft H, Kühn I, Lenzner B, Liebhold A, Mosena A, Moser D, Nishino M, Pearman D, Pergl J, Rabitsch W, Rojas-Sandoval J, Roques A, Rorke S, Rossinelli S, Roy HE, Scalera R, Schindler S, Štajerová K, Tokarska-Guzik B, Kleunen M van, Walker K, Weigelt P, Yamanaka T, Essl F (2017) No saturation in the accumulation of alien species worldwide. Nature Communications 8(1): 14435. https://doi.org/10.1038/ncomms14435
- Shea K (2002) Community ecology theory as a framework for biological invasions. Trends in Ecology & Evolution 17(4): 170–176. https://doi.org/10.1016/S0169-5347(02)02495-3
- Skaer Thomason MJ, Grewell BJ, Netherland MD (2018a) Dynamics of *Ludwigia hexapetala* Invasion at three Spatial Scales in a Regulated River. Wetlands 38(6): 1285–1298. https://doi. org/10.1007/s13157-018-1053-2
- Skaer Thomason MJ, McCort CD, Netherland MD, Grewell BJ (2018b) Temporal and nonlinear dispersal patterns of *Ludwigia hexapetala* in a regulated river. Wetlands Ecology and Management 26: 751–762. https://doi.org/10.1007/s11273-018-9605-z
- Tang Y, Horikoshi M, Li W (2016) ggfortify: unified interface to visualize statistical result of popular R Packages. The R Journal 8(2): 474–485. https://doi.org/10.32614/RJ-2016-060
- Thiébaut G, Thouvenot L, Rodríguez-Pérez H (2018) Allelopathic effect of the invasive *Ludwigia hexapetala* on growth of three macrophyte species. Frontiers in Plant Science 9: 1835. https://doi.org/10.3389/fpls.2018.01835
- Thouvenot L, Haury J, Thiébaut G (2013a) Seasonal plasticity of *Ludwigia grandiflora* under light and water depth gradients: An outdoor mesocosm experiment. Flora - Morphology, Distribution, Functional Ecology of Plants 208(7): 430–437. https://doi.org/10.1016/j.flora.2013.07.004
- Thouvenot L, Haury J, Thiébaut G (2013b) A success story: water primroses, aquatic plant pests. Aquatic Conservation: Marine and Freshwater Ecosystems 23 (5): 790–803. https://doi. org/10.1002/aqc.2387



- Tóth VR, Villa P, Pinardi M, Bresciani M (2019) Aspects of invasiveness of *Ludwigia* and *Nelumbo* in shallow temperate fluvial lakes. Frontiers in Plant Science 10: 647. https://doi.org/10.3389/fpls.2019.00647
- Troia A, Azzella MM (2013) *Isoëtes sabatina* (Isoëtaceae Lycopodiophyta), a new aquatic species from central Italy. Plant Biosystems 147(4): 1052–1058. https://doi.org/10.1080/11263504.2013.782902
- Vernay A (2022) Water primrose (*Ludwigia grandiflora* subsp. *hexapetala*) auto- and allogamy: an ecological perspective. Peer Community in Ecology, 100095. https://doi.org/10.24072/pci.ecology.100095
- Viciani D, Vidali M, Gigante D, Bolpagni R, Villani M, Acosta ATR, Adorni M, Aleffi M, Allegrezza M, Angiolini C, Assini S, Bagella S, Bonari G, Bovio M, Bracco F, Brundu G, Buffa G, Caccianiga M, Carnevali L, Ceschin S, Ciaschetti G, Cogoni A, Di Cecco V, Foggi B, Frattaroli AR, Genovesi P, Gentili R, Lazzaro L, Lonati M, Lucchese F, Mainetti A, Mariotti M, Minissale P, Paura B, Pellizzari M, Perrino EV, Pirone G, Poggio L, Poldini L, Poponessi S, Prisco I, Prosser F, Puglisi M, Rosati L, Selvaggi A, Sottovia L, Spampinato G, Stanisci A, Stinca A, Venanzoni R, Lastrucci L (2020) A first checklist of the alien-dominated vegetation in Italy. Plant Sociology 57(1): 29–54. https://doi.org/10.3897/pls2020571/04
- Wagner WL, Hoch PC, Raven PH (2007) Revised classification of the Onagraceae. Systematic Botany Monographs 83: 1–240.
- Wickham H (2016a) Data Analysis. Ggplot2. Use R! Springer International Publishing, Cham, 189–201. https://doi.org/10.1007/978-3-319-24277-4_9
- Wickham H (2016b) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 189– 201. https://doi.org/10.1007/978-3-319-24277-4_9
- Zhang L, Chen A, Li Y, Li D, Cheng S, Cheng L, Liu Y (2022) Differences in phenotypic plasticity between invasive and native plants responding to three environmental factors. Life 12(12): 1970. https://doi.org/10.3390/life12121970

Supplementary material 1

List of sampled sites with coordinates

Authors: Emanuele Pelella

Data type: xlsx

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Supplementary material 2

Category-explanation tables for water transparency (a) and disturbance level (b)

Authors: Emanuele Pelella

Data type: xlsx

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Supplementary material 3

Loadings from PCA regarding quantitative environmental data

Authors: Emanuele Pelella

Data type: xlsx

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Supplementary material 4

Loadings from PCA regarding qualitative environmental data

Authors: Emanuele Pelella

Data type: xlsx

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Supplementary material 5

Environmental parameters in invaded (a) and uninvaded (b) sites

Authors: Emanuele Pelella

Data type: xlsx

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