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Short communication

Survival and expansion of *Pistia stratiotes* L. in a thermal stream in Slovenia

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Abstract

We report about successful winter survival of a tropical plant *Pistia stratiotes* in a natural thermal stream Topla in Slovenia in temperate climate zone in central Europe. Only 2 years after its first occurrence in 2001 *P. stratiotes* managed to cover most of the water body where the thermal springs cause an elevated temperature (>17 °C year round). Enhanced biomass production of this invasive species took place in spring and summer and new stolons were formed at the end of the vegetation season. Over the winter older rosettes decayed and only small rosettes survived besides new rosettes formed from stolons. Plants developed flowers in April through August. Observations in December revealed viable seed production and seed presence in the sediment.

Keywords: *Pistia stratiotes*; Alien species; Thermal water; Temperate zone; Winter survival; Plant trait

1. Introduction

Pistia stratiotes L. (family Araceae) is a free-floating freshwater macrophyte and a perennial herb widely distributed in tropical and sub-tropical regions. In many countries it is known as one of the most important pantropical aquatic weeds (Labrada and Fornasari, 2002). Until recently, occurrence was recorded on all continents except Europe and Antarctica (Labrada and Fornasari, 2002); however occasional reports of the temporary occurrence of *P. stratiotes* in the temperate zone were published. Plants have been spotted in water channels in the Netherlands, and there they have represented a regularly recurring problem since 1973, especially in summer (Mennema, 1977; Pieterse et al., 1981; Venema, 2001). Other sites with recent *P. stratiotes* infestation during summer include the French Jalle de Blanquefort near Bordeaux and Cadiz in SW Spain (García Murillo et al., 2005). Records exist also from Central Europe (Pyšek et al., 2002), and surprisingly even in many ponds and rivers in Moscow and its vicinity (Schanzer et al., 2003). Other distribution areas around Europe are mainly limited to thermally abnormal rivers. In the river Erft in Germany, where higher temperatures are a consequence of warm water discharge from mining, the occurrence of *P. stratiotes* was first recorded in 1981 (Diekjobst, 1984), but recent investigations (Hüssner and Lösch, 2005) do not mention *P. stratiotes* among the alien plant species found. Despite many sporadic finds in temperate regions, winter survival of plants outdoors was not reported. The only case of overwintering is assumed for the Kazachii channel of the Volga delta in Astrakhan, owing to warm water discharge from a heating and electric power station (Pilipenko, 1993).

Slovenia is a central European country that has a mainly temperate continental climate with typical warm to hot summers and cold winters with snowfall. Average January temperatures are between −3 and 0 °C, and temperatures in July reach 15–20 °C. The rainfall is higher in summer than in winter, ranging between 1000 and 1300 mm/year (Ogrin, 2004). Slovenia has 28 thermal springs with temperatures at least 5 °C higher than average local temperatures (Uhan and Krajnc, 2003), which could be suitable for warmth-requiring macrophytes. Information about alien aquatic plants from Slovenia is rare, although more than 60% of invasive alien species are associated with water or wet habitats (Jogan, 2005). Near Čatež, the thermal waters of a natural stream have been colonized by *P. stratiotes*.

Here we report the first recorded successful winter survival of *P. stratiotes* in central Europe. We measured and studied some of the most important plant traits over a year's cycle, to

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obtain data about plant fitness. The objectives of our study were (1) to document the environmental data from the study site that enable survival of the species; (2) to determine how clonal reproduction and biomass change through the year; and (3) to ascertain whether viable seeds develop.

2. Methods

2.1. Site description and history

The study site is located in eastern Slovenia (Čatež, 45°53′N, 15°37′E), along the river Sava. Average air temperature measured at the nearest meteorological station from December 2003 to November 2004 fell below 0 °C only in January. From November until March the lowest monthly values were below −5 °C, with maximum −14 °C in January. In July and August the highest average temperatures reached 20 °C.

The surplus water from the thermal spring and the outflow from the pools inside the Čatež health resort follows a natural riverbed called the Topla stream (including swamps, backwater and meanders). This stream is 4 km long from its source to its mouth on the river Sava. The water temperature at the source of the stream is 25 °C and declines along the river course to 6 °C at the mouth on the river Sava in the coldest months of the year. During warmer periods the difference between source and mouth is less pronounced.

2.2. Sampling methods

Plants were sampled three times (December 2003, April and August 2004) at four 1 m^2 quadrat sites equally distributed along the stream, with site 1 being the closest to and site 4 the furthest from the source of warm water. At each quadrat the number of *P. stratiotes* plants was counted, and the fresh and dry weight of the whole collected biomass was also determined. Only non-damaged plants were included in further morphometric studies, where we used the following traits: rosette diameter; number of leaves per plant; number of stolons attached to rosettes sampled; root length; and number of flowers per plant. In August, at the end of the maintenance growth phase (preliminary observations in 2003), we measured leaf traits of fully developed leaves: length, width, specific leaf area (SLA), and leaf size-index computed as log(leafwidth × leaflength) (Pillar, 1999).

In December 2003 and August 2004, water samples from four sites were collected for measurements of water chemistry and physical properties: temperature on the surface and 15 cm deep, pH, electrical conductivity (EC) at 20 °C, total Kjeldahl nitrogen (TKN), ammonia–nitrogen (NH_4–N), nitrate (NO_3–N), nitrite (NO_2–N), and orthophosphate (PO_4^{3-}–P). Because free floating species are able to obtain nutrients predominantly from the water column (Bini et al., 1999), these were only analyzed from filtered water. We performed additional temperature measurements in the buds of rosettes over 3 months (February–April) to gain insight into the temperature gradient from water to plant and in the air layer above it.

2.3. Data analyses

To determine if significant differences occurred among plants from the same sampling sites at each sampling month (season), a one-way analysis of variance (ANOVA) was performed, followed by a post-hoc mean separation by the Tukey Honest Significant Difference (HSD) test. Seasonal influences on various plant traits throughout different sampling sites were examined by multivariate analysis of variance (MANOVA). Relationships among plant traits, biomass, and temperatures were assessed using linear correlation.

3. Results

A few plants were spotted on water bodies 600 m from the source for the first time in 2001, and by the next year, a large part of the Topla stream was covered by *P. stratiotes* during winter. In spring and summer the plant managed to spread through shallow swamps into the backwaters and formed dense mats. After 2 years it had spread along 3 km on approximately 25 ha down to where the Topla joins the river Sava.

Water temperature, as the most important factor influencing the assemblage of tropical aquatic plants, was above 17 °C the whole year round. The canopy temperature gradient, within the most important interaction level (surface–plant bud–air 15 cm above plant) was quite steep (Fig. 1). The temperature in the rosette bud correlated significantly with the water temperature ($y = -0.1 + 0.95x$, $r^2 = 0.89$, $r = 0.95$, $n = 22$, $P < 0.05$), and we observed only an average loss of 1.1 ± 0.7 °C that did not differ between either samples or time period. In the air 15 cm above plants, the influence of the warm water was less

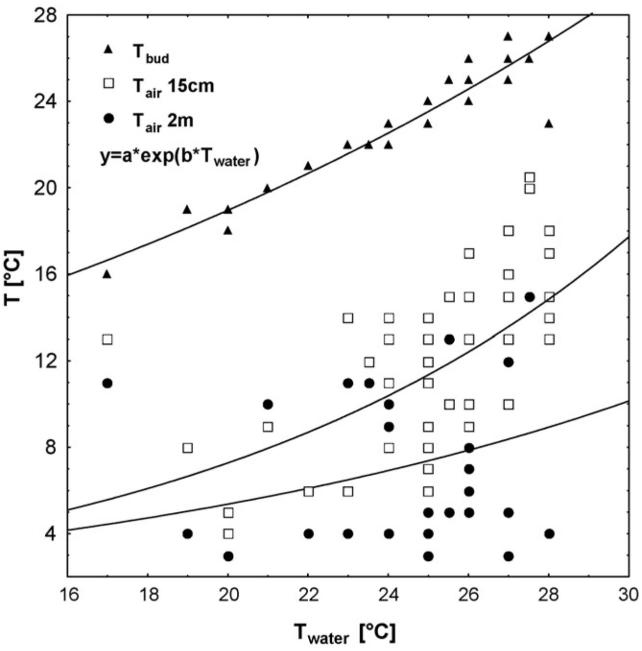

Fig. 1. Detailed temperature transect (water–plant bud–air 15 cm above plant–2 m above) measured from February to April 2004. Curves are exponentially fitted ($a = 7.98$, $b = 0.043$ for T_{bud}; $a = 1.2251$, $b = 0.089$ for T_{air} 15 cm; $a = 1.50$, $b = 0.064$ for T_{air} 2 m).

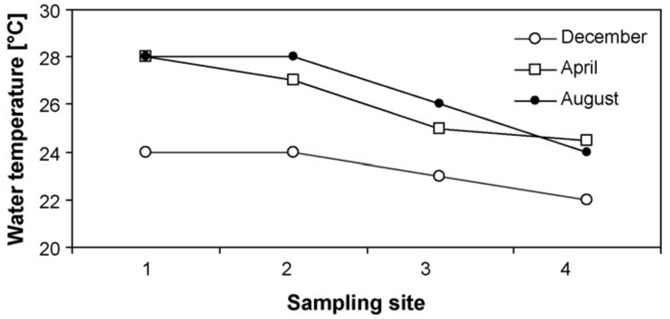

Fig. 2. Recordings of water temperatures from December 2003 to November 2004 at sampling sites equally distributed along the stream.

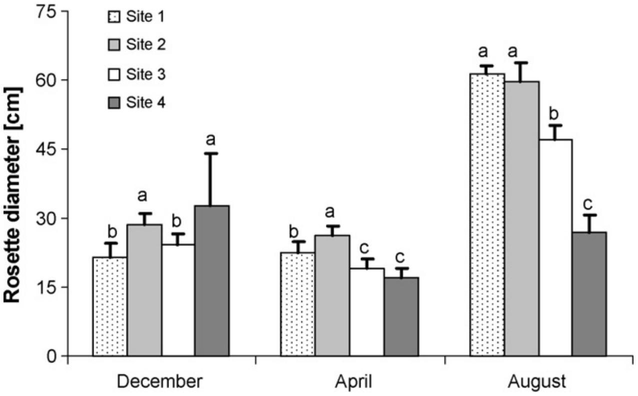

Fig. 3. Rosette diameter of plants from different sampling sites at different time periods differs significantly ($P < 0.05$) with season and between sampling sites (MANOVA; season $F_{2,117} = 295.43$; sampling site $F_{3,117} = 41.48$; season × sampling site $F_{6,117} = 41.00$). Differences revealed by ANOVA within sampling months (December $F_{3,68} = 13.97$; April $F_{3,32} = 33.76$; August $F_{3,18} = 126.75$) were significant. Columns (mean + 1S.D.) with different letters significantly differ from each other (determined by the Tukey HSD test at $P < 0.05$).

pronounced, while bigger differences were observed downstream, especially when the air temperatures were low.

At every sampling period water temperature declined along the stream course, but the difference did not exceed 4 °C between the upper and lower sampling sites (Fig. 2).

Little variation in pH was observed down stream or between seasons (average value 7.6). EC also did not change throughout the year, but increased slightly along with the stream (from 457 to 461 $\mu S\ cm^{-1}$ in December and from 423 to 458 $\mu S\ cm^{-1}$ in August). TKN was always below detection level. The nutrient loading ranged from eutrophic to hypereutrophic and changed during the vegetation period. The first two sampling sites showed about 60% less nitrate loading in December than at the growth peak in August, with [NO_3–N]: 1650 $\mu g\ L^{-1}$ and [NO_3–N]: 1810 $\mu g\ L^{-1}$ for sites 1 and 2, respectively, in August. Loadings for nitrite, ammonia–nitrogen and orthophosphate did not vary greatly between sampling sites, and the concentrations were low in December, but in August all loadings rose sharply ([NO_2–N]: 103.75 $\mu g\ L^{-1}$ [N–NH_4^+]: 159.5 $\mu g\ L^{-1}$ [PO_4^{3-}P]: 351.25 $\mu g\ L^{-1}$).

Biomass values increased during the vegetation period from 114 harvested in December to 308 $g\ m^{-2}$ in April, reaching 609 $g\ m^{-2}$ in August, with the highest values at the first two sampling sites (888 and 1137 $g\ m^{-2}$). Dry biomass per sampled square was negatively correlated ($y = 119.77 - 0.07x$, $r^2 = 0.07$, $r = -0.84$, $n = 12$, $P < 0.05$) with the number of rosettes on all plots throughout the season.

Plants from the different sampling sites significantly differed in selected plant traits within sites and across seasons. Specimens with the largest rosette diameters (Fig. 3) were observed in August, with bigger rosettes at the first two sites (ANOVA; $F_{3,18} = 126.75$, $P < 0.05$).

Bigger rosettes tend to have more leaves ($r = -0.82$, $n = 129$, $P < 0.05$). The recorded SLA and leaf size in August were negatively correlated ($r = -0.62$, $n = 69$, $P < 0.05$). ANOVA revealed differences in SLA ($F_{3,65} = 14.7$, $P < 0.05$) and leaf size ($F_{3,65} = 12.13$, $P < 0.05$) among plants from different sites. Plants from sites 3 and 4 had higher SLA values.

Clonal reproduction varied significantly within seasons among sites (ANOVA; December $F_{3,68} = 4.23$; April $F_{3,32} = 3.13$; August $F_{3,18} = 13.83$). Surprisingly, it was enhanced in December, when rosettes with the most stolons (4.1 ± 1.9) were observed as older plants started to decay. The number of stolons during the season from different sampling sites differed significantly according to the season and sampling site (MANOVA; season $F_{2,117} = 72.92$; sampling site $F_{3,117} = 17.83$; season × sampling site $F_{6,117} = 15.20$).

The length of roots did not differ between sampling sites in December (ANOVA; $F_{3,68} = 0.33$, $P = 8$), but in the spring and later in August it increased only at the first two sites (ANOVA for April: $F_{3,32} = 48.64$, $P < 0.05$ and August $F_{3,18} = 110.38$, $P < 0.05$), when we also observed altered root architecture. Plants from sampling sites 1 and 2, with higher nutrient status, had only a few long roots, in contrast to the plants from sites 3 and 4 with shorter, dense roots.

We found flowering plants in April with 3.4 ± 1.8 flowers per plant and until August the number of flowers per specimen increased to 6.6 ± 4.4 flowers. We observed the largest number of flowers per plant at site 2 (5.1 ± 1.6) in April and at site 1 in August (11 ± 2). No flowers were found in December, when we found seeds in the mucilage cover attached to the lower sides of older leaves. Collected seeds were viable and germinated successfully in laboratory conditions (submerged, 8 h/16 h light/dark photoperiod at 20 °C) after 3 weeks to 1 month. The seeds were already present in the sediment seed bank in the winter of 2004.

4. Discussion

Nowadays *P. stratiotes* is present in almost all tropical and subtropical zones, although its origin is uncertain, but may be South America (Howard and Harley, 1998). The introduction into Europe most likely occurred as an accidental release from aquaria (Pilipenko, 1993) or from horticulture, since it is cultivated widely as an ornamental plant (Venema, 2001; Schanzer et al., 2003). No records exist of how *P. stratiotes* might have been introduced to Slovenia.

Our measurements show that higher biomass values were observed in the period from March to August, with rosette size increasing by an increase in the number of leaves and in leaf size, while more new stolons were formed at the end of the vegetation season when no flowering but seeding was observed. The patterns in seasonal growth were similar to results presented by Dewald and Lounibos (1990), who made monthly observations in Florida. Those results are in contrast to Ghana, where additional decline of biomass from May to August and recovery from September to December occurred (Hall and Okali, 1974).

In our case the largest plants were found in August, with diameters of up to 65 cm at sampling site 2 (Fig. 3). In comparison, the largest plants observed in tropical region, growing in nutrient rich water, had diameters up to 45 cm (Den Hollander et al., 1999).

We observed no loss of plant biomass to herbivory or pathogens, nor damage to single plants. The only observable plants biomass reduction occurred in connection with winter cold between 8.12.2003 and 10.3.2004, with 41 nights of subfreezing temperatures, in spite of constantly high water temperatures (26.6 ± 1.1 °C) and a warm air layer above (6.8 ± 1.9 °C). During winter the number of leaves per rosette decreased and rosettes became smaller (Fig. 3). Older, bigger leaves decayed completely, becoming necrotic at the leaf margin; smaller, younger leaves remained undamaged, forming a very flat rosette, close to the water surface. Dewald and Lounibos (1990) observed a different rosette architecture, where younger leaves emerged vertically and the leaves' angle from the horizontal plane declined with leaf age. Small rosettes and those newly formed from stolons in December were probably able to survive the winter because of the flatness of the rosettes against the warm surface of the water.

Sexual reproduction of *P. stratiotes* in tropical zones was well known, but in subtropical regions (United States and Australia) it remained undiscovered until the nineties (Dray and Center, 1989; Harley, 1990). Flowering plants in the Topla stream were first observed in April (only in August in The Netherlands, Pieterse et al., 1981). The number of flowers counted in April was similar to observations made by Thawil and Mercado (1975) in tropical regions (3–8 flowers per plant), but an even higher number was counted in August. Seed production in tropical regions occurred rapidly when plants were exposed to unfavorable conditions (Den Hollander et al., 1999), while in Slovenia seeds were found on mature plants in December. Pieterse et al. (1981) concluded that frost-tolerant seeds may survive winter in the Netherlands; however, they also assumed that seed production and favorable conditions for germination would hardly occur. Our experiments have proven seed production, seed presence in the seed bank, and the viability of the collected seeds, but we did not find any seedlings occurring in natural conditions.

The invasiveness of *P. stratiotes* is very well known and has been studied in tropical and subtropical regions around the globe, and many strategies have been applied to cope with this invasive aquatic weed. In Slovenia, as early as 3 years after the first observation of *P. stratiotes*, native freshwater plants (*Ceratophyllum demersum* L., *Myriophyllum spicatum* L., *Najas marina* L. and *Trapa natans* L.) in this species-rich wetland habitat were on decline, because the whole water surface was covered with a dense mat that remained closed even during the winter (2004). Dissolved oxygen values declined by more than 50% when measured under the *P. stratiotes* cover, reaching only 2.5 mg L^{-1}, a critical value for fish survival (measured by the Fisheries Research Institute, Ljubljana 2003). The local fishermen try to remove the floating biomass at the end of every season, but since some of the stream banks and adjacent swamps are difficult to access, physical removal of the rosettes is only partly possible. Its explosive vegetative spread, beginning in early spring and its potential seed bank, persisting through drought and even through freezing temperatures lasting for several weeks (Pieterse et al., 1981) is making this species hard to exterminate. The Topla stream is now a potential source for further vegetative and sexual spread of *P. stratiotes*. We may assume that predicted climate change causing warmer conditions might create new problems: the species might extend its range.

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