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Temporary emersion enhances amphibious *Isoetes* production

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ABSTRACT

Temporary emersion enhances amphibious *Isoetes* production

Annual primary production of two amphibious quillworts was measured in two contrasting temporary environments in NE Spain: *Isoetes echinospora* growing in a high mountain semipermanent pool and *Isoetes delilei* growing in a Mediterranean pool. Leaf primary production showed a unimodal distribution with a peak in July for *I. echinospora* and in April for *I. delilei*. The population of *I. echinospora* produced 38.7 g DW m^{-2}y^{-1} (15.5 g C m^{-2}y^{-1}) of leaves the year of complete submersion, but leaf production increased to 49.3 g DW m^{-2}y^{-1} (19.7 g C m^{-2}y^{-1}) the year with two months of emersion (27.4% increase). Leaf production of *I. delilei* also increased significantly after emersion (the pool dries out annually) and reached an annual leaf production of 676.7 g DW m^{-2}y^{-1} (265.3 g C m^{-2}y^{-1}). *I. delilei* leaf production was 15 times higher than *I. echinospora* and much higher than the described range for submerged isoetids. The production increase of *I. echinospora* under temporal emersion together with the unusual high production of *I. delilei* thriving in temporary pools suggests that temporal emersion enhance amphibious isoetid production.

Key words: Leaf marking technique, NPP, Pyrenees, Albera Massif, soft-water lake, vernal pool, temporary pond, *Isoetes setacea*.

RESUMEN

La emersión temporal aumenta la producción de los Isoetes *anfibios*

En este trabajo se ha medido la producción primaria anual de dos isoetes anfibios en dos sistemas temporales diferentes en el NE de España: Isoetes echinospora, *en una charca semipermanente de alta montaña y* Isoetes delilei, *en una charca temporal mediterránea. La producción primaria de las hojas mostró una distribución unimodal con un máximo en julio para* I. echinospora *y en abril para* I. delilei. *La población de* I. echinospora *produjo 38.7 g PS m^{-2}y^{-1} (15.5 g C m^{-2}y^{-1}) en hojas el año de completa inmersión, pero la producción aumentó a 49.3 g PS m^{-2}y^{-1} (19.7 g C m^{-2}y^{-1}) el año en el que las plantas estuvieron emergidas durante dos meses (incremento del 27.4%). La producción de hojas de* I. delilei *también aumentó significativamente después de la emersión (la charca se seca anualmente) y alcanzó una producción anual de 676.7 g PS m^{-2}y^{-1} (265.3 g C m^{-2}y^{-1}). La producción primaria de hojas de* I. delilei *fue 15 veces mayor que la de* I. echinospora *y muy superior a los rangos descritos para los isoétidos submergidos. El incremento de producción de* I. echinospora *en condiciones de emersión temporal junto con la inusual y elevada producción de* I. delilei *en una charca temporal, sugieren que la emersión temporal aumenta la producción primaria en los isoetes anfibios.*

Palabras clave: Técnica de marcado de hojas, NPP, Pirineos, Macizo de la Albera, lago de aguas blandas, laguna temporal, Isoetes setacea.

INTRODUCTION

The quantification of primary production in aquatic macrophytes is important for evaluating ecological functions and has been used as an indicator of trophic status of aquatic systems (e.g. Krause & King, 1994; Penning *et al.*, 2008; Flor-Arnau *et al.*, 2015). Furthermore, the knowledge

on macrophyte primary production is essential to understand nutrient cycles and the role of macrophytes in global climate change and CO_2 fertilization (e.g. Ding *et al*., 2003; Saunders *et al*., 2014; Verspagen *et al*., 2014). In shallow environments, aquatic macrophyte production is often more important than other primary producers such as phytoplankton and epiphytes (Nõges *et al*., 2010). However, primary productivity differs significantly among continental aquatic systems (i.e. wetlands exhibit greater primary productivity than lakes and streams; Whittaker & Likens, 1973) and aquatic macrophytes (Madsen *et al*., 2002; Tamire & Mengistou, 2014). Helophytes generally produce more than hydrophytes (Tamire & Mengistou, 2014) and elodeids are more productive than isoetids (Madsen *et al*., 2002).

Despite a low primary production, isoetid populations are important worldwide in terms of abundance and functioning, especially in softwater lakes and also in temporary pools (Smolders *et al*., 2002; Bagella & Caria, 2012; Vila-Costa *et al*., 2016). The genus *Isoetes* or quillworts include about 100 species with 60% being aquatic. Quillworts are slow growing plants with leaves displayed in a helicoidal rosette over a bi- or trilobulate corm. Both *Isoetes echinospora* Durieu and *Isoetes delilei* Rothm. lose all their leaves during the disfavourable season, but the corm persists for several years, and both are amphibious plants. *I. echinospora* usually thrives submerged in shallow soft-waters from oligotrophic lakes in N-America and Europe (Boivin, 1961; Seddon, 1965). Spain is the southernmost distribution limit in Europe, where it is relatively abundant in oligotrophic high mountain lakes of the Pyrenees and is also found in semipermanent pools that may eventually dry out in summer (Gacia *et al*., 1994). *I. delilei* is a rare western Mediterranean quillwort that thrives in temporary pools with a relatively long inundation period (Molina, 2005). Mediterranean temporary pools represent unique species-rich habitats with many rare and endangered species (Deil, 2005; Bagella & Caria, 2012). Specifically, *Isoetes delilei* is a key species determining a priority habitat for the European Union (Habitats Directive 92/43/EEC) with declining populations, which qualifies for

a Nearly Threatened species in Europe (Rhazi, 2010). The decline is due to agricultural and development pressures as well as land abandonment (Rhazi, 2010) that favors the invasion of competing shrubs and trees (Rhazi *et al*., 2004).

Amphibious plants grow both in terrestrial as well as aquatic environments and, consequently, are well adapted to seasonal or fluctuating aquatic ecosystems. Water has been considered as a less suitable habitat for plant growth than the terrestrial environment (Maberly & Spence, 1989) because of reduced light and CO_2 availability. However, to our knowledge, the primary production of seasonal habitats and amphibious macrophyte species has not been studied. Nielsen & Sand-Jensen (1997) and Robe & Griffiths (1998) study the growth rate and other adaptations of *Littorella uniflora* to an amphibious life, but primary production is not calculated.

The aim of this work is to calculate the primary production of *I. echinospora* growing in a semipermanent pool (occasionally dries in summer) and *I. delilei* in a temporary pool (dries every summer). We used a leaf marking technique for in situ measurements of primary production (Gacia & Ballesteros, 1991) supplemented by a description of the leaf growing pattern (Gacia & Ballesteros, 1994).

MATERIALS AND METHODS

Study sites and species

The studied *I. echinospora* population thrived in Baciver pool, a small (ca. 20 m^2) semipermanent pond adjacent to Lake Baciver (Spanish Pyrenees, 42°41′46″N, 0°59′1″E) situated at 2120 m a.s.l. under a subalpine climate with high precipitation (> 1100 mm/y). The surface of the pool remains frozen half of the year and a large amount of snow accumulates during winter and early spring, similarly to the adjacent lake (Catalan *et al*., 1990; Gacia & Ballesteros, 1994). Some years the shallow pool stayed flooded over the summer, while it dried during summer on other years. Lake Baciver was dammed after the fieldwork finished and the semipermanent pool

no longer exists but populations of the same species exist in nearby pools.

The studied *I. delilei* population thrived in Sendu pool (Northeastern Spain, 42°22′50″N, 2°56′30″E), a relatively small (5000 m²) temporary pond situated at 113 m a.s.l. under a Mediterranean temperate climate with relatively low precipitation (ca. 600 mm/y). The pool generally floods in autumn or winter and dries out in spring. This species was previously identified as *Isoetes setacea* Bosc ex Delile (non Lamarck), a name that is not valid, as Lamarck previously described *Isoetes setacea* Lam., a completely different species that now is under synonymy with *Isoetes lacustris* (Greuter & Troia, 2015). In the same study, Greuter & Troia (2015) concluded that the correct name of the Mediterranean species generally misnamed *I. setacea* is *I. delilei*.

Environmental factors

Water samples were collected at the central part of each pool and kept frozen until analysis. A total of 6 abiotic variables were considered: pH, alkalinity, nitrate, nitrite, ammonia and phosphate (Table 1). Water chemistry variables were analyzed according to the standard methods described in Chappuis *et al.* (2014) for Sendu pool and as described in Gacia & Ballesteros (1993) and Gacia *et al.* (1994) for Baciver pool. pH was measured in situ with an electrode, alkalinity was determined by titration and nutrients were mainly measured by colorimetric analysis.

Production

Leaf production and loss (number of leaves) were determined using a leaf marking technique (Gacia & Ballesteros, 1991) during the full growing season of the species. *I. echinospora* was marked from June 1988 to October 1989 (two growing seasons) and *I. delilei* was followed from January 2009 to June 2009 (one growing season). Even though the production of both species was measured in different years, the data is still comparable as the differences between climate types (subalpine vs. Mediterranean temperate climate) is much greater than the annual fluctuations. The linear leaves of the *Isoetes* species are easy to mark with small metallic rings and, as new leaves are produced at the center of the rosette, leaf production and loss over time is easy to track (Gacia & Ballesteros, 1991). Thirty-two individuals of each species were identified by a knot code in a short nylon thread, firmly attached to the sediment by a small nail. All leaves of each individual were marked with 4 mm diameter copper wire rings. About one month later, the remaining marked leaves (old) and unmarked leaves (new) were counted for each individual. The number of lost leaves was estimated from the difference between initial and remaining marked leaves. The process was repeated 6 times per growing season and species (12 measures for *I. echinospora* and 6 for *I. delilei*).

Leaf biomass production was estimated based on leaf production and loss following Gacia & Ballesteros (1994). The biomass of the new leaves

Table 1. Water chemistry variables at the two studied sites. *Variables químicas del agua en los dos sitios de estudio.*

| Variable | Units | Baciver pool *Isoetes echinospora* | | Sendu pool *Isoetes delilei* |
		Min	Max	Mean
pH		5.56	8.51	7.28
Alkalinity	μeq/l	17	150	693
NO_3^-	μM	0	3.92	2.46
NO_2^-	μM	0.016	0.09	0.30
NH_4^+	μM	0.64	14.06	0.96
PO_4^{3-}	μM	0.02	0.24	1.01

produced and the elongation of the remaining old ones in a certain time interval allow us to estimate the production of leaf biomass (g DW). The population density of individuals was determined in situ with three replicates of 20 × 20 cm. Tissue carbon content was analyzed for both species in order to also express production in terms of g C. Clean leaf tissue of several individuals were dried and homogenized prior to analysis. *I. echinospora* carbon content was determined with a Carlo-Erba 1500 Analyser and *I. delilei* carbon content was determined by EA-IRMS (Elemental Analyzer –Isotope Ratio Mass Spectrometer) using a PDZ Europa ANCA-GSL elemental an-
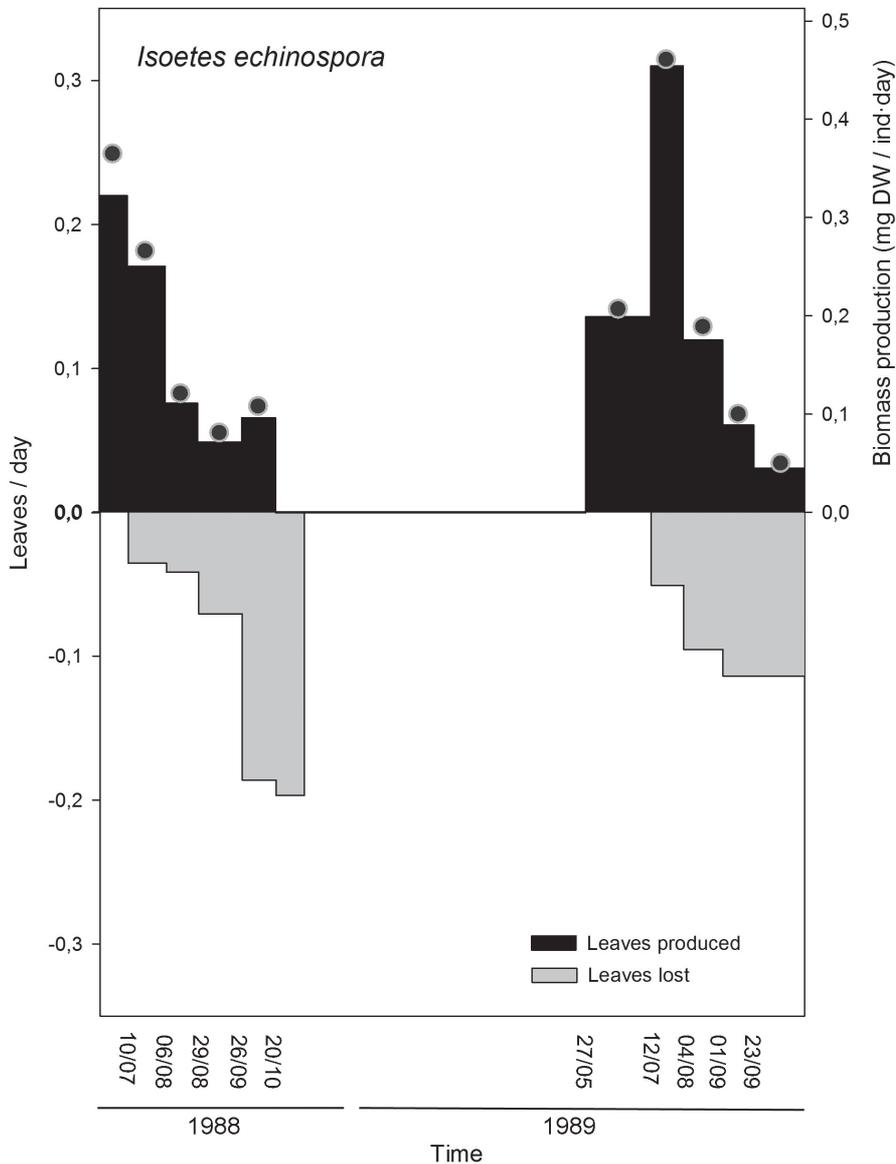

Figure 1. Leaf production and loss rates for *Isoetes echinospora* individuals during the growing seasons of 1988 and 1989. The average biomass production rate per individual is also shown as dark grey dots. *Tasas de producción y pérdida de hojas para individuos de* Isoetes echinospora *en los períodos de crecimiento de 1988 y 1989. La tasa promedio de producción de biomasa por individuo se muestra con puntos de color gris oscuro.*

alyzer interfaced to PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd, Cheshire, UK). All plots and regressions were performed with SigmaPlot 12.0.

RESULTS

Baciver pool is a soft-water pond with pH concentrations that varied greatly (see detailed daily profiles in Gacia & Ballesteros, 1993) as shown in Table 1. Dissolved inorganic nitrogen also varied greatly but always within the oligotrophic state. Ammonia showed the highest concentration among DIN forms (Table 1), probably due to sporadic cattle presence. Dissolved phosphorus fluctuated but within the low range that corresponds to oligotrophic waters (Wetzel, 1981; Catalan *et al.*, 2006). After ice melting, water level was rather constant at 50 cm over the summer and autumn of 1988. In contrast, water level gradually decreased and the pool dried out at the end of July 1989, but soil and plants were always moist until the pool flooded again in early October. Water transparency was high and light always reached the bottom of the pool.

Sendu pool shows harder waters than Baciver pool and a rather neutral pH (Table 1). Dissolved inorganic nitrogen values were within oligotrophic ranges but dissolved phosphorus was slightly within the eutrophic range (Wetzel, 1981) as shown in Table 1. The pool flooded around New Year and the highest water level was measured in February with a maximum depth of 55 cm. Plots with marked plants were situated at intermediate depths (33 cm of water depth in February). Water level gradually decreased and plots were dry by the end of April but still moist. Soil and plants were dry in May and June except for occasional rain. Water transparency was high and light always reached the bottom of the pool even if some filamentous algae appeared in March and April.

Both species completely changed the leaves every year and showed a highly seasonal leaf production. *I. echinospora* started producing leaves around June (Fig. 1), depending on the ice cover thawing. Maximum leaf production (both in terms of number and biomass) was reached in July, but the peak was higher in 1989, when the plants were emerged, than in 1988, when the plants were submerged (Fig. 1). Leaf production was low in autumn and all leaves were dead by November (Fig. 1). *I. delilei* started producing leaves in January, shortly after pool inundation (Fig. 2). Even if the production rate of new leaves was rather constant, the biomass production peaked in April due to an important leaf elongation that occurred right when the plots dried out. Leaf loss peaked at the end of the growing season (May and June) when the pool and soil were completely dry and all leaves were dead by mid June (Fig. 2).

I. echinospora showed the same biomass per leaf distribution within the rosette along both growing seasons and both years. Consequently, one general hyperbolic regression was calculated for this species (Fig. 3). In contrast, we found two distributions of biomass per leaf within the rosette along the growing season for *I. delilei*. From January to March, coinciding with the submerged period, *I. delilei* plants were small and had leaves with low biomass (Fig. 3). In April, the plots dried out and all leaves elongated with a consequent important biomass increase (Fig. 2 and 3). Therefore, two hyperbolic regressions were fitted for *I. delilei* corresponding to these two differentiated periods (Fig. 3). *I. echinospora* showed much lower maximum biomass per leaf (0.002 g DW, Fig. 3) than *I. delilei* (0.009 g DW for early season plants and 0.03 g DW for late season plants, Fig. 3).

Leaf turnover rates were slightly higher for *I. echinospora* (Table 2) but leaf production was slightly faster in *I. delilei* (leaf plastochrone interval of 9.2 days compared to 12.6 days, Table 2). Hence, *I. delilei* produced a total of 18.2 leaves per plant on average and 16.6 leaves for *I. echinospora* plants during their annual growing period of about 5 months. A slightly higher leaf production combined with higher leaf biomass resulted in higher annual biomass production for *I. delilei* (0.29 g DW per plant) than for *I. echinospora* (0.03 g DW per plant).

Plant density varied greatly for *I. echinospora* and increased through the growing season for

both years. Plant density started as low as 1242 plants/m^2, ended up with 2663 plants/m^2 and had an average of 1948.9 plants/m^2. Considering plant density, *I. echinospora* population had an average annual leaf production of 44.0 g DW/m^2 (Table 2). Leaf production was a 27.4% higher the year of emersion (1989) than the year without

emersion (1988). Plant density was stable for *I. delilei* with an average value of 2307.5 plants/m^2, which yields to an annual leaf production of 676.7 g DW/m^2 (Table 2). Carbon content is similar for both species: 40.0% for *I. echinospora* and 39.2% for *I. delilei*. Hence, the annual leaf production of *I. echinospora* was 17.6 g C/m^2
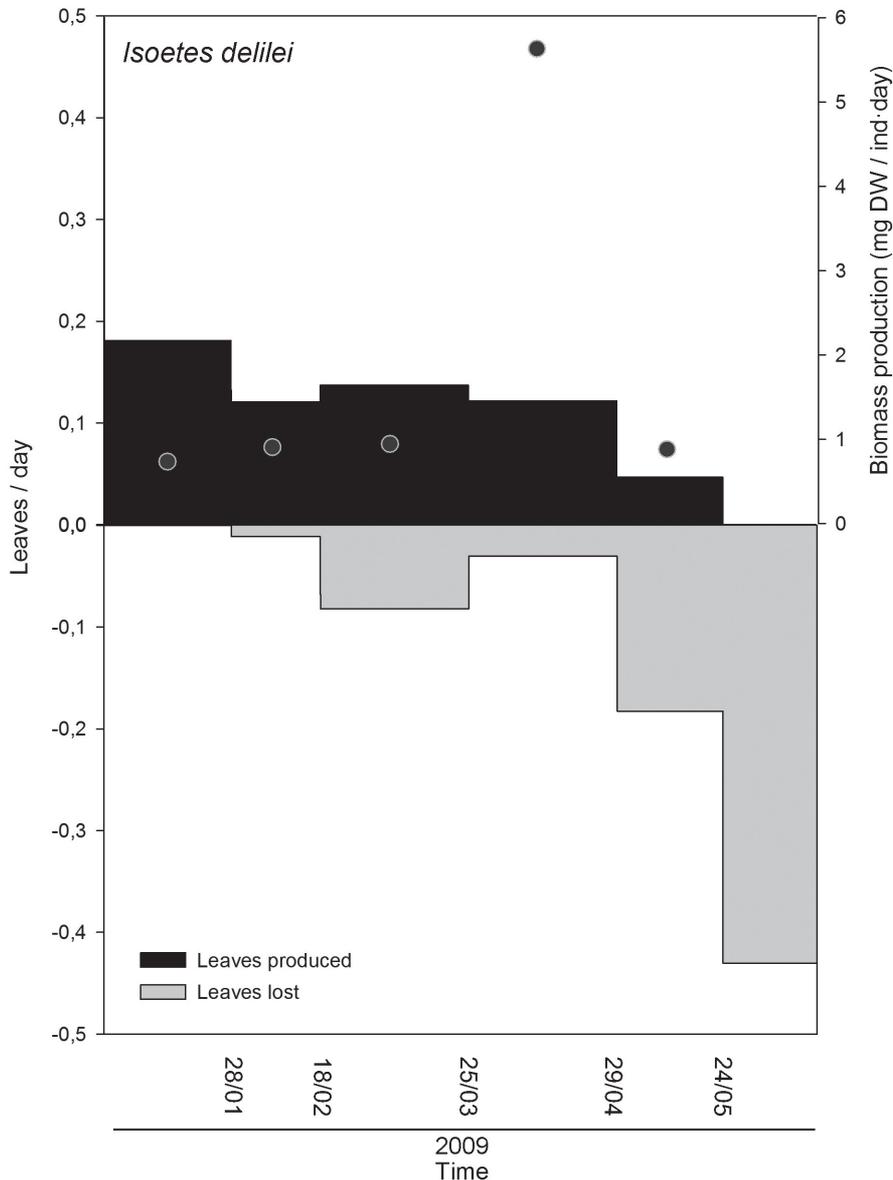

Figure 2. Leaf production and loss rates for *Isoetes delilei* individuals during the growing season of 2009. The average biomass production rate per individual is also shown as dark grey dots. Note the different scales of y-axes used in comparison to figure 1. *Tasas de producción y pérdida de hojas para individuos de* Isoetes delilei *en el período de crecimiento de 2009. La tasa promedio de producción de biomasa por individuo se muestra con puntos de color gris oscuro. La escala del eje y es distinta a la de la figura 1.*
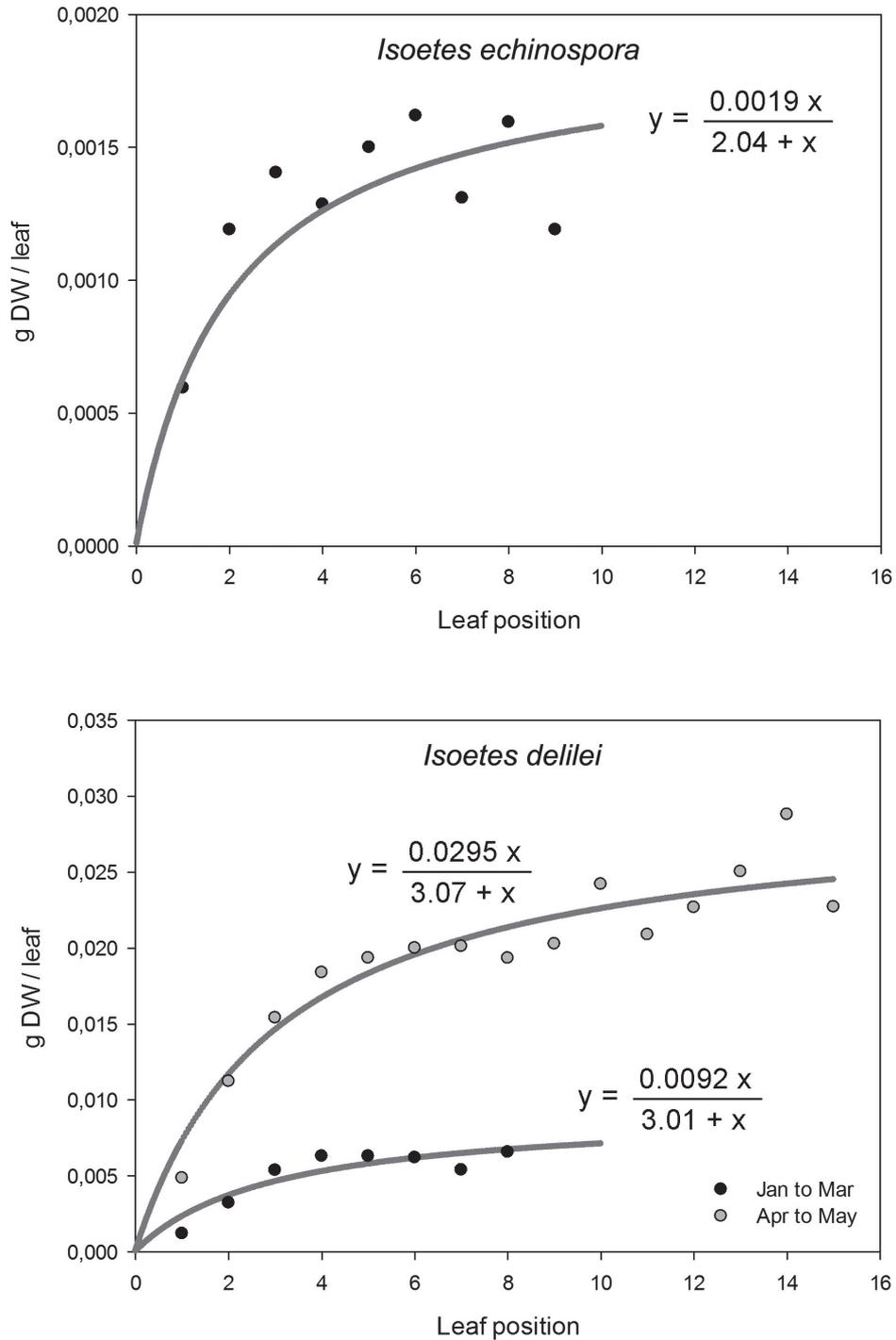
Figure 3. Leaf biomass (in dry weight [*DW*], *y*) *versus* its position in the shoot (*x*). Significant hyperbolic regressions and the fitted formula are shown. Note the different scale of *y*-axis used for each species. *Biomasa de las hojas (en peso seco [PS], y) versus su posición en la roseta (x). Se muestran las regresiones hiperbólicas significativas y sus fórmulas. La escala del eje y es distinta para cada especie.*

Table 2. Leaf turnover rates (annual), leaf plastochrone interval (growing season) and leaf production (annual) for *Isoetes echinospora* and *Isoetes delilei* populations. *Tasa de renovación de hojas (anual), intervalo plastocrónico de las hojas (periodo de crecimiento) y producción de hojas (anual) para poblaciones de* Isoetes echinospora *e* Isoetes delilei.

Species	Year	Leaf turnover rate (y^{-1})	Leaf plastochrone interval (d)	Leaf production (g DW $m^{-2}y^{-1}$)	Leaf production (g C $m^{-2}y^{-1}$)
Isoetes echinospora	1988	1.62	14.0	38.7	**15.5**
	1989	1.52	11.6	49.3	**19.7**
Isoetes delilei	2009	1.41	9.2	676.7	**265.3**

and of *I. delilei* was 265.3 g C/m^2 (Table 2). Thus, *I. delilei* leaf production per square meter was 15-fold higher than in *I. echinospora*.

DISCUSSION

Growth of both species showed a unimodal pattern that followed water availability but also irradiance and temperature (Gacia & Ballesteros, 1994). The *I. echinospora* population thrives in a high altitude pool under a cold high mountain climate and, consequently, the highest leaf and biomass production concentrates in summer as observed for other aquatic isoetids (Madsen *et al*., 2002). The annual production of *I. echinospora* was significantly lower than the shallow *I. lacustris* population occurring in the neighbouring Baciver lake (75 g DW/m^2.y at −0.8 m, Gacia & Ballesteros, 1994). Annual production was higher the year of emersion, which confirms that this species is well adapted to the amphibious life and suggests that emersion enhances productivity and growth rates in amphibious isoetids (Robe & Griffiths, 1998).

In contrast, *I. delilei* thrives in Mediterranean temporary pools that generally dry out in summer. Consequently, the highest leaf biomass production was observed in early spring after emersion at the same time that temperature and irradiance increased. However, the same population of *I. delilei* was visited several times the previous year (winter and early spring of 2007-08) when the pool almost did not flood and we observed an extremely reduced plant production and growth period length. Hence, the presence of an initial relatively long flooding period seems to be a key factor for *I. delilei* production, as observed for other amphibious species (Casanova & Brock, 2000).

The increased leaf production with emersion observed for both species could have several possible causes. First, an increased CO$_2$ supply could enhance photosynthesis. Submerged *Isoetes* obtain a considerable amount of CO$_2$ from the sediment pool through root absorption, which allow them to ameliorate the low CO$_2$ bulk water concentration (Madsen *et al*., 2002). However, *Isoetes delilei* leaves have stomata (Pfeiffer, 1922), which allow the use of CO$_2$ from the air when emerged. Analyzed European *I. echinospora* had no stomata (Pfeiffer, 1922), in contrast with some of the American plants. However, *I. echinospora* is usually found submerged in Europe and leaves of emerged individuals should be analyzed. Actually, Gacia & Ballesteros (1993) found lower titrable acidity (i.e. loss of CAM metabolism) in emerged compared to submerged *I. echinospora* leaves that could indicate the presence of stomata and the use of aerial CO$_2$. Second, mineral nutrient supply may have increased under higher aerobic conditions of the sediment. Actually, the population of *Isoetes lacustris* thriving in the adjacent Baciver Lake was nutrient limited, especially by phosphorus, and showed an increased growth after nutrient addition (Gacia & Ballesteros, 1994). Baciver pool had similar nutrient levels to the lake, *I. echinospora* and *I. lacustris* thrive in the same or similar lakes (Gacia *et al*., 1994) and, consequently, *I. echinospora* could also be nutrient limited. Sendu pool exhibited eutrophic levels of phosphorus but nitrogen was relatively low and the N:P ratio was of about 4:1. Therefore, *I. delilei* might be nitrogen limited and might respond positively to a nutrient increase. Third, higher irradiance could contribute

to higher photosynthetic rates. However, submersed plants of *I. lacustris* do not seem to be limited by light (Chappuis *et al*., 2015) and that could also be the case for the species studied here. And last, higher maximum temperatures reached in the air also could enhance photosynthesis rates (Pokorný & Květ, 2003). Probably more than one factor helped to increase the photosynthetic rate that resulted in higher leaf production associated with emersion but we believe that the increased CO_2 supply plays a major role since CO_2 is a largely reported limiting factor for productivity in extremely soft-water lakes (Murphy, 2002; Madsen *et al*., 2002) and emergent leaves have a much larger availability of CO_2 than submerged ones (Sand-Jensen & Frost-Christensen, 1999).

Isoetids have low productivity as a result of a relatively low biomass and low leaf turnover rates. Their annual productivity has been described to range from 5 to 425 g DW/m^2 in the review of Madsen *et al*. (2002). The annual pro-
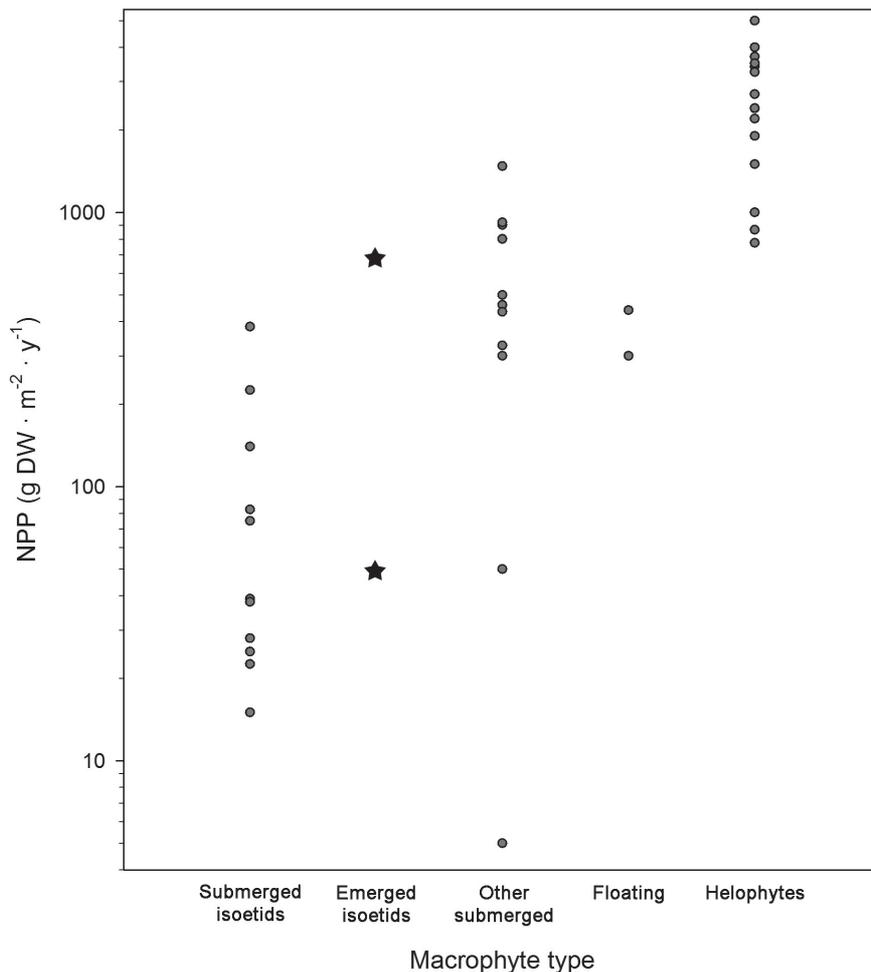

Figure 4. Annual net primary production (NPP) of different groups of macrophytes. Note that y-axis is in log scale. Different sources were used: Boston & Adams (1987), Boston *et al*. (1989), Gacia & Ballesteros (1994), Silva *et al*. (2009), Yu *et al*. (2010), Obrador & Pretus (2010), Miller & Fujii (2010), Tamire & Mengistou (2014). Black stars: data provided by this study. *Producción primaria neta anual (NPP) de distintos grupos de macrófitos. La escala del eje y es logarítmica. Distintas fuentes fueron usadas: Boston & Adams (1987), Boston* et al. *(1989), Gacia & Ballesteros (1994), Silva* et al. *(2009), Yu* et al. *(2010), Obrador & Pretus (2010), Miller & Fujii (2010), Tamire & Mengistou (2014). Estrellas negras: datos obtenidos en el presente estudio.*

duction of *I. echinospora* (44 g DW m^{-2}y^{-1}) falls in the lower end of the range (Fig. 4). Actually, we did not estimate the underground production and the actual NPP would be higher (Gacia & Ballesteros, 1994 estimated the underground production as acounting for 30-50 % of the total *Isoetes lacustris* production), but would still remain a low production value. In contrast, *I. delilei* leaf productivity (677 g DW m^{-2}y^{-1}) is much higher and similar to the range described for other submerged species of 300-1500 g DW m^{-2}y^{-1} (Madsen *et al*., 2002 and references therein) as shown in figure 4. The higher *I. delilei* production could be related to higher nutrient availability in the studied pool in comparison to the oligotrophic soft-water lakes where other isoetids often dominate and to a higher degree of adaptation to emersion. Emersion not only seems to enhance productivity for this amphibious *Isoetes* but also for helophyte species that generally show high annual production (Yu *et al*., 2010; Silva *et al*., 2013; Tamire & Mengistou, 2014).

The important production increase of both studied *Isoetes* species when emerged may have future consequences under a climate change scenario with rainfall regime shifts and temperature increase. The rainfall reduction expected in the Mediterranean region could negatively affect *I. delilei* thriving in temporary pools, which already have low rainfall, if the initial wet phase does not occur or is too short. In contrast, a rainfall reduction in the Pyrenees, which is currently high, could enhance the creation of more temporary environments and potentially increase *I. echinospora* production. *Isoetes* spore production only occurs above a minimum temperature of about 10 °C (Vöge, 2006) and percent of germination increases with temperature (Ctvrtlikova *et al*., 2012; Ctvrtlikova *et al*., 2014). However, high temperatures (17 °C) desynchronize the phenology of germination (Ctvrtlikova *et al*., 2012). Hence, depending on the ecosystem and the temperature increase, plant density and primary production will be whether enhanced or reduced.

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