Herbivory by spadefoot toad tadpoles and reduced water level affect submerged plants in temporary ponds

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ABSTRACT
In temporary ponds, submerged plants have adapted to recurrent periods of drought. These ponds often lack the impact of large herbivores but can harbor large numbers of herbivorous tadpoles. Tadpoles of the Iberian spadefoot toad (Pelobates cultripes) may reach large body size and consume large quantities of macrophytes during their long larval period, which is shortened with risk of pond drying. We conducted a mesocosm experiment to analyze the interplay between hydroperiod and impact of herbivorous tadpoles on the abundance and life history of 3 species of submerged plants common to temporary ponds. We observed differences in growth among plant species resulting in lags in timing of maximum cover, probably related to interspecific competitive interactions. Some plants responded to pond drying with a remarkable increase in the production of flowers. Tadpoles greatly reduced the plant cover, although their impact varied across plant species. Differential plant consumption by these large tadpoles can substantially modify the internal structure and complexity of temporary ponds, and tadpoles can also interfere with plant responses to shortened hydroperiod.

Introduction
Submerged plants are important components of freshwater ecosystems and modify the physical and chemical environment (e.g., Carpenter and Lodge 1986, Lodge 1991). They also provide structural complexity that serves as refuge, food, and support for other aquatic organisms (Cronk and Fenessy 2001). Herbivores may alter aquatic habitat structure (Lodge et al. 1998, Bakker et al. 2016), with impact increasing with biomass consumed. Many aquatic invertebrates feed mainly on periphyton or phytoplankton, whereas others, such as gastropods and large crustaceans, graze submerged macrophytes (Carpenter and Lodge 1986). Among vertebrates, waterfowl and many fish are important herbivores, grazing large quantities of aquatic plants and affecting the structure of these habitats. Aquatic turtles also graze aquatic vegetation as part of their omnivorous diet (Lodge et al. 1998, Pérez-Santigosa et al. 2011). In the case of anuran amphibians, although post-metamorphic stages rarely consume plants, tadpoles are commonly herbivorous (Altig et al. 2007, Montaña et al. 2019). Taking into account the proportion of aquatic macrophytes consumed by vertebrates, Lodge et al. (1998) considered that this group could have a higher impact on vascular plants than any other taxonomic group, even though the impact of amphibians was not considered in their study.

Tadpoles may reach high abundances (Gibbons et al. 2006, Pinero-Rodriguez et al. 2020) and have an important ecological role, contributing to nutrient cycling, altering the trophic web, and even causing bioturbation by modifying the distribution of sediments (Costa and Vonesh 2013, Cortés-Gómez et al. 2015, Kloh et al. 2019). They can potentially decrease food resources, including submerged plant biomass (Arribas et al. 2014). Tadpoles are often primary consumers or even omnivorous, with diet mostly consisting of algae, phytoplankton, and periphyton. Nevertheless, they can shift their diet in response to the availability of resources or as a result from competition and predation risk (Altig et al. 2007, Arribas et al. 2018, Montaña et al. 2019). Tadpole diets of some Iberian species have been described in detail (Díaz-Paniagua 1985). They mainly feed on algae and detritus and also include a high proportion of macrophytes in their diet (Díaz-Paniagua 1985). An isotopic analysis revealed that submerged macrophytes were the most assimilated food source in some species (Arribas et al. 2015). The macrophyte contribution to tadpole diet may differ considerably among species and also in relation to the body size of tadpoles.
Anurans with small tadpoles can favor the growth of aquatic plants by preferentially grazing the periphyton attached to their submerged stems (Arribas et al. 2014). By contrast, large tadpoles, such as those of the western spadefoot toad, *Pelobates cultripes*, can have major impacts on aquatic systems, largely decreasing or even exhausting the macrophyte biomass (Arribas et al. 2014, 2015).

Temporary wetlands have aquatic species that can resist drying (Grillas et al. 2004, Williams 2006). Many amphibian species develop as larvae in temporary ponds and are adapted to the fluctuations in hydroperiod by either having a short developmental time or the ability to perceive the risk of pond drying and accelerating their development accordingly (Richter-Boix et al. 2006). In Mediterranean temporary ponds, the emergence of aquatic organisms is synchronized with the beginning of the wet cycle, as detected for zooplankton (Florencio et al. 2020) and macrophyte communities (Fernández-Zamudio et al. 2017). Mediterranean temporary ponds are particularly rich in macrophytes (Grillas et al. 2004, Rhazi et al. 2006, Pinto-Cruz et al. 2009, Bagella and Caria 2012, Díaz-Paniagua et al. 2015), and the emergence of seedlings is conditioned by the timing of pond inundation, independent of season (Fernández-Zamudio et al. 2017). The life cycle of most plants in temporary ponds is annual, decaying when the water temperature increases and the ponds are close to drying out. In the subsequent aquatic phase, the plant assemblage is reestablished by means of a persistent seed bank (Bonis et al. 1995, Brock et al. 2003, Aponte et al. 2010). However, how plants respond to pond drying and the extent to which amphibian larvae can interfere with that response are largely unknown.

In our study area in southwestern Spain, some amphibians start breeding immediately after pond inundation (Díaz-Paniagua 1992). In particular, the western spadefoot toad lays eggs immediately after pond inundation and extends its larval phase until the ponds are about to dry out. It is the largest tadpole in all of the Iberian Peninsula. The diet of spadefoot toad tadpoles is omnivorous, and although they are predominantly herbivorous, feeding on algae and macrophytes, they also commonly ingest detritus and invertebrates (Díaz-Paniagua 1985, Arribas et al. 2015, Escoriza et al. 2016), exploiting primary production in the ponds and consuming large quantities of macrophyte biomass that results in modification of the structure of these aquatic habitats (Arribas et al. 2014, 2015).

We examined the interplay between the impact of large herbivorous tadpoles and risk of pond drying on submerged plants to assess the potential interference of herbivory on the plant responses to pond drying. We conducted a mesocosm experiment simulating the annual wet cycle of temporary and permanent ponds while manipulating the presence/absence of spadefoot toad tadpoles. We hypothesized that (1) tadpoles would differentially reduce the biomass of the plant species included in the experiment, (2) plants would respond to pond drying by advancing their reproductive cycle, and (3) tadpole herbivory would interfere with the plants’ responses to pond drying.

**Materials and methods**

We conducted a mesocosm experiment at the Doñana Biological Reserve (37°00′N, 6°38′W) within Doñana National Park, located in southwestern Spain close to the Atlantic coast. Temporary ponds are abundant in this area and are the main breeding site for 7 of the 11 amphibian species present in the park. After a summer dry phase, the wet phase of the ponds starts when the accumulated rains raise the groundwater table, which may occur in autumn, winter, or spring. At the end of spring or during summer, increased water temperature and evaporation cause pond drying (a detailed description of the inundation cycle of this pond network is given in Díaz-Paniagua et al. 2010, 2015). The variation of temperature and rainfall of the area was recorded during the annual cycle in which the experiment was carried out (Fig. 1).

We established mesocosms using 40 round 500 L tanks (96 cm high and 120 cm in diameter). Each tank received 50 L of sand plus a homogenized mixture of 4 L of pond sediment from several nearby ponds to provide them with propagules (seeds, spores, or zooplankton cysts), enabling plants and zooplankton to grow naturally in the tanks upon inundation. The tanks filled naturally with rainfall in the autumn, and we added additional well water until the water level reached the 400 L mark over the thick layer of substrate. We left the mesocosms undisturbed until February 2015, when we first visually inspected the species and abundance of plants in the tanks. The vegetation of the tanks was mainly composed of 3 macrophyte species: *Callitriche obtusangula*, *Myriophyllum alterniflorum*, and *Ranunculus peltatus*. These are common macrophytes in Doñana temporary ponds, where they can reach high density, providing spatial complexity to the water column (Fernández-Zamudio et al. 2016). Other species were rare (*Elatine macropoda* or some charophytes). Macrophytes grew naturally in the tanks from the seed bank present in the pond sediment. We removed or transplanted plants among tanks to homogenize the

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relative abundance of the 3 most abundant submerged macrophytes. All tanks received transplanted plants to homogenize the effect of this perturbation, and all plants were grown from the seed bank in the tanks.

We used a 2 × 2 factorial design, considering the effect of 2 factors: hydroperiod and herbivory. The effect of hydroperiod was tested at 2 levels, simulating either permanent ponds with a constant high water level (400 L, PERM) or temporary ponds where we gradually decreased the water level (TEMP). To analyze the effect of herbivory, we manipulated the presence/absence of herbivorous tadpoles. In mesocosms assigned to the herbivory treatment (Pcul), we introduced 2 tadpoles of *Pelobates cultripes*, which are large-sized and macrophyte grazers (Arribas et al. 2014), whereas tadpoles were absent from the remaining tanks (No Pcul). Based on our previous experience with herbivory of *P. cultripes*, we only added 2 tadpoles per tank because a higher number of tadpoles per tank would have quickly removed the macrophyte biomass. Each treatment (PERM-NoPcul, PERM-Pcul, TEMP-NoPcul, and TEMP-Pcul) was replicated 10 times.

The experiment began when we introduced the tadpoles on 26 February 2015. Average tadpole body mass (standard deviation) was 0.21 (0.02) g, total length was 29.39 (2.41) mm, and Gosner developmental stage was 25 (Gosner 1960). We kept tadpoles in the tanks until 14 April, at which time they had reached an average body mass of 15.43 (4.46) g and total length of 111.11 (13.17) mm and were in Gosner developmental stage <35. To maintain the target water level in each treatment, we checked the tanks weekly. In permanent tanks, we added water as needed to maintain the 400 L level. In tanks simulating temporary ponds, 15 days after tadpole introduction, we removed 25 L of water weekly until the tanks reached the 250 L mark on 15 April and maintained this reduced level until 5 June 2015, when we terminated the experiment.

We estimated plant cover over the water surface of the tanks by counting the number of each plant species in a sampling quadrat of 6 × 6 cells (cell size 10 cm × 10 cm). These data were recorded 3 times: 25 February (initial data), 26 March, and 10 April. We obtained phenological data of *M. alterniflorum* and *R. peltatus*, recording the day when we observed the first flower of each species in each tank, and estimated the length of the period from the first to the last flower observation. We could not obtain similar data for *C. obtusangula* because its submerged flowers were difficult to detect, and also because plants produced few flowers due to the early consumption of the stems by tadpoles. We recorded plant phenological data weekly, from 7 March to 22 May (13 weeks total). We also quantified variation in the abundance of flowers using a 12 × 12 cells quadrat (cell size 5 cm × 5 cm) and counting the number of cells containing flowers. Also, in April we marked 3 individuals of each plant species per tank and monitored them weekly until the end of the experiment. We could only monitor individuals of *M. alterniflorum* (from 8 Apr) and *R. peltatus* (from 2 Apr), because of the early consumption of *C. obtusangula* in the tanks. We counted the number of the monitored plants surviving (still present in the tank) each week, to generate survival curves through the remaining experimental period.

**Statistical analyses**

To compare plant cover among treatments across the 3 sampling times, we used generalized linear mixed models (GLMM) with binomial error distribution on the number of grid cells containing each plant species and the number of cells without them. We included hydroperiod (PERM/TEMP) and herbivory (present/absent) as fixed factors. To account for nonindependence of the repeated measures over time within each tank, we included tank as a random effect in our analyses. We
performed a similar analysis to compare the number of grid cells containing flowers throughout the experiment. Likewise, we then used GLMMs, with a binomial error distribution on a response variable containing the number of cells with flowers and the number of cells without them, herbivory, and hydroperiod as fixed factors, and tank as a random factor. We also included the initial plant cover as a covariable to control for differences in plant densities among tanks. To analyze the time to the first flower blooming, we fitted a generalized linear model with negative binomial error distribution using data of the first flower in each cell of the quadrat per tank as variable response, hydroperiod and herbivory as predictors, and initial plant cover as the covariable. To test for differences in the length of the flowering period, we fitted a generalized linear model with Gaussian error distribution and the same predictor variables after checking that the model met parametric assumptions. We studied the effect of herbivory (Pcul/NoPcul) and hydroperiod (PERM/TEMP) on plant survivorship, analyzing the survival of individually monitored plants by the end of the experiment (data from 30 Apr for R. peltatus and from 22 May for M. alterniflorum). We fitted a GLMM with binomial error distribution, using tank as the random factor and herbivory and hydroperiod as fixed factors. The response variable was a vector including, for each monitored plant, if it was still present (1) or if it had been completely consumed and did not survive (0). We used R 3.6.3 (R Core Team 2020) for all analyses.

Results

Effect of tadpole herbivory and pond drying on plant cover

At the beginning of the experiment, the species with the highest plant cover was C. obtusangula, present on average in 99% of the water surface across all tanks, whereas the cover of M. alterniflorum was 60% and that of R. peltatus was 30%. We observed a gradual reduction in the cover of C. obtusangula in March and April in all tanks, significantly affected by tadpole herbivory ($\chi^2 = 7.403, p = 0.006$) and by the interaction of herbivory and hydroperiod ($\chi^2 = 4.3944, p = 0.036$). The presence of tadpoles caused the highest reduction in plant cover in tanks simulating temporary hydroperiod, with a 54% reduction in the cover of C. obtusangula. The other 2 species showed a slow increase in cover in March, but in April M. alterniflorum showed only a low decrease in cover in the presence of herbivores and a slight increase in the absence of herbivores, not exhibiting significantly different treatment effects (Fig. 2, Table 1a).

Phenology of plant reproduction

Number of flowers

Throughout the experiment, the presence of tadpoles significantly decreased the number of flowers of R. peltatus ($\chi^2 = 29.26, p < 0.0005$), whereas hydroperiod did not affect it. In the case of

Figure 2. Variation in the average plant cover (and standard error bars) occupied by each of the 3 submerged macrophyte species in the presence or absence of herbivorous spadefoot toad tadpoles and in constant high or decreased water levels. Plant cover was estimated as the number of cells with each plant species in a sampling quadrat of 36 cells of 10 cm × 10 cm each. (PERM: permanent hydroperiod, TEMP: temporary hydroperiod; Pcul: presence of Pelobates cultripes tadpoles; NoPcul: absence of P. cultripes tadpoles).
**Table 1.** Statistical results for (a) generalized linear mixed models ($\chi^2$ Wald tests) with binomial error distribution on plant cover of the 3 macrophyte species (quantified contrasting a vector containing the number of cells with macrophytes against one reflecting the number of cells without macrophytes); (b) generalized linear mixed models with binomial error distribution on flower production (vector with number of cells with flowers, number of cells without them); (c) generalized linear models with negative binomial error distribution on the time to the first flower of *Myriophyllum alterniflorum* and *Ranunculus peltatus*; (d) generalized linear models, with Gaussian error distribution, on the length of the flowering period of *M. alterniflorum* and *R. peltatus*; (e) generalized linear mixed models on survival of individually monitored plants using a binomial error distribution. Plant survival was analyzed as a vector with data on survival (1) or not (0) of each monitored plant. In all the models, the presence of tadpoles (herbivory) and hydroperiod (PERM/TEMP) were used as fixed factors. In generalized linear mixed models, tank was used as random factor, and variance, standard deviation, and intraclass correlation coefficient for the random effect “tank” were also provided.

(a) Plant cover

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *C. obtusangula* | Hydroperiod | 1 | 0.550 | 0.458 |
|           | Herbivory   | 1 | 7.403 | 0.007 |
|           | Hydroperiod x herbivory | 1 | 4.394 | 0.036 |
|           | Residuals   | 115 |       |       |
|           | Tank        | 2.74 | 1.65  | 45.4 |

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *M. alterniflorum* | Hydroperiod | 1 | 0.183 | 0.669 |
|           | Herbivory   | 1 | 1.602 | 0.206 |
|           | Residuals   | 116 |       |       |
|           | Tank        | 1.61 | 1.27  | 32.8 |

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *R. peltatus* | Hydroperiod | 1 | 0.068 | 0.795 |
|           | Herbivory   | 1 | 4.511 | 0.034 |
|           | Residuals   | 116 |       |       |
|           | Tank        | 2.32 | 1.52  | 41.4 |

(b) Flower production

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *M. alterniflorum* | Hydroperiod | 1 | 4.082 | 0.026 |
|           | Herbivory   | 1 | 5.938 | 0.015 |
|           | Residuals   | 307 |       |       |
|           | Tank        | 1.14 | 1.07  | 25.8 |

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *R. peltatus* | Hydroperiod | 1 | 1.149 | 0.284 |
|           | Herbivory   | 1 | 29.257 | <0.0005 |
|           | Residuals   | 439 |       |       |
|           | Tank        | 0.46 | 0.66  | 11.7 |

(c) Time to the first flower

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *M. alterniflorum* | Hydroperiod | 1 | 9.309 | 0.002 |
|           | Herbivory   | 1 | 5.361 | 0.021 |
|           | Hydroperiod x herbivory | 1 | 2.935 | 0.087 |
|           | Residuals   | 35 |       |       |

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *R. peltatus* | Hydroperiod | 1 | 1.423 | 0.233 |
|           | Herbivory   | 1 | 2.718 | 0.1   |
|           | Residuals   | 33 |       |       |

(d) Length of the flowering period

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *M. alterniflorum* | Hydroperiod | 1 | 8.441 | 0.006 |
|           | Herbivory   | 1 | 4.917 | 0.033 |
|           | Residuals   | 34 |       |       |

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *R. peltatus* | Hydroperiod | 1 | 0.004 | 0.948 |
|           | Herbivory   | 1 | 13.059 | 0.001 |
|           | Residuals   | 32 |       |       |

(e) Survival of individually monitored plants

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *M. alterniflorum* | Hydroperiod | 1 | 2.586 | 0.108 |
|           | Herbivory   | 1 | 2.112 | 0.146 |
|           | Residuals   | 116 |       |       |
|           | Tank        | 1.19 | 1.09  | 26.6 |

|           | df | $\chi^2$ | p     |
|-----------|----|----------|-------|
| *R. peltatus* | Hydroperiod | 1 | 0.001 | 0.972 |
|           | Herbivory   | 1 | 24.801 | <0.0005 |
|           | Residuals   | 116 |       |       |
|           | Tank        | 0.18 | 0.42  | 5.1  |

*M. alterniflorum*, tadpoles also reduced the number of flowers ($\chi^2 = 5.94, p = 0.015$), but pond drying produced an increase in flowers ($\chi^2 = 4.08, p = 0.026$). The interaction of both factors was not significant (Fig. 3, Table 1b).

**Effects of herbivory and pond drying on the flowering period**

*Ranunculus peltatus* began producing flowers on 7 March in tanks with permanent hydroperiod and in the absence of herbivorous tadpoles. On 20 March,
flowers were detected in all treatments, reaching the highest values between 1 and 23 April (Fig. 3). However, neither herbivory ($\chi^2 = 2.718$, $p = 0.099$) nor hydroperiod ($\chi^2 = 1.423$, $p = 0.233$) significantly affected the date of the first flower bloom in *R. peltatus*.

*M. alterniflorum* flowered later than *R. peltatus*, starting on 1 April in tanks with temporary hydroperiod, with a gradual increase in flower production. Flowering peaked in mid-May in all treatments. In contrast to *R. peltatus*, hydroperiod ($\chi^2 = 9.86$, $p = 0.002$) and herbivory ($\chi^2 = 5.68$, $p = 0.017$) significantly affected the beginning of the flowering period in *M. alterniflorum*. The earliest flowers appeared in the tanks with the highest flower abundance, which occurred in temporary hydroperiod tanks with no tadpoles (Fig. 3, Table 1c). The interaction of both factors was not significant ($\chi^2 = 2.93$, $p > 0.05$).

Herbivory significantly affected the length of the flowering period in *R. peltatus* ($\chi^2 = 13.06$, $p = 0.0003$), with flower production finishing 4–5 weeks earlier in tanks with tadpoles than in the other treatments (Fig. 3, Table 1d). We did not record the complete flowering period of *M. alterniflorum* because we still detected flowers during the last week of our experimental period, despite the reduced number of plants remaining.

### Individual plant monitoring

#### Survival

Herbivory strongly determined the survivorship of *R. peltatus* individuals ($\chi^2 = 24.80$, $p < 0.0005$), whereas hydroperiod and the interaction of both factors did not (Table 1e). In the tanks with herbivorous tadpoles, plant survival decreased from the first week. By the end of the experiment, only 6.7% of *R. peltatus* plants survived in the presence of tadpoles, whereas survivorship was 73.3% in tanks without them (Fig. 4, Table 1e).

For *M. alterniflorum*, the lowest plant survival was observed in the tanks containing tadpoles and also experiencing pond drying (~37%), and tadpole herbivory had little impact on plant survival at continuously high water level (Fig. 4, Table 1e). At the end of the experiment, hydroperiod and herbivory showed no significant effects on plant survival (Table 1e).
Discussion

The 3 macrophyte species included in this study commonly form dense and complex masses in Mediterranean temporary ponds (Fernández-Zamudio et al. 2016). We observed broad changes in the composition of the plant assemblage in our experimental setup caused by the herbivory by spadefoot toad tadpoles and also due to pond drying. In tanks with permanently high water level, *C. obtusangula* was the most abundant species in the initial phases of the experiment but was followed by a steep reduction, whereas the other 2 macrophytes increased in cover. These opposing trends indicate that *C. obtusangula* may be affected by competition from the other 2 species, either for light or nutrients. Interspecific competition may be important in the structure of aquatic macrophytes (Grace 1991), and the morphological characteristics of some species may confer a competitive advance over others in competition for common resources (McCreary 1991). For instance, species with floating leaves may be directly exposed to sunlight while shading other macrophytes. Thus, in dense and extensive masses of aquatic vegetation, some species only predominate if they have earlier and faster growth than the other macrophytes that will predominate later in the season (Larson 2007). In our study, *C. obtusangula* was the smallest species, with thinner and fewer stems. However, it was also the earliest developing species and prevailed while the other species were still small-sized and not as abundant.

Herbivory by tadpoles had a strong impact on the cover and volume of the 3 submerged macrophyte species. The most affected was *C. obtusangula*, especially in tanks with a permanent hydroperiod where tadpoles reduced its cover to <25%. Tadpoles showed a feeding preference toward *C. obtusangula* compared to the other 2 macrophytes, possibly associated with the higher initial abundance of this species. This plant may also be easier for tadpoles to ingest because of its thin stems and its simple and poorly branched structure, which may be particularly important when tadpoles are still small and unable cut or shred the thicker or harder stems or leaves of other species. The effect of herbivorous tadpoles was higher in permanent than in temporary tanks, probably because in a larger volume of water, *C. obtusangula* reached higher biomass than in the gradually decreasing volume of temporary tanks. Pond drying constrained the growth of this plant to the extent that the cover and volume reached by *C. obtusangula* in tanks with decreasing water level was similar to those observed in permanent tanks but exposed to tadpole herbivory.

By contrast, tadpoles consumed *M. alterniflorum* least. This plant was also the slowest to grow and to produce flowers, consistent with previous studies also detecting that this macrophyte is consumed in low amounts by tadpoles (Arribas et al. 2014) and can even have lethal effects on them when it is the only food available (Cabrera-Guzmán et al. 2020). Some *Myriophyllum* species contain allelopathic compounds (Leu et al. 2002), and therefore *M. alterniflorum* might also contain them, presumably as a defense mechanism to avoid or reduce its consumption by herbivores, including tadpoles. These effects may also explain why tadpoles fed on this plant mainly at the end of the experiment, when the biomass of *R. peltatus* had been reduced to a few survivors that no longer produced flowers. Our experiment demonstrates that *P. cultripes* tadpoles have a strong impact through herbivory on submerged macrophytes and therefore may alter the structure of aquatic ecosystems. However, this conclusion should not be generalized to other tadpole species. Indeed, small tadpoles, even if also herbivorous, can favor the growth of macrophytes by removing periphyton growing over their stems or leaves (Arribas et al. 2014). In particular, the negative effect of *P. cultripes* herbivory is mainly due to the high volume of food they need to ingest in the late phases of their larval period when they reach a large body size. Natural abundance and density of tadpoles may be much greater than that used in our mesocosms (7/m²; Pinero-Rodríguez et al. 2020).

Hydroperiod also affected *M. alterniflorum*, although we only detected a reduction in cover for this species at the end of the experiment due to reduced water level. Growth of submerged macrophytes is also constrained by light and nutrient availability, so the higher plant density associated with the reduced volume of water may have limited access to these resources (Cronk and Fennessy 2001). In temporary ponds, the extent and volume of the ponds is variable and gradually decreases from spring to summer. When the water depth decreases, macrophytes density increases, reducing the availability of light to individual plants. In this late phase of the inundated period, higher temperature or lower oxygen content may become additional stressors (Williams 2006), favoring the deterioration of the plants and accelerating their senescence and death.

Phenological changes

Herbivory is detrimental to plants, and they may respond by increasing growth and flower and fruit production or elevating photosynthetic rate to compensate the loss of consumed plant parts (Belsky 1986). These compensatory effects have been described mainly for
terrestrial species, whereas studies about the pressure of herbivory on aquatic species mainly refer to changes in the distribution, diversity, and abundance of macrophytes in the ponds (Sheldon 1987, Wood et al. 2016).

In this study we detected shifts in plant phenology in response to herbivory and pond drying. Thus, *R. peltatus* shortened its flowering period when exposed to herbivorous tadpoles but maintained its flower production despite its shorter lifespan. In response to pond drying, however, *R. peltatus* markedly augmented its flower production, hence increasing the probability of successful plant reproduction when at risk of pond drying and improving its resilience by securing persistence in future inundation cycles.

By contrast, *M. alterniflorum* showed similar flowering periods across treatments. In our experiment, *M. alterniflorum* produced flowers when the ambient temperature was >30 °C, whereas *R. peltatus* flowered in the 20–30 °C temperature range. The beginning of the flowering period and flower production in *M. alterniflorum* occurred earlier in tanks with decreased water levels in the absence of tadpoles. Such a phenological shift could constitute an adaptive plasticity response to pond drying, coincidentally occurring in the species with delayed growth and maturation.

In general, tadpole herbivory did not affect the flower production of *M. alterniflorum*, although it did impact plant survivorship. Because of its delayed growth, *M. alterniflorum* is commonly exposed to herbivory from spadefoot toad tadpoles that have been growing for a long time and have therefore attained large sizes. Tadpole herbivory may therefore have played a role in the association between delayed growth and flowering of this macrophyte species and its production of allelopathic compounds.

It is important to consider the temporal variation in macrophyte abundance in relation to the reproductive phenology and growth patterns of potential herbivores (Lodge et al. 1998). In our study, we detected different peaks of abundance among the 3 species. *C. obtusangula* was the earliest growing species and the most affected by herbivory. *R. peltatus* was intermediate and maintained a high flower production in a shorter flowering period to secure a successful reproduction under the pressure of herbivory. *M. alterniflorum* was the latest growing species and was the least affected by herbivory, which could be associated with its ability to produce allelopathic compounds.

The herbivory exerted by *P. cultripes* tadpoles on submerged macrophytes may be as great as the effect of large herbivores because they can remove all plant biomass in the mesocosms in a short period of time (Arribas et al. 2014). Its effect may be comparable to that exerted by the exotic red swamp crayfish, *Procambarus clarkii* (Arribas et al. 2014), and to terrestrial megaherbivores like cows, horses, or deer, which only occasionally come into ponds to feed on aquatic plants (Azorit et al. 2012). In aquatic ecosystems, plant removal involves important changes in the physical and chemical characteristics of the water that affect the composition of the aquatic communities by increasing phytoplankton abundance and also altering nutrient fluxes and reducing habitat complexity, with consequent reduction of shelter and food resources for other species (Scheffer 2001). Our study reveals that large tadpoles consume large amounts of plant biomass and can thus be considered among the main herbivores in temporary ponds, modifying the life history of the submerged macrophytes and the spatial complexity of the water column.

**Acknowledgements**

We are grateful to M. Comas and R. Arribas for their help in the field, and to ICTS-RBD for the facilities provided for the development of the experiment.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

This study was funded by the Spanish Ministry of Economy and Competitiveness [project CGL2014-59206-P] and a fellowship to MJP-R [BES-2013-064954].

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