Acta Oecologica 91 (2018) 73–80

Contents lists available at ScienceDirect

Acta Oecologica

journal homepage: www.elsevier.com/locate/actoec

Plant response to overcrowding – *Lemna minor* example

Lech Kufel[a,*], Małgorzata Strzałek[a], Anna Przetakiewicz[b]

[a] Department of Environmental Studies and Biological Education, Siedlce University of Natural Sciences and Humanities, Prusa 12, 08-110, Siedlce, Poland
[b] Department of Plant Pathology, Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, 05-870, Błonie, Poland

ARTICLE INFO

Keywords:
Competition
Density-dependence
Growth rate
Frond area
Abscission
Colony size

ABSTRACT

Plants adopt various strategies in response to increasing density. We tested that response in two populations of *Lemna minor* L. – a free floating aquatic plant that frequently experiences intraspecific competition for space. Surface area of fronds and colonies, colony size (the number of fronds per colony), the rate of reproduction (based on the number of produced fronds) and growth rate (enlargement of surface area of all colonies) were the analysed factors presumably affected by density. The study was performed in natural stands and in experimental conditions with the use of two contrasting plant densities. Plants growing in natural conditions produced fronds of smaller and less variable surface area as a response to overcrowding but the number of fronds per colony was unrelated to plant density. Stable experimental conditions facilitated formation of fronds and colonies larger than in the field but frond detachment decreasing colony size was more intensive at high than at low density. This strategy allowed plants to more efficiently occupy limited available space. No self-thinning was observed during experimental cultures. Due to increasing frond area in cultures, growth rate was always higher than the rate of plant reproduction. Both were strongly negatively affected by high density. Performed calculations indicate that density-dependent growth inhibition starts when *L. minor* colonies cover the available water surface with a mono-layer mat. Some types of responses were found to significantly differ between analysed populations, which was also shown by genetic differences tested with he ISSR-PCR technique. Possible causal relationship between plant strategies and their genomic structure needs, however, further studies.

1. Introduction

Overcrowding may appear in plants and in some sessile animals (barnacles, bivalves). Among plants the phenomenon is quite common in species that reproduce asexually from rhizomes (common reed), runners (strawberry) but may also happen in plants producing large and relatively heavy seeds (Morschhauser et al., 2009), especially when animal seed dispensers are absent for some reasons (Babweteera et al., 2007). High density of seedlings (fronds, ramets) obviously exerts negative impact on future plant development by triggering the density-dependent factors (see review in Herrando-Pérez et al., 2012). The factors operate stronger within than between populations (Johnson et al., 2012) and result in accelerated intraspecific competition and finally in increased mortality. The relationship between plant biomass and density is usually explained with the use of self-thinning concept. In short, this concept posits parallel increase of individual or stand biomass of plants and decrease of plant density at a cost of higher mortality of smaller individuals. Self-thinning was analysed in both terrestrial (De Kroon and Kalliola, 1995; Li et al., 2013; Mithen et al., 1984) and, much less frequently, in aquatic (Creed et al., 1998; Scrosati and DeWreede, 1997) environments. Results of studies on self-thinning were critically reviewed and commented (Scrosati, 2005; Weiner, 1995) since some papers gave inconclusive or misinterpreted results and other (Scrosati and DeWreede, 1997) did not find self-thinning at all.

Self–thinning concept is best evidenced in even-aged dense monospecific populations observed for a long period of time. Population development intensifies within-population competition there, which ends up with suppression of less competitive (usually smaller) individuals. This happens when faster growing seedlings overshadow and finally eliminate the smaller ones. Competition for light in such a case may affect not only stand biomass but also size structure in a population (Falińska, 1991). Increasing density of plant population may increase or decrease size variability but multiple environmental and intrinsic factors make the final effect hardly predictable (Pfister and Stevens, 2002). Moreover, light is only one of environmental requisites plants may compete for. One may imagine, for example, the competition for water or nutrients in soil. In such a case, elongation of shoots at a cost of above-ground biomass would be the option for competing individuals and the final outcome would not necessarily be manifested in changes of total biomass as plant response to high density.

* Corresponding author.
E-mail address: lech.kufel@uph.edu.pl (L. Kufel).

https://doi.org/10.1016/j.actao.2018.06.007
Received 23 March 2017; Received in revised form 15 December 2017; Accepted 13 June 2018

For our study of plant response to high density we selected *Lemna minor* L. – one of the most rapidly growing free floating aquatic macrophyte (Ziegler et al., 2015). Its reproduction is mainly vegetative. Adult frond produces alternately daughter fronds from two side pouches giving rise to a colony composed of mother and several (often 3–4) offspring (Landolt, 1986). Daughter fronds are connected with mother frond with the stipes and the connection may persist for many generations thus increasing size of the colony. Many biotic and abiotic factors affect the process of detachment (abscission) so that colonies of quite variable number of fronds may be found both in natural and experimental conditions (Henke et al., 2011; Landolt, 1986). Moreover, the size of individual frond of *L. minor* decreases as the mother frond ages (Landolt, 1986) and varies among different clones (Vasseur et al., 1995). This apparent plasticity allows for supposition that *L. minor* may adopt various strategies at a level of fronds, colonies and the whole population to minimize negative effects of crowding. And crowding is quite common in duckweed populations growing in small ponds, midfield water holes and puddles if only sufficient nutrient and light supply permits vigorous growth. In fact, the expansion of duckweed in a water body leads primarily to the competition for space. *L. minor* was found to produce dense mats of several layers of fronds overlapping each other (Clatworthy and Harper, 1962). Under such conditions the production of lower frond layers is limited by light but the presence of sugars in medium and a switch to heterotrophic production (Landolt and Kandeler, 1987 and literature cited therein) might mitigate light limitation.

In this study we aimed at testing the response of two populations of *L. minor* to high frond density in natural and experimental conditions. Our working hypotheses were: 1) *L. minor* will produce smaller fronds at high densities, 2) frond detachment will be more intense at high than in low plant density and 3) high density will decrease reproduction and growth rate of duckweed. Moreover, we tested whether the type of response might differ between populations and whether this is reflected in their genetic background.

2. Material and methods

Samples of *L. minor* for measurements and experiments were collected from two small and shallow ponds: pond Krypy (52°22′59.81″ N and 21°59′12.66′ E) and pond Miednik (52°31′34.24′ N and 21°57′36.37′ E) in east-central Poland named after the villages situated nearby. Both are isolated ponds fed only with water from rainfall and surface runoff. Pond Krypy (about 0.18 ha) is surrounded by intensively managed meadows, whose fertilisation ensured relatively high concentrations of nutrients in pond water. Population of *L. minor* accompanied by *Spirodela polyrhiza L.* covered about ¼ of the surface area of this pond. Pond Miednik (about 0.06 ha) is surrounded from two sides by deciduous forest and supplied with nutrients from treated domestic sewage delivered from a small forest ranger lodge. Summer plant available nutrient concentrations were 1.24 mg $N\text{-}NO_3$ dm^{-3} and 0.050 mg $P\text{-}PO_4$ dm^{-3} in pond Krypy and 0.09 mg $N\text{-}NO_3$ dm^{-3} and 0.111 mg $P\text{-}PO_4$ dm^{-3} in pond Miednik (unpubl. data).

In order to follow seasonal changes in population features, 40 randomly sampled colonies of *L. minor* were taken in March, May and July from each pond. In the lab the number of fronds per colony was counted and every colony was photographed with Sony α500 camera equipped with macro lens DT 2.8/30.

In the middle of July experiments were set up to determine the effect of density on frond size, number of fronds per colony and the growth rate of *L. minor*. Bulk mass of about 1 kg fresh weight of plant material was taken from each pond and transported to the laboratory in original pond water. In the laboratory, *L. minor* plants were spread in large plastic containers filled with tap water to form a monolayer of floating plants. Colonies of *L. minor* taken at random from containers were planted into marked perforated transparent pots 50 mm of inner diameter (surface area = 1962.5 mm^2) and 40 mm high made of polyethylene terephtalate (PET). Applied planting densities were 5 colonies (hereinafter termed low density variant) and 100 colonies (high density variant) per pot, each in 40 repetitions. The latter number of colonies was chosen on the assumption that the total area of plants in the end of experiment would exceed available surface area in pots. The number of fronds was counted in each pot and the content of pot was photographed. Pots of contrasting densities of plants from the same pond (80 pots in total) were randomly placed in a large common cuvette filled with modified APHA medium (PN-EN ISO 20079, 2006). The modification consisted in changing the original P and N concentration to more realistic values of 2.1 mg $N\text{-}NO_3$ dm^{-3} and 0.185 mg $P\text{-}PO_4$ dm^{-3} with other mineral components left unchanged. The culture lasted 16 days. No attempts were undertaken to keep the culture aseptic. Every 4 days the medium was exchanged for a new one and pots were replaced at random within cuvette. Light was provided by fluorescent tubes and the intensity of radiation was about 80 μM photons m^{-2} s^{-1} in the 14:10 h light: dark regime. In the end of experiment, the number of grown colonies and individual fronds was counted in every pot. Fronds extending from mother frond but not fully developed were counted as ½ of frond.

In the end of experiments some fronds were found dead (devoid of chlorophyll or completely translucent) in both high and low density variants. Their number did not exceed 3.5% of the total number of fronds in a pot. Such fronds were not taken into account in the calculations of frond and colony numbers and in measurements of their surface areas. Based on three assumptions we did not consider this mortality as density-dependent. First, the dead fronds appeared in similar proportions in low and in high density variants. Second, dead fronds were always fully developed and relatively large once. Third, the average lifespan of *L. minor* (after various authors summarised in Landolt (1986)) is 5–7 weeks. Therefore, we assumed that observed dead fronds represented those that aged and passed their lifetime during the 16-day-long experiment.

In view of expected negative impact of plant density on growth, application of commonly used first-order kinetic equation to calculate growth rate would be inappropriate. Instead, we calculated the relative growth rate based on increments of the total surface area of colonies according to equation: $RGR_S = (S_t - S_0)/S_0 * t$, where S_0 and S_t are the surface areas of colonies at the beginning and in the end of experiment and t is time (16 days). Additionally, we calculated growth rate based on the number of fronds: $RGR_N = (N_t - N_0)/N_0 * t$, where N_0 and N_t are the numbers of fronds at the beginning and in the end of experiment and t = 16 days. The latter index may be termed relative vegetative reproduction rate of *L. minor* (Lemon et al., 2001) in contrast to the former, which includes also possible changes in the area of daughter fronds during culture.

All photographs were processed to measure the surface area of a single colony (seasonal changes) or of all colonies before and after experimental cultures. The content of pots from high density experimental variants was divided into 3 parts photographed separately to avoid overlapping of fronds. To make a photo, plants were transferred to a rectangular transparent plastic vessel of known dimensions, which were later used for scaling. Obtained photos were processed with Corel Photo-Paint X5 programme and the surface area of colonies was measured with the help of ImageJ free software.

Plants for the analyses of polymorphism were obtained from the ponds, sterilized with 1% NaOCl in a laboratory, rinsed with distilled water and inoculated into 200 ml flasks with 100 ml Steinberg medium modified by Altenburger (PN-EN ISO 20079, 2006) – one 3-frond *Lemna* colony per flask. Medium and distilled water were autoclaved before use at 121 °C for 20 min. A laminar flow cabinet was used to perform the inoculation and plants were transferred to a new medium every 7 days under aseptic conditions. The cultures were incubated under 68–119 μmol s^{-1} m^{-2} PAR emitted by fluorescent tubes (14:10 h light:dark) at19–22 °C in the growth chamber. Duckweeds were cultured for 52 days. So obtained plant material was disintegrated in liquid