

# A Statistical Analysis of Vegetative Reproduction Rate in Two Different Types of Weeds: *Gracilaria Verrucosa* and *L. Minor*

Dr. Himansu Bhusana Panigrahy<sup>1</sup>, Sunita Barik<sup>2</sup>, Sasmita Hota<sup>3</sup> and Sarala Prasanna Pattanaik<sup>4</sup>  
<sup>1,3</sup>Gandhi Institute For Technology, Bhubaneswar  
<sup>2,4</sup>Gandhi Engineering College, BBSR, Odisha

## Abstract

The rate of vegetative development was estimated in *Gracillaria verrucosa* and *Lemna minor*, by measuring the number of daughter fronds produced over the life span of mother fronds. Under the same constant environmental conditions, plants of *L. minor* lived the longest (31.3 days) and produced the most daughter fronds (14.0), yet *Gracillaria verrucosa* had the highest reproduction rate (0.62 fronds per day). This translates to a higher rate of population growth for *Gracillaria verrucosa*. Plants of *Gracillaria verrucosa* had the shortest life span, produced the least number of daughter fronds (1.1), and thus had the lowest frond production rate (0.08 fronds per day). When *Gracillaria verrucosa* was experimentally induced to release daughter fronds at maturity, and not well past maturity (which is usually the case), mother fronds produced three times more daughter fronds with no effect on their longevity. Presumably different retention times are associated with different costs and benefits, however frond longevity appears unrelated to retention time. Vegetative propagule production in the Lemnaceae forms a continuum from *Gracillaria verrucosa*, which develops relatively small (0.5–1.5 mm) and numerous propagules that are released before maturity, to *Spirodela*, which develops fewer yet relatively large propagules (4–12 mm) that are retained well past maturity. The different rates of propagule production likely represent different reproduction strategies, from an opportunistic strategy (*Gracillaria verrucosa*), to a strategy of increased competitive ability.

**Keywords:** *Gracillaria verrucosa*, duckweed, demography, vegetative reproduction, population growth.

## Introduction

Plants of the duckweed family (Lemnaceae) are small floating or submerged aquatic whose populations expand nearly exclusively by the recruitment of asexual propagules (Landolt, 1986). The development of propagules occurs in one way, by the branching and subsequent fragmentation of the shoot into separate units called fronds (Lemon and Posluszny, 2000), but results in a diversity of population growth rates. *Lemna minor* has been reported to live 4–5 weeks and produce between 4 and 12 daughter fronds (Ashby et al., 1949). Unfortunately, population growth in these plants is rarely expressed in terms of frond demography, obscuring aspects of development that regulate frond production.

The objective of this study was to examine how rates of shoot development influence vegetative reproduction in the Lemnaceae. This was accomplished by examining frond production rates and its regulation in the species: *Lemna minor* and *Gracillaria verrucosa borealis*. These species are useful

as they have a common developmental plan, but the manner in which development (i.e. number of new fronds produced over time and the length of time new fronds remain attached to a parent frond) regulates population growth differs widely. To address this objective, we considered the following specific questions: (1) how do the life span, number of daughter fronds produced, and the rate of frond production in *S. polyrhiza*, *L. minor*, *Gracillaria verrucosa* compare; (2) how

### Materials and methods

Plants of *Gracillaria verrucosa* L., *L. minor* L., and *Gracillaria verrucosa* (Engelm. ex Hegelm.) Landolt were collected locally at a small pond in Morriston, Ontario near the junction of Hwy. 6 south and Hwy. 401 (43° 33'N 80° 7'W, *S. polyrhiza*) and a small pond attached to Bronte Creek, north of Freelon, Ontario along Hwy. 6 (43° 35'N 80° 3'W, *L. minor* and *W. borealis*), transferred into axenic culture, and cultured in a growth chamber. Voucher specimens of each species have been placed in the herbarium at the University of Guelph (OAC) (accession 83390 (*S. polyrhiza*), 83389 (*L. minor*), and 83388 (*W. borealis*)).

Cultures of each species were isolated and sterilized by following the methods of Bowker et al. (1980). Plants were grown in small (15 mm 60 mm), sterile, plastic Petri dishes, sealed with Parafilm®. Petri dishes were filled half-full with sterile (autoclaved) 33% v/v

strength Hutner's medium, adjusted to pH 6.5 (Hutner, 1953). Plants were transferred into new Petri dishes with fresh solution every 5 days. The growth cabinet was set to 24°C with a 12 h photoperiod and a photo irradiance of 180–210  $\mu\text{m}^{-2} \text{s}^{-1}$ .

does variation in life span, number of daughter fronds produced, and the rate of frond production among *S. polyrhiza*, *L. minor*, and *Gracillaria verrucosa* influence vegetative reproduction at the level of a population and (3) is the production of fronds increased in plants where daughter fronds are removed and not allowed to be retained past maturity (i.e. *L. minor* and *S. polyrhiza*)?

Uncontaminated duckweed fronds were acclimated and allowed to vegetatively multiply for 2 months. During this time one clone of each species was randomly selected for study. A single clone was used as representative of each species since much more variation occurs among species for the parameters estimated than within species (Landolt, 1986). In August 1997 fronds of each species that had just been released from their mother frond (fronds producing new (daughter) fronds) the previous day were separated into individual Petri dishes. In this way all fronds began at the same developmental stage of not having released any daughter fronds.

The removal treatment for *L. minor* and *Gracillaria verrucosa* was applied when a daughter frond developed past the point of maturity, i.e. when the daughter fronds were fully-grown and their own daughter fronds began to extend out of the pocket. The removal occurred by holding the daughter frond with a pair of tweezers and then brushing the mother frond against a probe. Daughter fronds normally detached easily and minimal pressure had to be applied to the mother frond.

Clones of mother fronds were examined under

a laminar flow hood each morning of each day until the death of the mother frond. In cultures where the mother frond had produced a daughter frond, or the daughter frond was removed from the mother frond, the daughter frond was removed from the Petri dish. The time (to the nearest day) each daughter frond was produced and the life span of the mother frond was recorded for each mother frond. From these data, the total number of daughter fronds produced for each mother frond, and the rates of frond production (total number of daughter fronds divided by the life span of the mother frond) for each mother frond were calculated.

Frond production rates over the life span of mother fronds were first compared by plotting the daughter frond number onto the time of production (measured in days) for each treatment. The linearity of this relationship was confirmed using a lack of fit test (SAS Institute, 1994) with a significance (alpha) level of 0.05. Since this relationship was linear, it can be assumed that frond production was not hindered by nutrient availability or ambient CO<sub>2</sub> concentrations, and an average production rate could describe patterns of frond production.

## Results and discussion

### *Variation in vegetative reproduction among species (controls)*

Statistically significant differences were

found among the three species for all three variables (Table 1). Fronds of *L. minor* had a significantly longer life span than *Gracillaria verrucosa*

**Table 1**

The ANOVA and linear contrasts for each of total daughter fronds released, mother frond life span, and frond production rate<sup>a</sup>

Source	d.f.	MS	F-value	Probability >F
<b>Life span</b>	4	793.180	78.845	0.000
Error	36	10.060		
Linear contrasts				
<i>Lemna</i> : control vs.1 treatment		30.400	3.022	0.091
<i>Spirodela</i> : control vs.1 treatment		3.772	0.375	0.544
<b>Total Daughter fronds</b>	4	276.397	105.501	0.000
Error	36	2.620		
Linear contrasts				
<i>Lemna</i> : control vs.1 treatment		20.889	7.974	0.008
<i>Spirodela</i> : control vs.1 treatment		25.150	9.600	0.004

treatment					
<b>Fronnd production rate</b>	4		0.305	85.305	0.000
Error	36		0.004		
Linear contrasts					
<i>Lemna</i> : control vs.1			0.005	1.356	0.252
Treatment					
<i>Spirodela</i> : control vs.1			0.215	60.094	0.000
Treatment					

The degrees of freedom (d.f.), mean square (MS), *F*-value, and *P*-value (probability >*F*) are reported.

and *Gracillaria verrucosa* (Table 2). There was no significant difference between the life span of *Gracillaria verrucosa* and *Gracillaria verrucosa* (Table 2). The mean number of daughter fronds produced was significantly different among all three genera. *L. minor* and *Gracillaria verrucosa* produced the most daughter fronds (mean 14.0) and fewest (mean 1.1), respectively (Table 2). Compared with the other two species, the number of daughter fronds produced in *Gracillaria verrucosa* was extremely low, ranging from 0 to 3 (Table 2), however in many cases *Gracillaria verrucosa* retained its daughter

fronds and formed connecting chains of fronds. The vegetative reproduction rate was also significantly different among the three species. *Gracillaria verrucosa* reproduced at the fastest rate (mean 0.62 fronds per day), while *Gracillaria verrucosa* had the slowest reproduction rate (mean 0.08 fronds per day) (Table 2).

The results for life span and total daughter fronds produced for *L. minor* are comparable with those found in other studies (see Section 1). One known estimate of the life span of *Gracillaria verrucosa* was 33 days (Boss et al., 1964), which is much greater than the 12 days reported in this study. The differences between this study and others for *L. minor* and especially *Gracillaria verrucosa*.

**Table 2**

Mean (±S.E.) for life span of mother fronds, number of daughter fronds produced, and vegetative reproduction rates for *Gracillaria verrucosa* (*n* = 5), *L. minor* (*n* = 9), and *Gracillaria verrucosa* (*n* = 10)<sup>a</sup>

Taxa	Lifespan (day)	Daughter fronds (number)	Production rate (fronds per day)
<i>W. borealis</i>	15.8 (1.5)	9.8 (0.7)	0.62 (0.03)
<i>L. minor</i>	31.3 (1.1)	14.0 (0.5)	0.45 (0.02)
<i>S. polyrhiza</i>	12.1 (1.1)	1.1 (0.5)	0.08 (0.02)

<sup>a</sup>Means for each species were compared using and ANOVA and Tukey–Kramer HSD multiple comparison tests. All values, except for lifespan of *Gracillaria verrucosa* and *L. minor*, are significantly different at  $P < 0.05$  and at  $P < 0.01$ . *polyrhiza* may be due to environmental conditions, and illustrate the phenotypic plasticity of these plants (Landolt, 1986). This study is the first to estimate the life span and number of daughter fronds produced for *Gracillaria verrucosa*.

These results can be used to estimate how variation in life span and daughter frond production among the three species affects vegetative reproduction at the population level. For example, which species would have a greater population growth rate ( $r$ ), *L. minor*, which produces more propagules and lives longer, or *Gracillaria verrucosa*, which has a higher frond production rate. Either case could be possible depending on the absolute values for life span and daughter frond production.

The exponential rate of population growth ( $r$ ) can roughly be estimated in the relatively simple system of the duckweeds by converting the time it takes for a population to double ( $t_2$ ) into  $r$ , where  $r = \ln 2/t_2$ . The doubling time of a population is the inverse of the reproduction rate (rp) minus the death rate (dr). This is measured in years and represented by the formula  $t_2 = [1/(rp - dr)]/365$ , where dr is the inverse of the life span. Thus,  $r$  for *L. minor*, *Gracillaria verrucosa*, and *Gracillaria verrucosa* would be 104, 151, and 2.1, respectively. Under the conditions of this study *Gracillaria verrucosa* has a faster population growth rate than *L. minor*, not

**Vegetative reproduction in removal experiments**

Statistically significant differences were

because it produces more daughter fronds over its life span, but because it produces daughter fronds at a faster rate than the other two species.

The differences between population growth rates in *L. minor* and *Gracillaria verrucosa* illustrate the significance of comparing the demographic characteristics of frond production and life span of individual fronds when studying vegetative reproduction. At the level of the individual frond, differences in reproductive rates can be the result of a faster development and release of daughter fronds and/or a longer life span. This level of understanding is not achieved in most studies on duckweed population growth since they only measure multiplication rates or changes in biomass over time (Clatworthy and Harper, 1962; Hodgson, 1970; Tillberg et al., 1979; Markarova et al., 1995).

These estimates of  $r$  must be considered in the context of this study. The absolute values are oversimplified and overestimate real population growth rates by excluding the influences of immigration, emigration, seasonal influences on both death and reproduction rates, interspecific variation, as well as herbivory, plant density, and competition. This study was conducted under conditions that are favorable for vegetative reproduction and as a result represent one estimate of  $r$ . This simplified model does, however, have heuristic value and could be used as a basis for a more complete model of population growth.

found among the treatments and controls for some of the variables estimated in *L. minor* and *Gracillaria verrucosa* (Table 1). There was no significant difference in frond life

span between the control versus the removal treatment for *L. minor* or *Gracillaria verrucosa* (Table 3). The mean number of daughter fronds produced in the control versus the treatment for both *L. minor* and *Gracillaria verrucosa* was significantly different (14.0 versus 11.9 and 1.1 versus 3.6, respectively); however, the differences were

marginal in *L. minor* (Table 3). The vegetative reproduction rate was significantly different between the control and treatment for *Gracillaria verrucosa* (0.08 versus 0.31 fronds per day, respectively), but not for *L. minor* (0.45 versus 0.41 fronds per day, respectively) (Table 3).

**Table 3**

Mean ( $\pm$ S.E.) for life span of mother fronds, number of daughter fronds produced, and vegetative reproduction rates for the control and treatment in *L. minor* ( $n = 9$  and  $n = 10$ , respectively) and *Gracillaria verrucosa* ( $n = 10$  and  $n = 7$ , respectively)<sup>a</sup>

Taxa	Lifespan (day)	Daughter fronds (number)	Production rate (fronds per day)
<i>L. minor</i>			
Control	31.3 (1.1)	<b>14.0</b> (0.5)	0.45 (0.02)
Treatment	28.8 (0.9)	<b>11.9</b> (0.6)	0.41 (0.01)
<i>S. polyrhiza</i>			
Control	12.1 (1.1)	<b>1.1</b> (0.5)	<b>0.08</b> (0.02)
Treatment	11.1 (1.4)	<b>3.6</b> (0.5)	<b>0.31</b> (0.03)

<sup>a</sup> Means for each species were compared using ANOVA and linear contrast tests. Contrast in bold are significantly different at  $P < 0.05$ . The general lack of significant differences between the control and removal treatment for *L. minor* is not surprising since only 7% of the total daughter fronds in the treatment reached maturity (the point subjectively assigned as when a granddaughter frond projected out of the daughter frond pocket) and were physically removed early. The decrease detected in the mean number of daughter fronds produced in the removal treatment is likely not due to the treatment, and is not detectable when compared using the less sensitive Tukey–Kramer HSD test. More importantly, the frond production rate (a combination of life span and daughter fronds produced) was not significantly

different. In contrast to *L. minor*, all of the daughter fronds produced in the treatment for *Gracillaria verrucosa* were physically removed which significantly increased the rate and total number of daughter fronds produced, but had no effect on the life span of the mother frond. Therefore, what normally occurs is that daughter fronds, which are not released until well after maturity, produce an ‘apical dominance’ effect, preventing the development of subsequent daughter fronds. Viewed in another way, mother fronds do not normally live up to their full production capacity since the removal treatment resulted in a more than threefold increase (from 1.1 to 3.6) in the number of daughter fronds produced. Two previous studies, those of Wangermann (1952) and Kasinov (1981), assessed the effects of prematurely removing

the first daughter frond of *L. minor* on the future reproduction and longevity of mother fronds. Wangermann (1952) found that the total number of daughter fronds produced decreased by about half but had no effect on the life span of the mother frond. Kasinov (1981) found no change in the total number of daughter fronds produced, but a significant shortening of the life of the mother frond. While presenting contradictory conclusions, which in itself is very peculiar, both report some trade-off in the early release of daughter fronds, which was not seen in this study. McLay (1976) found similar results to this study under natural conditions in plants of *Lemna perpusilla* Torrey. Different plants within Lake Los Carneros (CA, USA)

### **Reproductive strategies**

Relative to each other, the three species of Lemnaceae examined have very different reproductive strategies, especially in terms of frond retention times (Table 4). In *Spirodela*, this leads to question: what is the advantage of retaining daughter fronds past maturity and not producing more? It seems plausible that the different retention times are associated with different costs and benefits. In *S. polyrhiza*, longevity of the mother fronds seems unrelated to retention time, but other trade-offs are still apparently operating. Short retention times result in relatively small plants but high frond production rates (i.e. *Gracillaria verrucosa*). Long retention times result in relatively larger plants and slower frond production rates (i.e. *Spirodela*). Most likely neither is 'best' for all conditions. Short retention species like *Gracillaria verrucosa* may represent a kind of opportunistic strategy that allows for rapid population growth when resources are plentiful and competition is minimal (an r-

remained attached to their daughter fronds for different lengths of time. The plants that fragmented into single fronds reproduced at a faster rate relative to those that formed connected chains when grown both in vivo and in vitro. It is interesting to speculate on the effect that the artificial increase in daughter frond production has when extrapolated to the population level. There can be many environmental disturbances to a *Gracillaria verrucosa* population that could induce the premature release of daughter fronds, such as wind and wave action, predation, animals, and humans (via boats). It is plausible that a more heterogeneous environment would increase population growth rates in *S. polyrhiza*.

strategist). Long retention species like *Spirodela* may represent a strategy that favors large plants (even if they are formed through a network of many fronds) with a superior competitive ability but slower population growth rates (a K-strategist).

These results indicate that changes in population growth rates in the Lemnaceae are due to variations in development at the level of the individual frond. In order to understand these changes the demographic characters of reproduction and longevity need to be measured. Vegetative propagule production forms a continuum in the Lemnaceae from *Gracillaria verrucosa*, which develops relatively small and numerous propagules (Bernard et al., 1990) to *Spirodela*, which develops fewer yet relatively large propagules. Understanding propagule development in these species has helped to understand and make inferences about the population growth strategies of these prolific plants.

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