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A statistical analysis of vegetative reproduction rate in two different types of weeds: Gracilariaverrucosa and L. minor

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Abstract: *The rate of vegetative development was estimated in Gracillariaverrucossaand Lemna minor, by measuring the number of daughter fronds produced over the life span of mother fronds. Under the same constant environ- mental conditions, plants of L. minor lived the longest (31.3 days) and produced the most daughter fronds (14.0), yet Gracillariaverrucossahad the highest reproduction rate (0.62 fronds per day). This trans- lates to a higher rate of population growth for Gracillariaverrucossa. Plants of Gracillariaverrucossahad 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 Gracillariaverrucossawas 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 Gracillariaverrucossa, which develops relatively small (0.5–1.5 mm) and numerous propagules thatarereleased before maturity,to Spirodela, which developsfeweryetrelativelylargepropagules (4–12 mm) that are retained well past maturity. The different rates of propagule production likely represent different reproduction strategies, from an opportunistic strategy Gracillariaverrucossa), to a strategy ofincreasedcompetitiveability.*
Keywords*: Gracillariaverrucossa; Duckweed; Demography; Vegetative reproduction; Population growth*

I. Introduction

Plants of the duckweed family (Lemnaceae) are small floating or submerged aquat- ics whose populations expand nearly exclusively by the recruitment of asexual propag- ules (Landolt, 1986). The development of propagules occurs in one way, by thebranching 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 fronddemography, obscuringaspects ofdevelopmentthatregulatefrondproduction.

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 productionratesanditsregulationinthespecies:Lemna minorandGracillariaverrucossaborealis.Thesespeciesareuseful astheyhaveacommondevelopmentalplan,butthemannerinwhichdevelopment(i.e.numberofnewfrondsproducedove rtime and the length of time new fronds remain attached to a parent frond) regulates popula- tiongrowth differs widely.Toaddressthisobjective,weconsideredthefollowingspecific questions: (1) how do the life span, number of daughter fronds produced, and the rate of frond production in S. polyrhiza, L. minor, Gracillariaverrucossacompare; (2) how does variation in life span, number of daughter fronds produced, and the rate of frond productionamongS. polyrhiza, L. minor, and Gracillariaverrucossainfluence 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)?

II. Materials andmethods

PlantsofGracillariaverrucossaL.,L.minorL.,andGracillariaverrucossa(Engelm.exHegelm.)Landoltwerecollectedloca llyatasmallpondinMorriston,OntarionearthejunctionofHwy.6southandHwy.401(43°33ʳN80°7ʳW,S.polyrhiza)anda smallpondattachedtoBronteCreek,northofFreelton,OntarioalongHwy.6(43°35ʳN80°3ʳW,L.minorandW.borealis),tr ansferred into axenic culture, and cultured in a growth chamber. Voucher specimens of eachspecieshavebeenplacedintheherbariumattheUniversityofGuelph(OAC)(accession83390(S. polyrhiza), 83389 (L. minor), and 83388 (W.borealis)).

CulturesofeachspecieswereisolatedandsterilizedbyfollowingthemethodsofBowker 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 top H6.5 (Hutner,1953). Plantsweretransferredinto newPetridisheswithfreshsolutionevery5days.Thegrowthcabinetwassetto24°Cwith a12h photo period andaphotoirradianceof180–210μmm^{-2}s^{-1}.
Uncontaminatedduckweedfrondswereacclimatedandallowedtovegetativelymultiplyfor 2 months. During this time one clone of each species was randomly selected forstudy. Asingle clone was used asrepresentativeofeachspeciessincemuchmorevariationoccursamongspeciesfortheparametersestimatedthanwithin species(Landolt,1986).InAugust 1997 fronds of each species that had just beenreleased from theirmotherfrond(frondsproducing new (daughter) fronds) the previous day were separated into individual Petri dishes.Inthiswayallfrondsbeganatthesamedevelopmentalstageofnothavingreleased any daughterfronds.

TheremovaltreatmentforL.minorandGracillariaverrucossawasappliedwhenadaughterfrond 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 againstaprobe Daughter frondsnormally detachedeasily andminimal pressurehad tobe applied to the motherfrond.

Clonesofmotherfrondswereexaminedunderalaminarflowhoodeachmorningofeach 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. Fromthesedata,thetotalnumberofdaughterfrondsproducedforeachmotherfrond,and the rates of frond production (total number of daughter fronds divided by the life span of the mother frond) for each mother frond werecalculated.
Frondproductionratesoverthelifespanofmotherfrondswerefirstcomparedbyplottingthedaughterfrondnumberontoth etimeofproduction(measuredindays)foreachtreatment. The linearity of this relationship was confirmed using a lack of fit test (SAS Institute, 1994) with asignificance (alpha) level of 0.05.Sincethis relationship waslinear, itcan be assumed that frond production was not hindered by nutrient availability or ambient CO_2 concentrations ,andanaverageproductionratecoulddescribepatternsoffrondproduction.

III. Results and discussion

Variation in vegetative reproduction among species(controls)

Statistically significant differences were found among the three species for all three variables (Table1). Frondsof L.minorhada significantly longerlifespanthanGracillariaverrucossa

Table 1 The ANOVA and linear contrasts for each of total daughter fronds released, mother frond life span, and frond production rate[a]

Source	d.f.	MS	F-value	Probability >F
Life span	4	793.180	78.845	0.000
Error	36	10.060		
Linear contrasts				
Lemna: control vs. treatment	1	30.400	3.022	0.091
Spirodela: control vs. treatment	1	3.772	0.375	0.544
Total Daughter fronds	4	276.397	105.501	0.000
Error	36	2.620		
Linear contrasts				
Lemna: control vs. treatment	1	20.889	7.974	0.008
Spirodela: control vs. treatment	1	25.150	9.600	0.004
Frond production rate	4	0.305	85.305	0.000
Error	36	0.004		
Linear contrasts				
Lemna: control vs. Treatment	1	0.005	1.356	0.252
Spirodela: control vs. Treatment	1	0.215	60.094	0.000

The degrees of freedom (d.f.), mean square (MS), F-value, and P-value (probability >F) are reported. and Gracillariaverrucossa(Table 2). There was no significant difference between the life span ofGracillariaverrucossaand Gracillariaverrucossa(Table 2). The mean number of daughter fronds produced was significantlydifferentamongallthreegenera.L.minorandGracillariaverrucossaproducedthemost daughter fronds (mean 14.0) and fewest (mean1.1), respectively (Table 2). Compared with the other two species, the number of daughter fronds produced in Gracillariaverrucossawas extremelylow,rangingfrom0to3 (Table2),howeverinmany casesGracillariaverrucossaretained its daughter fronds and formed connecting chains of fronds. The vegetative reproduction ratewasalsosignificantlydifferentamongthethreespecies.Gracillariaverrucossareproducedatthe fastest rate (mean0.62 fronds peer day), while Gracillariaverrucossahad the slowest reproduction rate (mean 0.08 fronds per day) (Table2).

TheresultsforlifespanandtotaldaughterfrondsproducedforL.minorarecomparablewiththosefoundinotherstudies(seeSection1).OneknownestimateofthelifespanofGracillariaverrucossawas33days(Bossetal.,1964),whichismuchgreaterthanthe12daysreported in this study. The differences between this study and others for L. minor and especiallyGracillariaverrucossa.

Table 2

Mean (±S.E.) for life span of mother fronds, number of daughter fronds produced, and vegetative reproduction rates for Gracillariaverrucossa(n = 5), L. minor (n = 9), and Gracillariaverrucossa(n = 10)[a]

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

[a]Means for each species were compared using and ANOVA and Tukey–Kramer HSD multiple comparison tests. All values, except for lifespan of Gracillariaverrucossaand L. minor, are significantly different at $P < 0.05$ and at $P < 0.01$.polyrhizamay 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 andnumber of daughter fronds produced for Gracillariaverrucossa.

These results can be used to estimate how variation in life span and daughter frond productionamongthethreespeciesaffectsvegetativereproductionatthepopulationlevel. For example, which species would have a greater population growth rate (r), L. minor, which produces more propagules and liveslonger,orGracillariaverrucossa,whichhasahigherfrondproductionrate.Eithercasecouldbepossibledependingontheabsolutevaluesforlifespan and daughter frondproduction.

Theexponentialrateofpopulationgrowth(r)canroughlybeestimatedintherelativelysimplesystemoftheduckweedsbyconvertingthetimeistakesforapopulationtodouble (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 andrepresented by the formula t_2 [1/(rpdr)]/365, where dr is the inverse of the life span. Thus, r forL.minor,Gracillariaverrucossa,andGracillariaverrucossawouldbe104,151,and2.1,respectively.Under the conditions of this study Gracillariaverrucossahas a faster population growth rate than L. minor, not because it produces more daughter fronds over its life span, but because it produces daughter fronds at a faster rate than the other twospecies.

ThedifferencesbetweenpopulationgrowthratesinL.minorandGracillariaverrucossaillustratethesignificanceofcomparingthedemographiccharacteristicsoffrondproductionandlifespan of individual fronds when studying vegetativereproduction.Atthelevelofthe individualfrond,differencesinreproductiveratescanbetheresultofafasterdevelopmentandrelease ofdaughterfrondsand/oralongerlifespan.Thislevelofunderstandingisnotachievedin most studies on duckweed population growth since they only measure multiplicationrates or changes in biomass over time (Clatworthy and Harper, 1962; Hodgson, 1970; Tillberg et al., 1979; Markarova et al.,1995).

Theseestimatesofrmustbeconsideredinthecontextofthisstudy.Theabsolutevaluesareoversimplifiedandoverestimaterealpopulationgrowthratesbyexcludingtheinfluences of immigration, emigration, seasonal influences on both death and reproduction rates, in- terspecific 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 representoneestimateofr.Thissimplifiedmodeldoes, however,haveheuristicvalueand could be used as a basis for a more complete model of populationgrowth.

Vegetative reproduction in removalexperiments

Statistically significant differences were found among the treatments and controls for some of the variables estimated in L. minor and Gracillariaverrucossa(Table 1). There was no significant difference in frond life span between the control versus the removal treatment forL. minoror Gracillaria verrucossa (Table3). Themeannumberofdaughterfrondsproducedinthe control versus the treatment for both L. minor and Gracillariaverrucossawas significantly different (14.0versus 11.9and 1.1 versus 3.6,respectively); however,thedifferenceswere marginal inL. minor (Table3). hevegetativereproductionratewassignificantlydifferent between the control and treatment for Gracillariaverrucossa(0.08 versus 0.31 fronds per day, respectively), but not for L. minor (0.45 versus 0.41 fronds per day, respectively) (Table3).

Table3 Mean(±S.E.)forlifespanofmotherfronds,numberofdaughterfrondsproduced,andvegetativereproductio n ratesforthecontrolandtreatmentinL.minor(n=9andn=10,respectively)andGracillariaverrucossa(n=10and n = 7,respectively)[a]

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

[a] Means for each species were compared using and 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.minorisnotsurprisingsinceonly7%ofthetotaldaughterfrondsinthetreatmentreached maturity (the point subjectively assigned as when a granddaughter frond projected out of thedaughterfrondpocket)andwerephysicallyremovedearly.Thedecreasedetectedinthemeannumberofdaughterfrond sproducedintheremovaltreatmentislikelynotduetothetreatment,andisnotdetectablewhencomparedusingthelesssens itiveTukey–KramerHSD test. More importantly, the frond production rate (a combination of life span anddaughter fronds produced) was not significantlydifferent.

IncontrasttoL.minor,allofthedaughterfrondsproducedinthetreatmentforGracillariaverrucossawerephysic allyremovedwhichsignificantlyincreasedtherateandtotalnumberofdaughter 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 releaseduntilwellaftermaturity,producean'apicaldominance'effect,preventingthedevelopmentofsubsequentdaught er fronds.Viewedinanotherway,motherfrondsdonotnormallyliveuptotheirfullproduction capacity since the removal treatment resulted in a more than threefold increase (from 1.1 to 3.6) in the number of daughter frondsproduced.

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 repro- duction 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 contradictoryconclusions,whichinitselfisverypeculiar,bothreportsometrade-offinthe early release of daughter fronds, which was not seen in thisstudy.

McLay (1976) found similar results to this study under natural conditions in plants of LemnaperpusillaTorrey.DifferentplantswithinLakeLosCarneros(CA,USA)remained attached to their daughter fronds for different lengths of time. The plants that fragmented intosing lefrondsre produce data fasterraterelativetothosethatformedconnectedchains when grown both in vivo and invitro.

It is interesting to speculate on the effect that the artificial increase in daughter frond productionhaswhenextrapolatedtothepopulationlevel.Therecanbemanyenvironmenta isturbancestoaGracillariaverrucossapopulationthatcouldinducethepremature releaseofdaughter 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.

Reproductivestrategies

Relative to each other, the three species of Lemnaceae examined have very different reproductivestrategies,especiallyintermsoffrondretentiontimes(Table4).InSpirodela, thisleadstoquestion:whatistheadvantageofretainingdaughterfrondspastmaturity and notproducingmore?Itseemsplausiblethatthedifferentretentiontimesareassociatedwith differentcostsandbenefits.InS.polyrhiza,longevityofthemotherfrondsseemsunrelated to retention time, but other tradeoffsarestillapparentlyoperating.Shortretentiontimesresultinrelativelysmallplantsbuthighfrondproductionrate s(i.e.Gracillariaverrucossa).Longretentiontimesresultinrelativelylargerplantsandslowerfrondproductionrates(i.e.Sp irodela).Mostlikelyneitheris'best'forallconditions.Shortretentionspecies likeGracillariaverrucossamayrepresentakindofopportunisticstrategythatallowsforrapidpopulation growth when resources are plentiful and competition is minimal (an r-strategist). Long retention species like Spirodelamay representastrategythatfavorslargeplants(eveniftheyareformedthroughanetworkofmanyfronds)withasuperiorcomp etitiveabilitybut slower population growth rates (aK-strategist).

Theseresultsindicatethatchangesinpopulationgrowthratesinthe Lemnaceaeareduetovariationsindevelopmentatthele veloftheindividualfrond.Inordertounderstandthese changes the demographic characters of reproduction and longevityneedtobemeasured.VegetativepropaguleproductionformsacontinuumintheLemnaceaefromGracillariaverr ucossa,which develops relatively small and numerous propagules (Bernard et al., 1990) to Spirodela, which develops fewer yet relatively large propagules. Understanding propagule develop- ment in these species has helped to understand and make inferences about the population growth strategies of these prolificplants.

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