

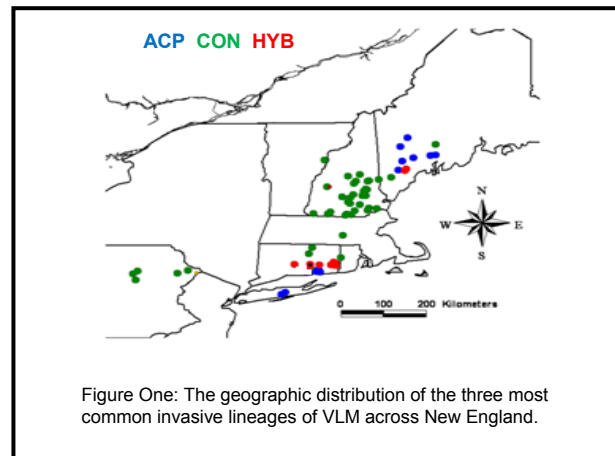
Implications of genetics and the environment for management: are
certain lineages of variable-leaf watermilfoil more apt to grow
invasively?

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INTRODUCTION

Closely related taxa are often difficult to distinguish morphologically. This problem has been confounding lake management efforts for decades. Due to convergent evolutionary pathways that maximize surface area of leaves and other structural characters that facilitate survival in aquatic environments, many aquatic plant species are morphologically similar (Moody, Les and Ditomaso 2008). For example, at a large scale (i.e.- whole plant beds), it is often hard to distinguish between species of watermilfoil, but upon fine scale inspection, we see distinguishing differences in growth form and floral characters. This problem of species identification is further confounded by the genetic variation we see within a species which results from the presence of several distinct genetic lineages (i.e.- distinct genetic groups). It stands to reason that these genetically distinct taxa will respond differently to management if lineages differ morphological and ecologically, as most management regimes are geared toward a target species (e.g.- selective herbicides).

Myriophyllum heterophyllum (Variable-leaf Watermilfoil, VLM) is native to most of the Central and Southern United States, but has recently become a high profile invader in the Northeast (Moody and Les 2002). Though there are several invasive genetic lineages, most of the invasive populations can be traced back to the Atlantic Coastal Plain (ACP, Southern coastal states, Blue in Figure One), Continental region (CON, Great Lakes or Ozark mountain region, Green in Figure One), or a hybrid taxa (HYB, also from Southern coastal states, *M. heterophyllum* x *M. laxum*, Red in Figure One). These genetic lineages have distinct geographic origins and appear to remain distinct within the invaded range (multiple lineages do not often occupy the same lake, and when they do they do not appear to interbreed, though further genetic analysis is needed to confirm this). Using the genetic lineages identified by Moody and Les (2002), I designed a multi-facet experiment that would answer the following questions:



1. Is the genetic diversity we see between these three main invasive lineages biologically relevant?
 - a. Are they morphologically distinct?
 - b. Do they display different growth patterns in the field, as well as in the lab (i.e.- does one grow more “invasively?”)?
2. How can we utilize any ecological diversity to create more specialized management regimes and predict spread?

METHODS

In order to address the above questions, I undertook both a field observational study and a common garden experiment to address growth and morphological patterns in the field and under controlled laboratory conditions.

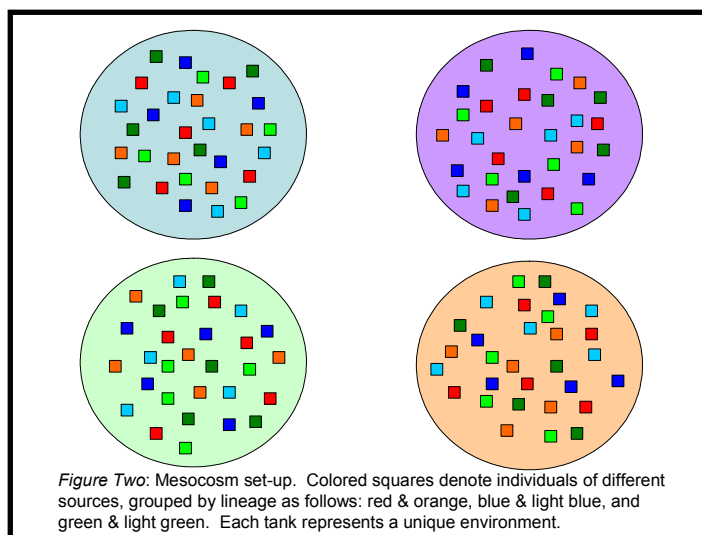
Field Observational Study: Using previously identified genetic lineages (see Figure One) seven lakes were chosen that had been invaded by each of the three most common lineages (ACP, CON, and HYB), for a total of 21 study lakes in New England. These lakes were chosen based on size (under ~1000 acres), ease of access, and distribution across the invaded range (i.e.- I tried to choose lakes from all around New England, not just one localized area). For a list of study lakes, see Appendix A.

Within each lake, we mapped out all VLM beds using a Trimble GPS unit. Beds were identified, circled to assess extent, assigned a density value, and mapped accordingly. In situations where the boat could not pass through the bed (e.g.- shallow water), beds were hand drawn on lake maps to be later projected in ArcView. Once we had mapped out all beds, I returned to the three densest patches of VLM and laid a 25 meter transect. We recorded GPS coordinates for the two endpoints of each transect and collected environmental data with a YSI meter. I snorkeled each 25 meter transect, counting root crowns (individual plants) to assess density and harvested 3 representative individuals to be analyzed in the lab (for a total of 9 plants per lake). Back in the lab, we cleaned the plants, counted stems and flowers, harvested 5 leaves and photographed them to assess leaf area, and dried and weighted the above and below ground biomass of each plant separately.

Common Garden Experiment: From the field observational study lakes, two lakes were chosen containing each lineage to serve as source populations in the common garden experiment, for a total of six source lakes (denoted in Appendix A). Within each source lake, about 30 stems were harvested, cleaned, and mailed to Michigan where they were planted in two 300 gallon culture tanks and grown out for a month. We grew out source plants for this experiment to ensure that only new growth would be used, canceling out maternal effects (i.e.- advantage of some plants “raised” in a specific lake environment) that may skew the results of the study in favor of a certain source population.

We established four distinct environments using a light by nutrient cross to assess the success of each genetic lineage across a range of environments. 20 twelve centimeter stems were harvested from each

source population and allocated among the treatment tanks in a random grid (5 per source per tank, 30 stems total per tank- see Figure Two). Each stem was tagged for elongation rate using a small zip tie attached 3cm from the apical meristem (all stems had only one meristem) and planted in a 9” sapling pot (see Figure Two, sediment varied based on nutrient treatment). We established the following four



environments: high light- high nutrients, high light- low nutrients, low light- high nutrients, and low light- low nutrients. “High light” refers to the normal power of the full spectrum sodium lights available for use at the AWRI mesocosm facility (see Appendix B). One tablespoon of granular Osocote was added per bag of topsoil to establish the high nutrient environments. The low nutrient environment stems were planted in sand. Low light environments were established using 50% light reduction shade cloth. All tanks were set at 14:10 light:dark cycles to mimic summer solar exposure.

After a 30 day growth period, we broke down all experimental tanks, measured elongation rate (gained length), gained weight, branching events, and biomass allocation to root, stem, and leaf tissue for each stem. Allocation measurements were based on dry mass.

RESULTS

Using the above-described methodology, I sought to quantify the ecological differences we see between the three main invasive lineages of VLM. These differences can be placed into two categories: (1) morphological differences, and (2) differences in “invasiveness.”

Morphological Differences in the Field: In order to establish morphological distinction between these invasive lineages, we assessed number of branches per plant, individual plant mass, and leaf area. Number of branches per plant was highly influenced by both genetics and environment ($p < 2.2 \times 10^{-16}$ and $p = 1.9 \times 10^{-7}$ respectively). In post hoc pairwise comparisons, the hybrid lineage (HYB) had significantly more branches per plant than both the continental (CON) and Atlantic coastal plain (ACP) lineages (see Appendix C for all ANOVA tables and bar graphs). Individual plant size was dictated by genetics, environment, and location within environment ($p < 2.2 \times 10^{-16}$, $p < 2.2 \times 10^{-16}$, and $p = 1.5 \times 10^{-9}$, respectively). These results are to be expected because a plant’s individual success is often determined by the quality of its habitat and the space it has to grow. In post hoc comparisons, all three lineages were significantly different sizes, the hybrid being the largest, then the ACP lineages, and the CON lineage plants the smallest. Average leaf area was influenced by genetics and environment ($p = 0.0002$ and $p = 4.8 \times 10^{-10}$, respectively). The ACP and CON lineages differ significantly in this measurement, but the hybrid does not differ from either.

Morphological Differences in the Common Garden: To measure morphological differences in actively growing plants, we measured number of branches per planted stem, total gained length, and biomass allocation to stem, leaf, and root tissue. Branches per stem is dictated by genetics and environment ($p = 2.9 \times 10^{-11}$ and $p = 1.1 \times 10^{-13}$), as we see in the field, as well as the interaction term ($p = 0.0009$). All lineages are significantly different from one another. In the common garden experiment, the CON lineage had the most branches per stem, followed by the hybrid, then the ACP lineage. The increased branching seen in both the CON and hybrid lineages may attest to the life history differences we see between these two lineages and the ACP lineage (see discussion). Total gained length is controlled by genetics, environment, and the interaction ($p = 1.6 \times 10^{-13}$, $p = 2.2 \times 10^{-16}$, and $p = 2.8 \times 10^{-11}$, respectively) and all lineages are significantly different from each other. As would be expected from the amount of stems each plant sprouted, we see the most gained length in the CON lineage, followed by the hybrid, then the ACP lineage. Biomass allocation across all environments was significantly different for all lineages. Percent leaf tissue was dictated by genetics, environment, and the interaction ($p = 0.00053$, $p = 2.4 \times 10^{-5}$, and $p = 0.003$, respectively). Stem tissue allocation was solely dictated by genetics

($p=5.2E-07$), an interesting result in face of the life history differences we see between lineages. Root tissue allocation is dictated by environment and the genetics by environment interaction term ($p=2.86E-6$ and $p=7.8E-5$ respectively), which is often observed in studies where plants are put under nutrient stress.

Differences in “Invasiveness” in the Field: To quantify differences in “invasiveness,” we must first define the term. In this study, “invasiveness” represents the perceived density and volume (i.e.- clogging of the water column) of an invasive taxa. To address these aspects of invasiveness, we measured bed density (plants per square meter) and bed volume (biomass per meter, a function of individual size and plants per unit area). Bed densities are dictated by both genetics ($p=0.0006$) and environment ($p=0.02$). Due to their larger individual size, the hybrid plants occurred in lower densities than the CON and ACP lineages. Biomass per square meter is controlled by genetics only ($p=0.0003$). The hybrid lineage has the most biomass per meter and the CON lineage the least. The ACP lineage does not have a significantly different amount of biomass per meter than the other lineages.

DISCUSSION

This study has provided a case for both morphological distinction and differences in invasive characters between the main invasive VLM lineages. We see significant differences in branching patterns, leaf area, individual plant size, bed densities, biomass allocation, and biomass per meter. These differences could be due to different life history traits between the invading lineages. In the ACP lineage, we see a large investment in stem tissue, and few branching events per plant. Anecdotally, in the field this plant is almost always flowering and this investment in stem tissue and propensity to not branch as much as the other two lineages could point to a heavy investment in sexual reproduction via flowering. In the HYB lineage, we see the most branching of any of the lineages, as well as the most biomass per meter. This not only makes the hybrid lineage the most invasive by our perception, but also implies that flowering is not of high reproductive priority. The HYB lineage tends to form dense mats in the lakes it invades, and it is hypothesized that this lineage may reproduce through fragmentation. We also hypothesize that the CON lineage invests in fragmental dispersal as opposed to sexual reproduction as well. This hypothesis is supported by its performance in the common garden, where each stem represented a fragment of the original culture plants. The CON lineage presented the most branching and overall growth across all treatments. Though this experiment provides some evidence for each lineages’ success as an clonal propagator, more work is still needed to assess the true modes of reproduction of these lineages.

These differences rival those we see between recognized distinct species. Since herbicides and other management regimes are often formulated to work on a very selective basis, it stands to reason that these different genetic lineages may respond differently to a variety of management practices. In order to optimize management efficacy, it may be important to know what lineage of VLM is present. For example, if the ACP lineage is investing much of its energy in flowering and establishing a seed bank to further its invasion, it may be critical for management to take place early in the growing season to prevent seedset. On the other hand, when dealing with the HYB lineage, mechanical harvesting may not be the most effective practice, due to this lineage’s propensity to fragment as a means of spread.

Prioritization of management can also be gleaned from this study. The HYB lineage appears to be the most aggressive lineage in the field, based on biomass per square meter and also its propensity to

form dense mats of entangled branches. On the other hand, the ACP and CON lineages tend to grow as smaller plants that do not branch as often.

FUTURE RESEARCH

I am currently using GIS technology to compile total lake bed area data, so I can assess differences in the area of the littoral zone that each of these lineages occupies. This “axis of invasion” will be added to the others aforementioned, and will build a clear picture of how invasive each genetic lineage is based on individual plant size, bed densities, biomass per square meter, and total bed area.

As a continuation of this study, I hope to use the environmental data collected within each lake to test the hypothesis that these lineages invade distinct environments. If clear patterns exist in the data, managers could use this information in a predictive manner to identify habitats at risk for invasion.

Works cited

- Moody, M. L. & D. H. Les (2002) Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 14867-14871.
- Moody, M. L., D. H. Les & J. M. Ditomaso (2008) The role of plant systematics in invasive aquatic plant management. *Journal of Aquatic Plant Management*, 46, 7-15.

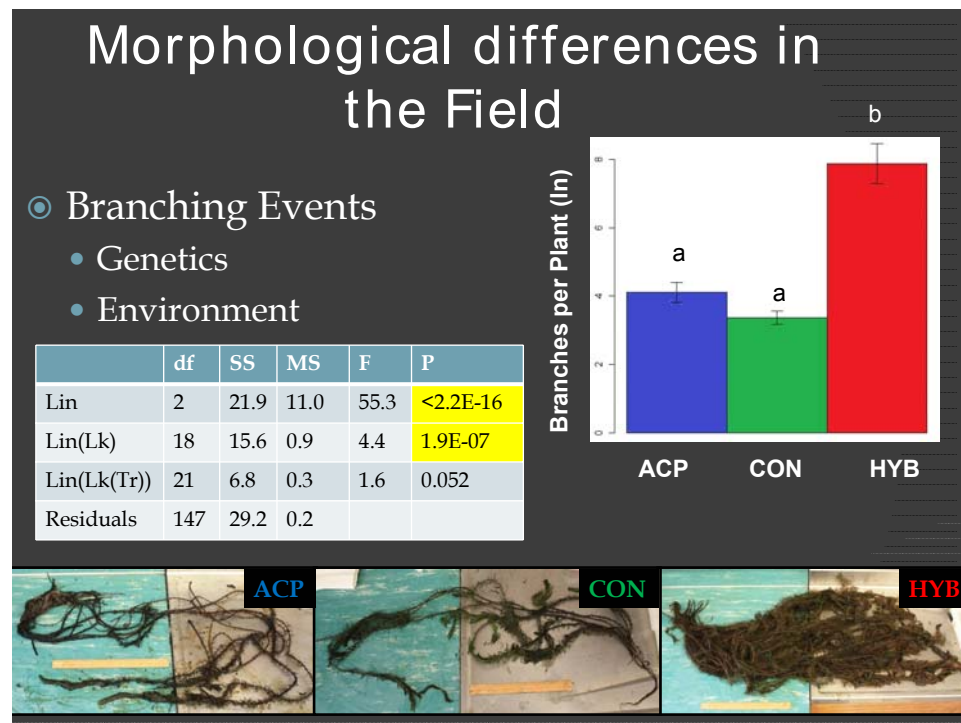
Appendix A: Names and locations of the lakes used for the field observational study organized by lineage. Asterisks denote source populations for the common garden experiment.

Lake Name	Lineage Present	City	County	State
Bryant Pond	ACP	Woodstock Twp	Oxford	ME
Carbuncle Pond	ACP	-	Kent	RI
Gorton Pond**	ACP	East Lyme	New London	CT
Pleasant Pond	ACP	Litchfield, Gardiner twps.	Kennebec, Sagadahoc	ME
Powers Lake**	ACP	East Lyme	New London	CT
Lower Yaphank Lake	ACP	Yaphank	Suffolk	NY
Lake Pattagansett	ACP/ HYB	East Lyme	New London	CT
Balch Pond/ Stump Pond	CON	Wakefield	Carroll (NH), York (ME)	NH, ME
Brindle Pond	CON	Barnstead	Belknap	NH
Contoocook Lake	CON	Jaffrey, Rindge	Cheshire	NH
Crystal Lake**	CON	Ellington, Stafford	Tolland	CT
Flints Pond**	CON	Hollis	Hillsborough	NH
Gorham Pond	CON	Dunbarton	Hillsborough	NH
Turtle Pond	CON	East Concord	Merrimack	NH
Amos Lake	HYB	Preston	New London	CT
Billings Lake	HYB	North Stonington	New London	CT
Black Pond**	HYB	Meriden, Middlefield	Middlesex	CT
Collins Pond	HYB	Windham Twp	Cumberland	ME
Gardner Lake**	HYB	Bozrah, Montville, Salem	New London	CT
Pickerel Lake	HYB	Colchester, East Haddam	New London	CT

Appendix B: The AWRI mesocosm facility during the common garden experiment.



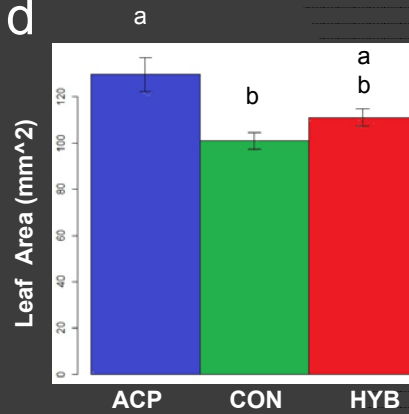
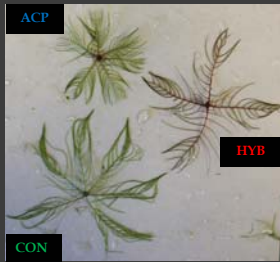
Appendix C: ANOVA table and bar graphs of each morphological and growth character measured as presented at the 2011 NEAPMS conference. Significant p-values are highlighted in yellow and significant pairwise comparisons are denoted by different letters on bar graphs.



Morphological differences in the Field

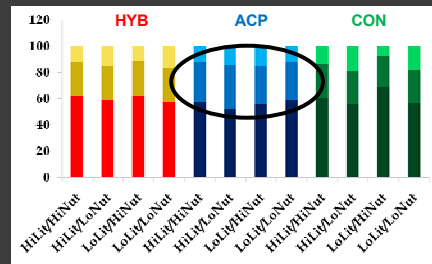
Leaf Area

- Genetics
- Environment



	df	SS	MS	F	P
Lin	2	1.4	0.7	9.4	0.0002
Lin(Lk)	18	7.6	0.4	5.9	4.8E-10
Lin(Lk(Trt))	21	4.2	0.01	1.4	0.088
Residuals	147	9.0	0.1		

Biomass Allocation Across Environments



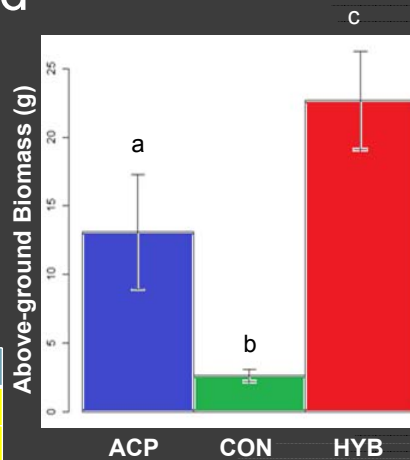
	Percent Leaf	Percent Stem	Percent Root
Lin	0.00053	5.2E-07	0.75
Trt	2.4E-05	0.36	2.86E-06
Lin(Sr)	0.06	0.87	0.034
Lin x Trt	0.003	0.16	7.8E-05
Lin(Sr) x Trt	0.043	0.051	1.1E-05

Measures of “Invasiveness” in the Field

Individual mass

- Genetics
- Environment
- Location

	df	SS	MS	F	P
Lin	2	128.6	64.3	200.3	<2.2E-16
Lin(Lk)	18	68.5	3.8	11.9	<2.2E-16
Lin(Lk(Tr))	42	53.0	1.3	3.93	1.5E-09
Residuals	126	40.4	0.32		

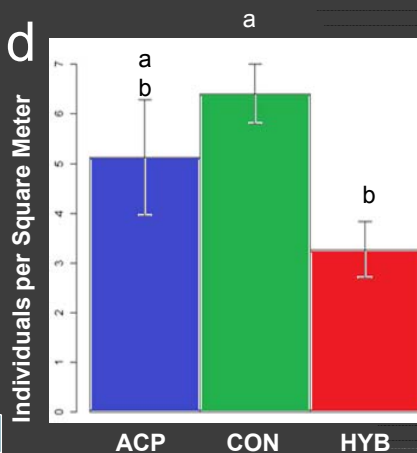


Measures of “Invasiveness” in the Field

Plants per meter

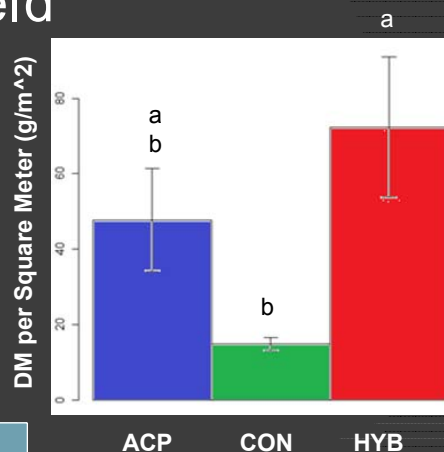
- Genetics
- Environment

	df	SS	MS	F	P
Lin	2	5.9	2.9	9.3	0.0006
Lin(Lk)	15	10.8	0.7	2.3	0.02
Residuals	36	11.4	0.3		



Measures of “Invasiveness” in the Field

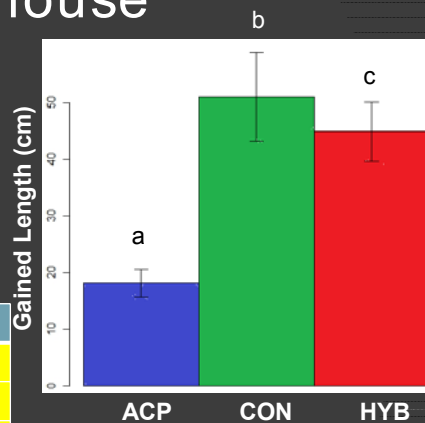
- ◉ Biomass per meter
 - Genetics
- ◉ Perception of water column “clogging”



	df	SS	MS	F	P
Lin	2	14.9	7.5	10.1	0.0003
Lin(Lk)	15	15.5	1.0	1.4	0.20
Residuals	36	26.6	0.74		

Measures of “Invasiveness” in the Greenhouse

- ◉ Total Growth
 - Genetics
 - Environment
 - Interaction



	df	SS	MS	F	P
Lin	2	25k	12k	40.7	1.6E-13
Trt	3	82k	27k	90.6	<2.2E-16
Lin(Sr)	3	7k	2k	7.34	0.0002
Lin x Trt	6	25k	4k	13.8	2.8E-11
Lin(Sr) x Trt	9	6k	653	2.17	0.031
Residuals	96	29k	1.8		