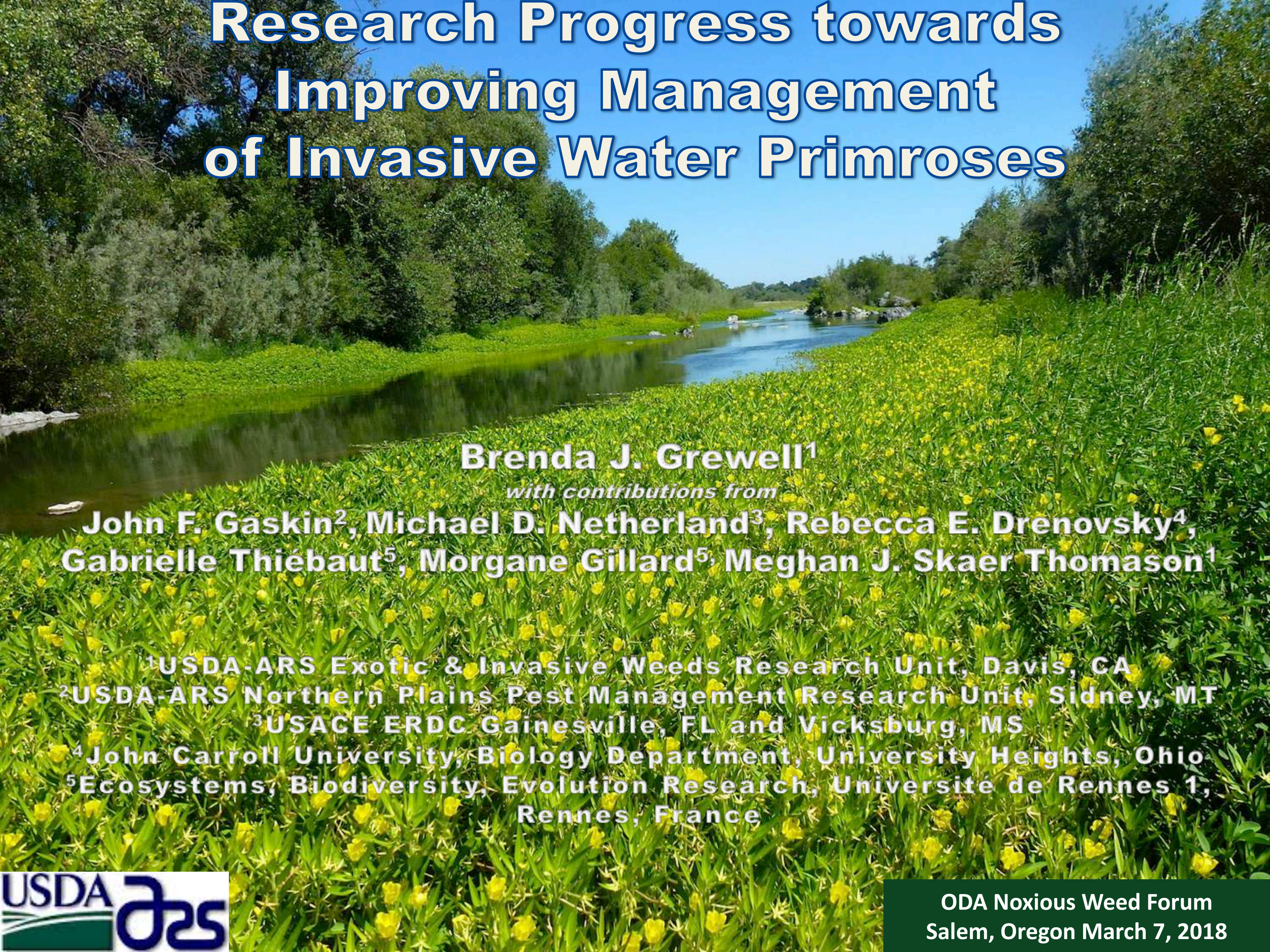


Research Progress towards Improving Management of Invasive Water Primroses

Brenda J. Grewell[1]

with contributions from

John F. Gaskin[2], Michael D. Netherland[3], Rebecca E. Drenovsky[4], Gabrielle Thiébaut[5], Morgane Gillard[5], Meghan J. Skaer Thomason[1]

[1]USDA-ARS Exotic & Invasive Weeds Research Unit, Davis, CA
[2]USDA-ARS Northern Plains Pest Management Research Unit, Sidney, MT
[3]USACE ERDC Gainesville, FL and Vicksburg, MS
[4]John Carroll University, Biology Department, University Heights, Ohio
[5]Ecosystems, Biodiversity, Evolution Research, Université de Rennes 1, Rennes, France

USDA ARS

ODA Noxious Weed Forum
Salem, Oregon March 7, 2018

Ludwigia hexapetala and *L. peploides* (Onagraceae): among world's worst invasive aquatic plants

***L. peploides* subsp. <u>*montevidensis*</u>**

2n=16; diploid

Napa River Watershed

Ludwigia hexapetala

2n=80; decaploid

Russian River Watershed

L. peploides is the ancestral diploid cytotype of aquatic *Ludwigia* sect. Jussiaea

MANAGEMENT CHALLENGE

Russian River CA

Kissimmee Chain of Lakes FL

Aggressive spread of *Ludwigia* populations is increasingly impacting aquatic and riverine ecosystems in **Pacific west states** and Florida. Economic and ecological **impacts are high**, and **ecosystem restoration projects are impacted**.

Need for research: The need for selective management approaches is growing, yet little is known about the basic biology distribution of the different invasive *Ludwigia* species, and how they each respond to varying environmental conditions in the United States.

Improved understanding of the biology and ecology of the species is critical for risk assessment and is the **foundation for developing effective management.**

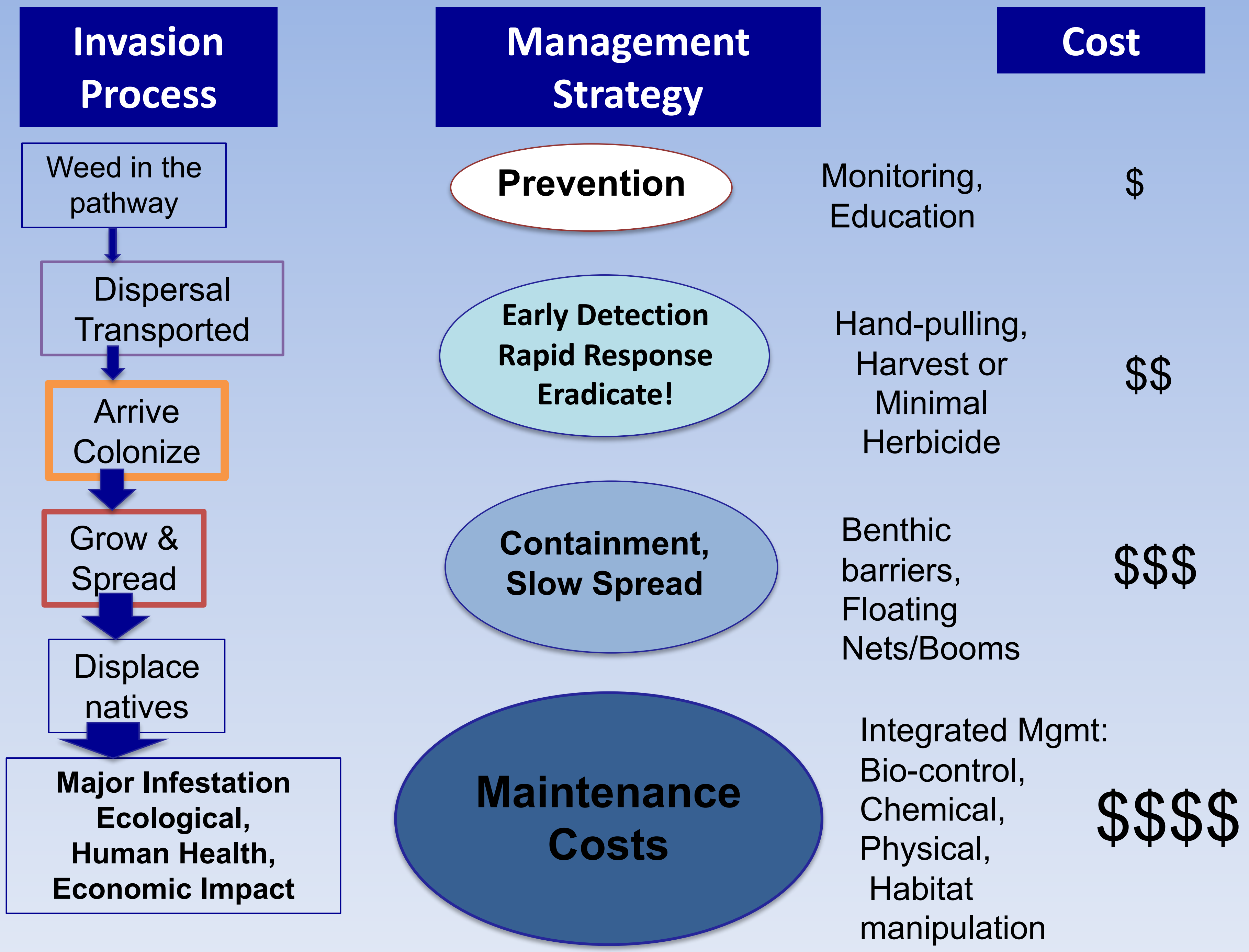
Understanding how invasions begin and progress through space and time is knowledge fundamental to managing and disrupting the process of invasion.

INTRODUCTION: Initial dispersal from the native range

ESTABLISHMENT: Colonization of self-sustaining populations. Priority stage when management if most effective

SPREAD: Increases in propagule pressure, dispersal and spatial spread. Management requires knowledge of dispersal processes

IMPACTS: Impacts of invasion on the recipient ecosystem.



Invasion Process

Weed in the pathway
Dispersal Transported
Arrive Colonize
Grow & Spread
Displace natives
Major Infestation Ecological, Human Health, Economic Impact

Management Strategy		Cost
Prevention	Monitoring, Education	$
Early Detection Rapid Response Eradicate!	Hand-pulling, Harvest or Minimal Herbicide	$$
Containment, Slow Spread	Benthic barriers, Floating Nets/Booms	$$$
Maintenance Costs	Integrated Mgmt: Bio-control, Chemical, Physical, Habitat manipulation	$$$$

Management of Aquatic Weed Invasions must be based on:

Careful Planning
Clear short term goals and long term objectives

→ **Context**
Understanding of the local hydrology/aquatic system

→ **Biology and ecology** of the specific target aquatic weeds and the resident aquatic plant community/ecosystem
Conservation/protection measures for sensitive species

Logistics
Local logistical and regulatory constraints

APPROACH
(i.e. mechanical, chemical, biological, integrated strategies)
should be based on all of the above

PROJECT OBJECTIVES

Multi-year studies 2014-present

1. **Accurate taxonomic identification of weed species is an essential first step for effective management.** Identify problematic *Ludwigia* species in Florida; compare to invasive populations in California and Oregon, and in native South American range to support biological control and integrated management strategies

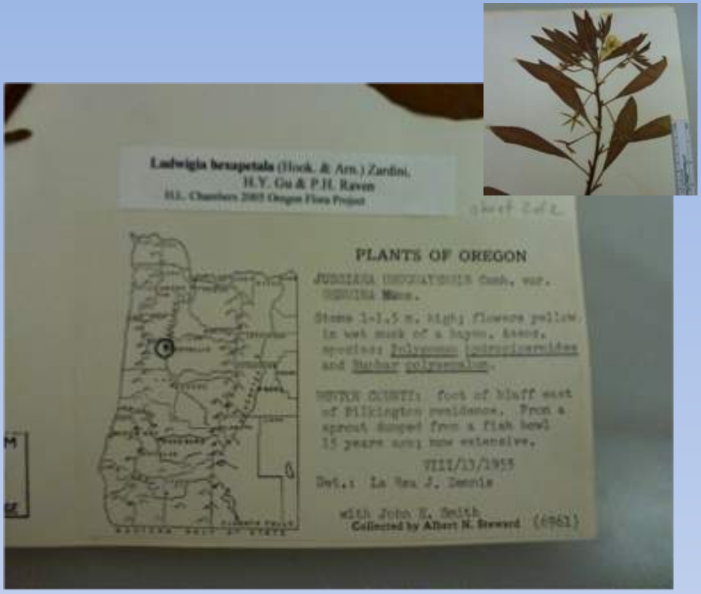
2. **Evaluate the response of *Ludwigia* cytoptypes to environmental conditions during establishment by clonal fragments**

3. **Investigate seed banks and germination ecology** and seed bank recruitment of *Ludwigia* species **under changing environmental conditions.**

4. **Investigate dispersal and patch expansion processes driving watershed invasion of *L. hexapetala*** including links between environmental factors, survival, colonization and spread

Ludwigia hexapetala
Pacific West Coast

Introduced as Ornamental & Aquarium Plant



Ludwigia hexapetala (Hook. & Arn.) Zardini,
H.Y. Gu & P.H. Raven
H.L. Chambers 2005 Oregon Flora Project

sheet 2 of 2

PLANTS OF OREGON

JUSSIAEA URUGUAYENSIS Camb. var.
GENUINA Munz.

Stems 1-1.5 m. high; flowers yellow.
In wet muck of a bayou. Assoc.
species: Polygonum hydropiperoides
and Nuphar polysepalum.

BENTON COUNTY: foot of bluff east
of Pilkington residence. From a
sprout dumped from a fish bowl
15 years ago; now extensive.

VIII/13/1955

Det.: La Rea J. Dennis

with John E. Smith
Collected by Albert N. Steward (6961)

Herbarium Record:
1955 (1949)
Corvallis, OR
(Extant)

Herbarium Record: **1956** Solo Slough WA **(Extant)**
1981 Eugene OR **(Extant)**

Blue Lake, Humboldt County CA: **1949** (Historic habitat destroyed, wetland filled)

Russian River Basin California 1st Record: 1960 **(Extant)**

Tiburon, California 1st Herbarium Record: **1945**

(Historic habitat destroyed)

San Diego, California

1st Herbarium Record: **1940** **(**Historic habitat destroyed, former pond now under a shopping center)

Can you identify the *Ludwigia* species in each photo?

Ludwigia Invasions: Implications for Management and Restoration


Riverine wetlands,


Rice Fields


Canals

Successful Colonization & Spread - Wide Range of Habitats

Oxbow Lakes


Managed Seasonal Wetlands (NWRs)

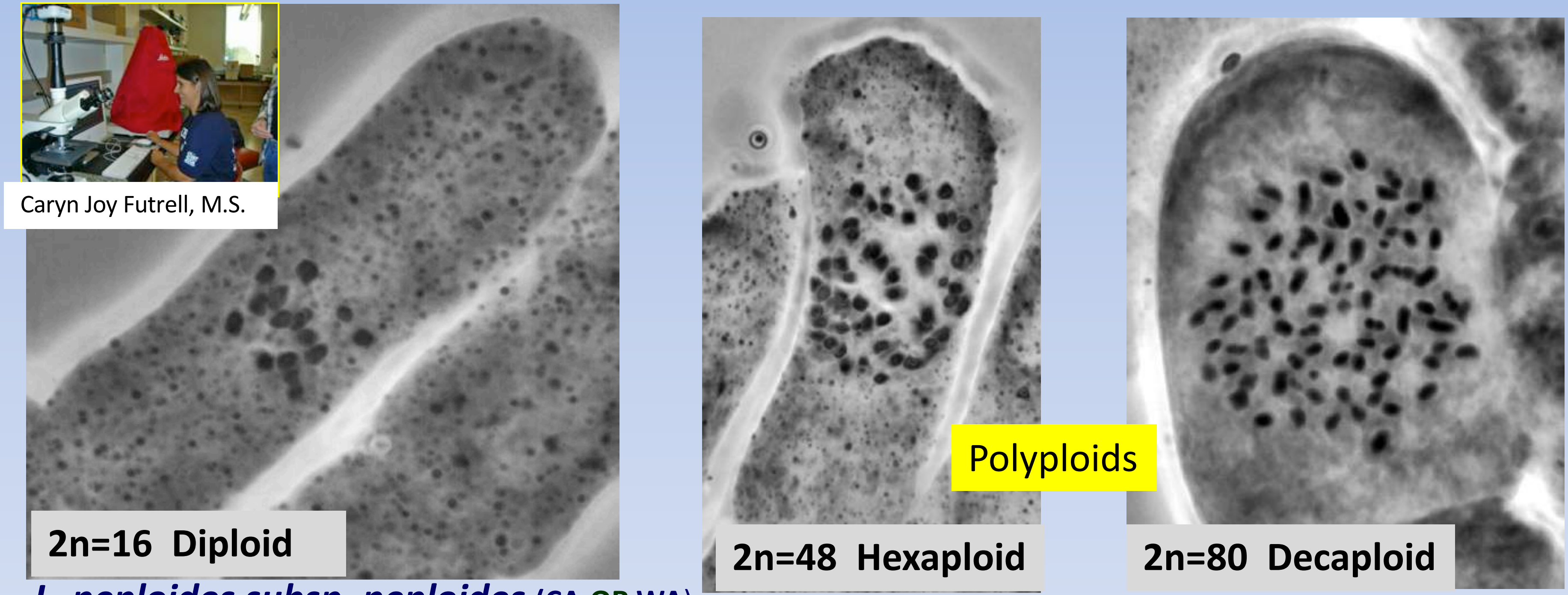

Floodplain Wetlands

Broad Ecological Tolerance
Phenotypic Plasticity

Purpose: Accurate taxonomic identification of weed species is an essential first step towards development of effective management strategies and supporting research.

- Evaluated chromosomes
- Morphometric Analyses
- Molecular Analysis & Ecology
- **Revised taxonomic treatments**

Photomicrographs (1,000x) of mitotic chromosome preparations:


Caryn Joy Futrell, M.S.

L. peploides subsp. peploides **(CA,OR,WA)**
L. L. p. subsp. montevidensis **(CA,OR,WA)**
L. decurrens **(2010, Butte C. CA ricefields)**

L. grandiflora **(?)**
(s.CA)

L. hexapetala **(CA,OR,WA)**

Cytological RESULTS: Three Chromosomal Races, 5 Taxa Pacific West Coast

*** Management Challenge ***

Distribution of Chromosomal Races *Ludwigia* sect. Jussiaea populations Pacific West

Ludwigia section ~~Oligospermum~~ **Jussiaea** (L.)*

● Creeping water primrose

Ludwigia peploides (Kunth) P. H. Raven subsp. *peploides*

Ludwigia peploides (Kunth) P. H. Raven subsp. *montevidensis* (Spreng.) P. H. Raven

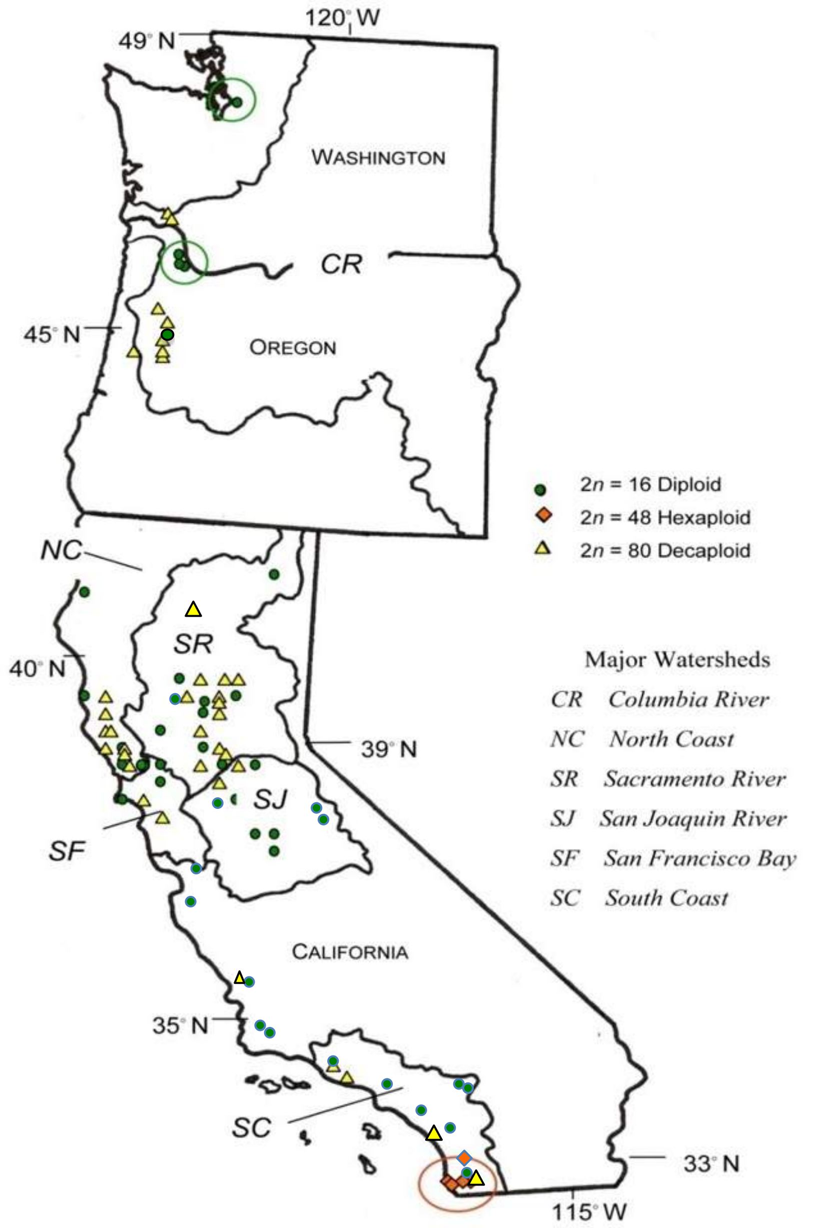
◆ Large-flowered primrose-willow
L. grandiflora

△ Uruguayan primrose-willow

Ludwigia hexapetala (Hook. & Arn.) Zardini, H. Y. Gu & P. H. Raven

Ludwigia section **Pterocaulon**

Winged primrose-willow
Ludwigia decurrens Walter



Peter Hoch, MBG

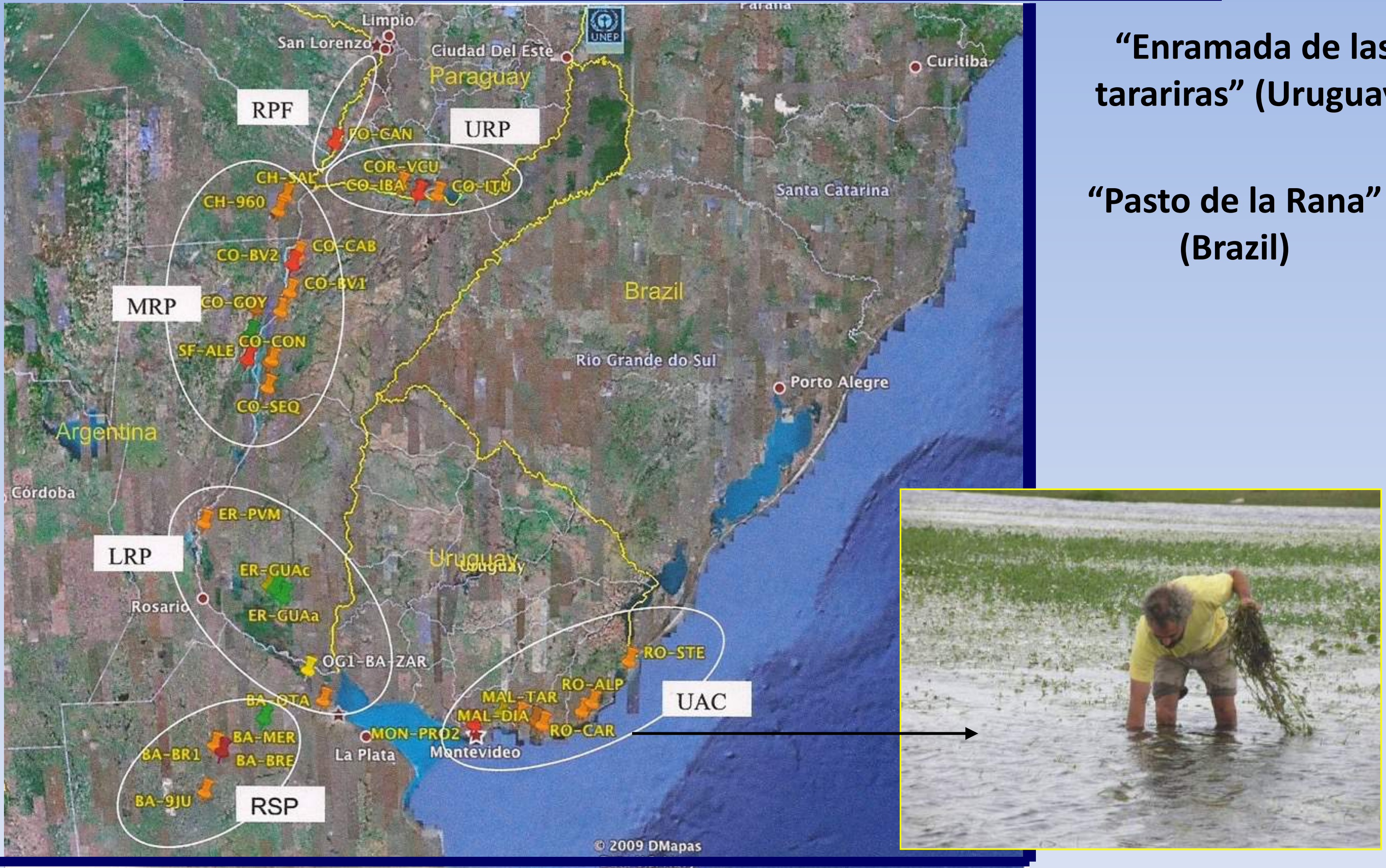
*Hoch PC**, Wagner WL, Raven PH. **2015.** The correct name for a section of *Ludwigia* L. (Onagraceae). PhytoKeys 50: 31–34.

Hoch PC, Grewell BJ. 2012. Onagraceae: *Ludwigia*. pp 948-949 In: Baldwin (ed.) Jepson Manual: Higher Plants of California, 2nd Edition, University of California Press.

Grewell BJ, Hoch PC. 2013. *Ludwigia* (Onagraceae). *In press*: Vol 3. Taxonomic treatment for *Oregon Flora*, OregonFlora Project

Ludwigia populations sampled for molecular comparison - Native Range

Research support, evaluation of biological control agents



"Enramada de las tarariras" (Uruguay)

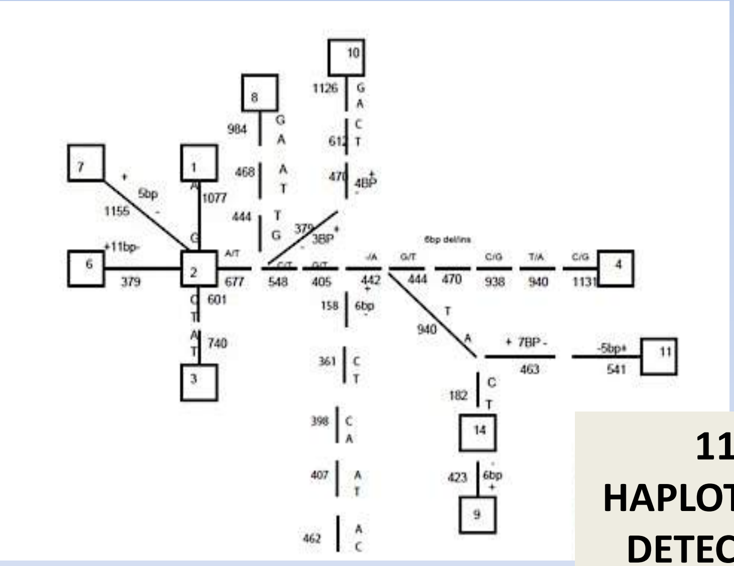
"Pasto de la Rana" (Brazil)

USDA United States Department of Agriculture
Agricultural Research Service
ars Your Future Grows Here

UCDAVIS
PLANT SCIENCES

Identify problematic *Ludwigia* species using chromosome numbers and molecular methods

Molecular Analyses *in progress* support species identification and origin.
Chloroplast DNA was sequenced to distinguish haplotypes (haploid genotypes) from inherited genes and lineage relationships among samples. Data are being compared to AFLP and cpDNA data from invasive *Ludwigia* spp. in Florida, California, **Oregon** and South America.



11 HAPLOTYPES DETECTED

Chloroplast DNA Sequences

All sampled octoploid and decaploid genotypes from FL, AL, CA, OR are the same haplotype, and the same haplotype as several samples evaluated from multiple populations in Argentina and Uruguay.

Three haplotypes were detected from **diploid** samples

Hexaploid samples are represented by at least **two haplotypes** with close relationship to samples evaluated from Argentina, Uruguay and/or a subset of Florida and California populations.

Preliminary Results: AFLP Genotypes

DNA extraction from plant tissue and **AFLP genotyping** was performed, and in progress: **ITS marke**rs are being evaluated now to further elucidate phylogenetic relationships and assess potential recent hybridization.

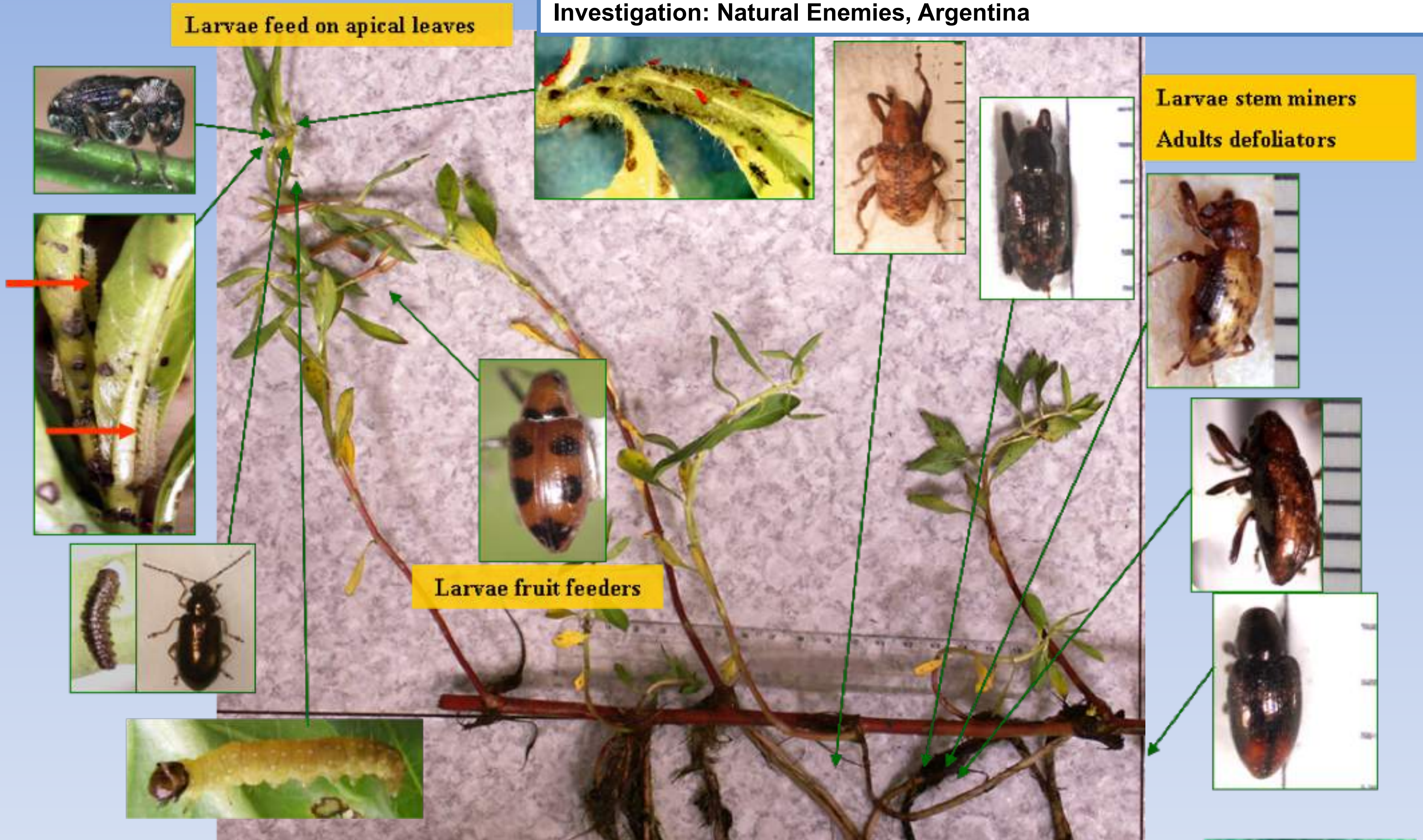
AFLP markers evaluated for n = 351 *Ludwigia* plants; 48 genotypes detected
Genotypes cluster by ploidy levels.

Decaploid *L. hexapetala* is most prevalent (n=182, 17 genotype. 96 specimens from Alabama (coll. N Harms), Florida and California are a single genotype that **will be evaluated for host specificity with potential biological control insects**.

Sampled **diploid *L. peploides* taxa have highest genetic diversity** (n=82, 22 genotypes)

Newly detected octoploid hybrids in Florida, **and all hexaploid (i.e. samples *L. grandiflora*)** will be further evaluated to assess potential recent hybridization and to resolve uncertainty in identification and origin.

Investigation: Natural Enemies, Argentina



USDA - ARS
SABCL
South American Biological Control Laboratory

Dr. M. Cristina Hernández, Dr. Guillermo Cabrera Walsh
Foundation for Study of Invasive Species, Argentina

Liothrips ludwigi Zamar, sp. nov. (Thysanoptera: Phlaeothripinae).

Ludwigia hexapetala

Attempts to Import for quarantine testing in progress: Funding coalition needed to support study

Paul Pratt, ARS Albany

BIOMASS ALLOCATION DYNAMICS

Allocation & recycling of biomass are fundamental aspects of plant competitiveness and invasive characteristics of aquatic plants.

Objective: Evaluate seasonal variation in growth, biomass allocation, and C storage capacity for regrowth patterns of biomass production and C storage reserves of *Ludwigia hexapetala* across water depth and nutrient availability gradients *to identify weak points in life cycle for targeted management*

METHODS

4 River Reach Sites X 6 Seasons X 4 water depths X 5 random transects

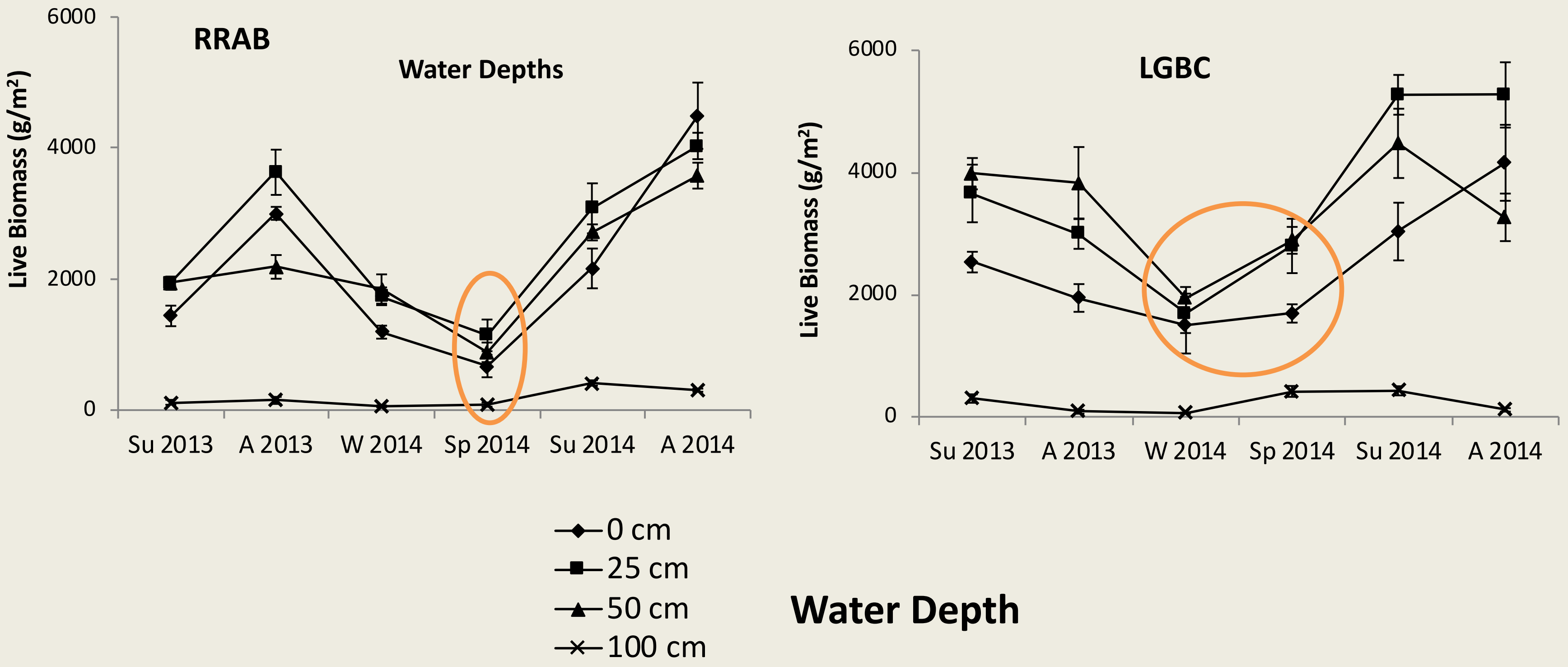
5 Random Transects per each River Site

Sample biomass along water depth gradient: * 0, 25, 50, 100 cm water depth;
* above water, in water column, below ground/sediment

Analyses: Plant biomass allocation, tissue nutrient concentrations,
Root/Rhizomes: total nonstructural carbohydrates
Water Quality, Sediment Nutrients

Partial RESULTS –

2. BIOMASS ALLOCATION DYNAMICS



Total Live Biomass of *Ludwigia hexapetala*)
Above Water Line (0cm) and at Three Water Depths

RRAB *= Russian River at Asti;*
LGBC *= Laguna de Santa Rosa at Blucher Creek*

n = ~2,500 plant biomass samples (all sites) collected, processed by anatomy, weighed

Seasonal changes at a biomass allocation field site, Russian River near Asti (Middle Watershed)


Winter 2014


Spring 2014

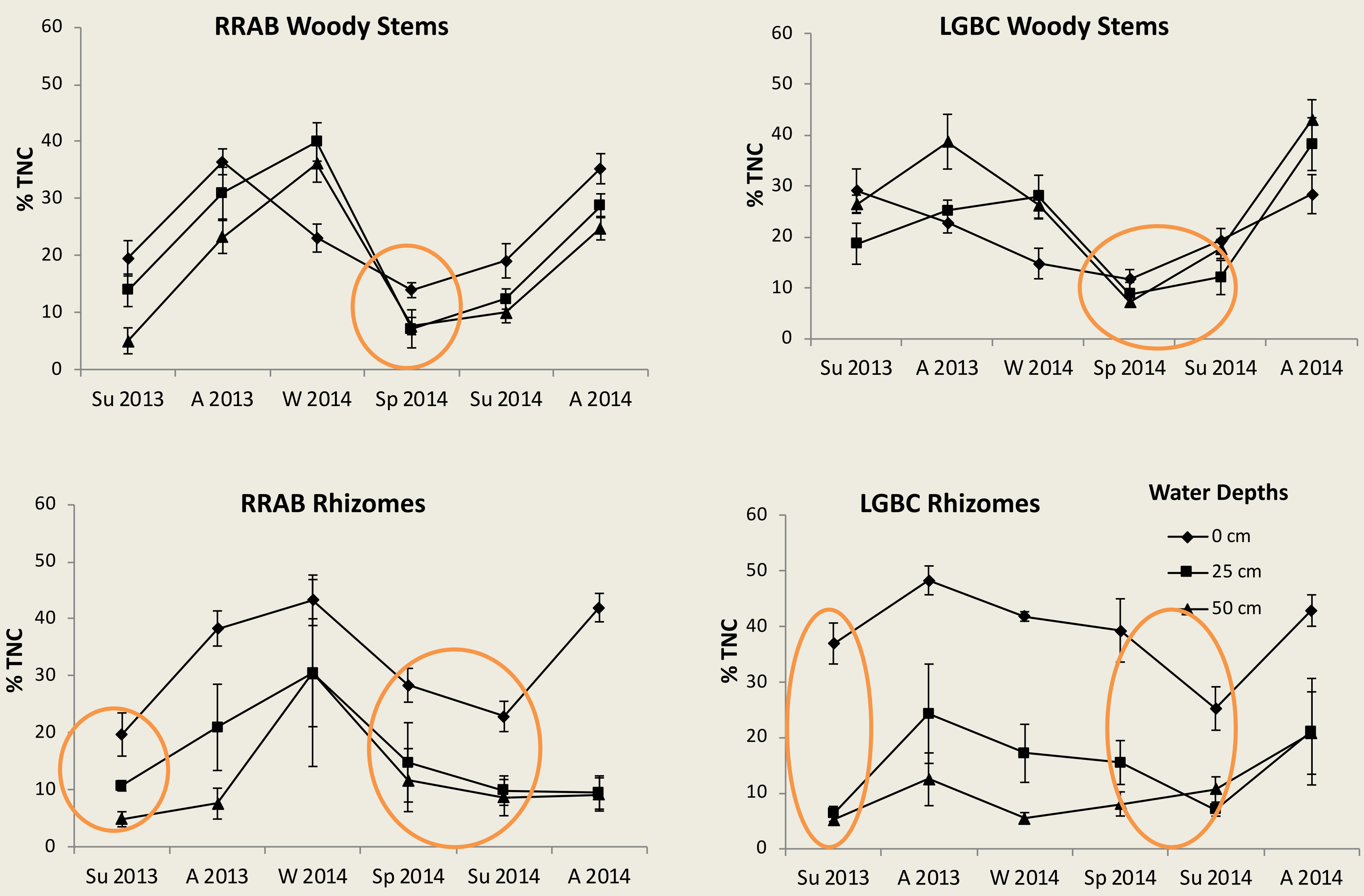

Summer 2014


Autumn 2014

C Storage Supports Resprouting Capacity

n=720 samples

Nonstructural Carbohydates (Storage Capacity/Reserves)



RRAB = Russian River at Asti

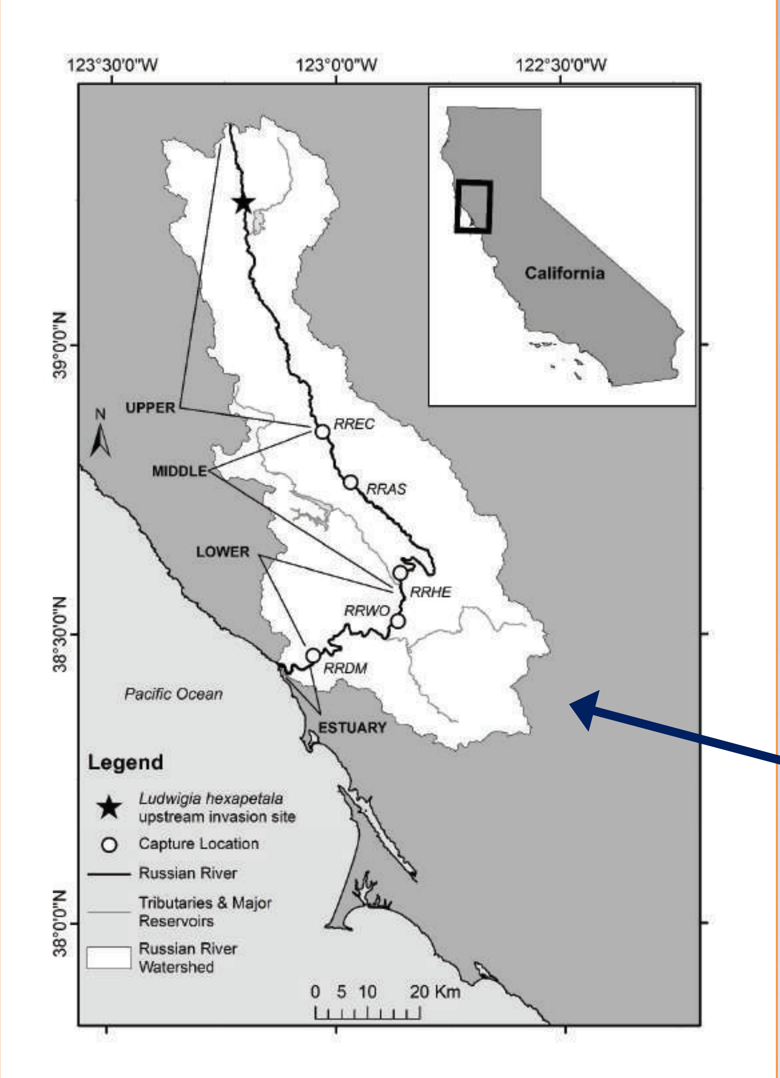
LGBC = Laguna de Santa Rosa@Blucher Creek

Spread rapidly by hydrochorous dispersal of asexual fragments or buoyant capsules

Okada, Grewell, Jasieniuk 2009 *Aquatic Botany* 91:123-129

Temporal and nonlinear dispersal patterns of *Ludwigia hexapetala* in a regulated river

Capture Sites Russian River



Evaluate factors associated with dispersal of clonal shoot fragments via hydrochory

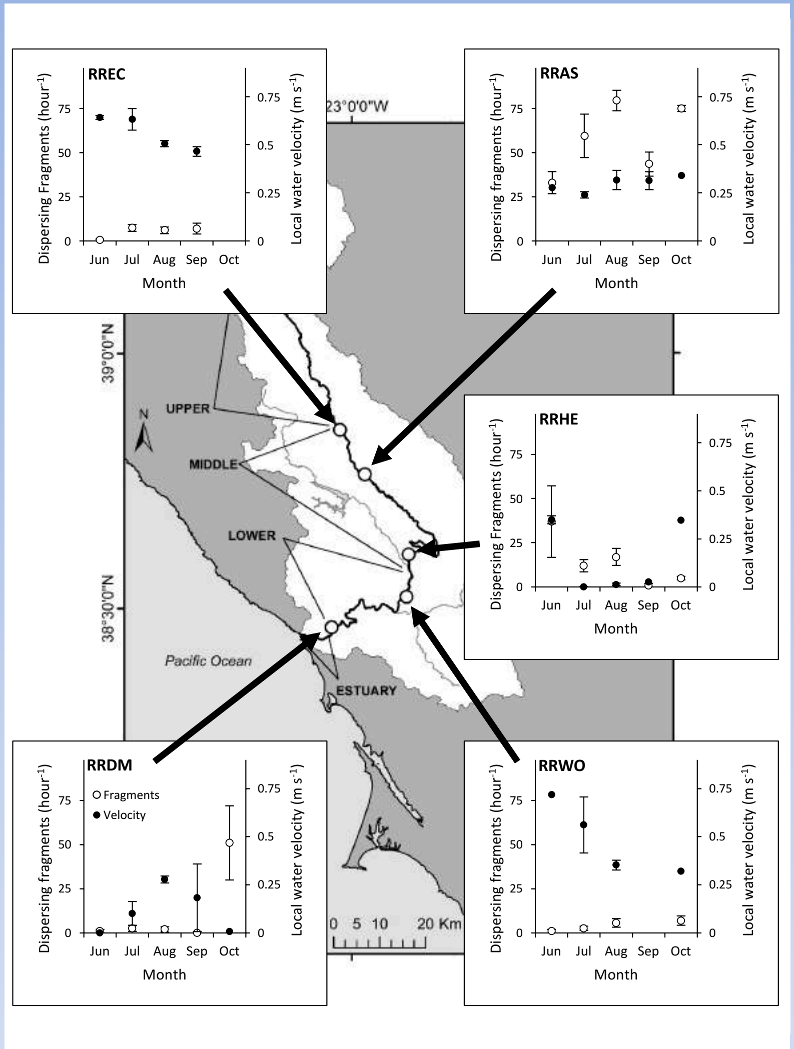
- **Time:** month, season
- **Hydrology:** flow, velocity
- **Watershed context:** reach, propagule pressure

Meghan Skaer Thomason

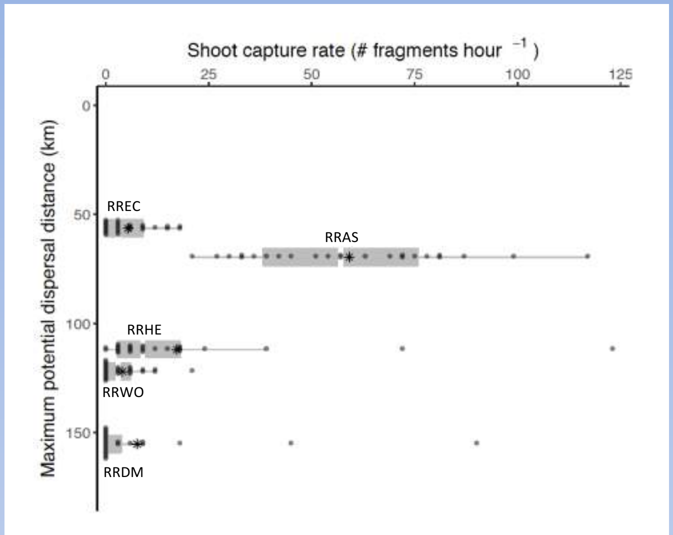
Timed collections of floating shoot fragments:
9 surveys, 5 months

5 survey sites
1 upper, 2 middle, 2 lower river
cross-section transects

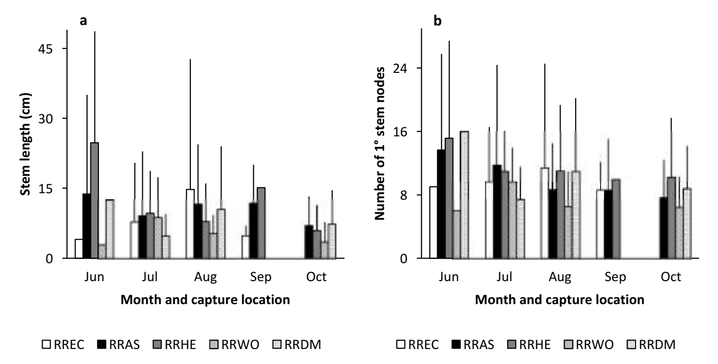
3 to 6 Collectors (to capture river width)



Dispersing propagule pressure was nonlinear: more shoot fragments captured in middle river reach where total abundance and disturbance highest, rather than lower river.



Highly variable capture counts suggest importance of pulse disturbance events initiating local dispersal



Estu
sho
into
inva

Captured fragments in the middle river were 2X longer than fragments captured in lower river and bore 83% more stem nodes, characteristics associated with greater establishment success.

Results support development of spatially targeted management, outreach, and prevention efforts that could lead to decreased dispersal and spread of asexual propagules

Mechanisms enabling establishment promote effective recruitment & reproduction

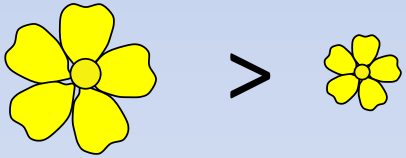
How can we use these functional traits to improve management strategies?

Does polyploidy promote a growth advantage?

Whole genome duplication:

- Increased genetic diversity and plasticity
- Promotes success of invasive species

Leading us to predict…



Decaploid *L. hexapetala*
will out-perform diploid *L. peploides*

Superior ability to maximize resource uptake & use

Vegetative regeneration of invasive Ludwigia cytotypes from clonal bud banks across resource gradients

Phase 1: Response of colonizing shoot fragments to contrasting light and nutrients

Full factorial experiment in blocked, split-plot

2 ploidy levels (diploid, decaploid *Ludwigia* spp.)

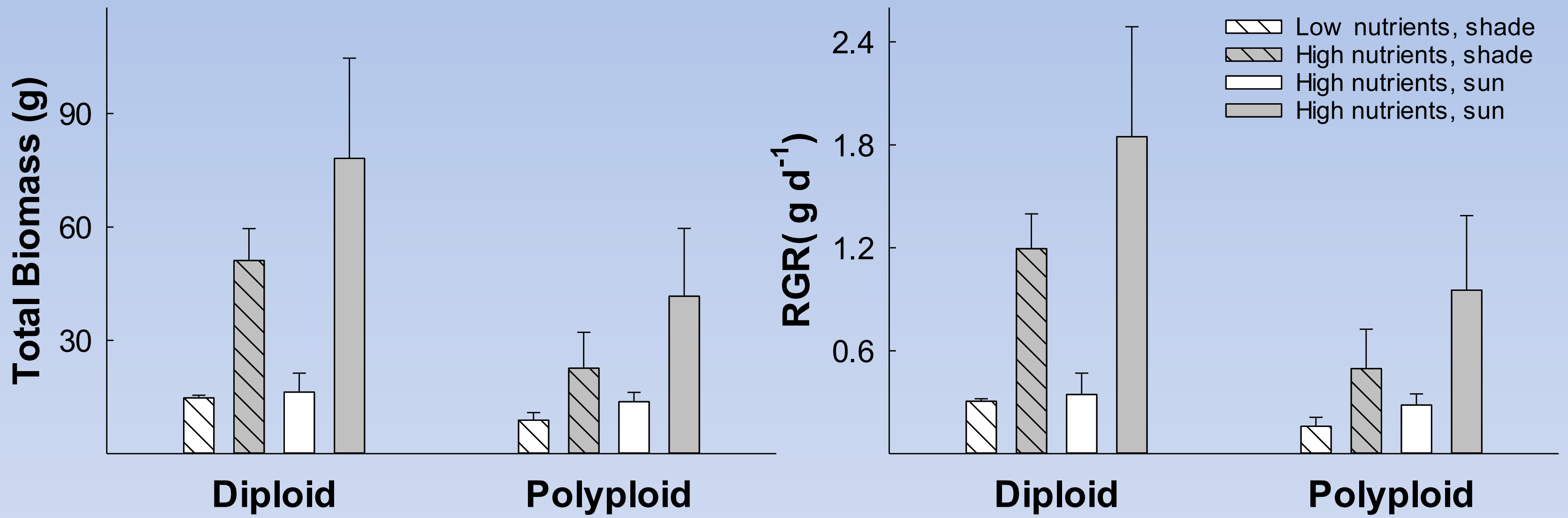
2 light regimes (full sun, 80% shade)
2 soil nutrient levels (high, low)


Week 1


Week 6

Grewell & Drenovsky

Phase 1: Counter to predictions, diploid outperformed decaploid



Irrespective of light availability:

Diploid produced more biomass, had higher RGR, and flowered earlier

Alternate strategy for establishment & success

More results: **Grewell et al. 2016. AoB Plants; doi: 10.1093/aobpla/plw014** **OPEN ACCESS**

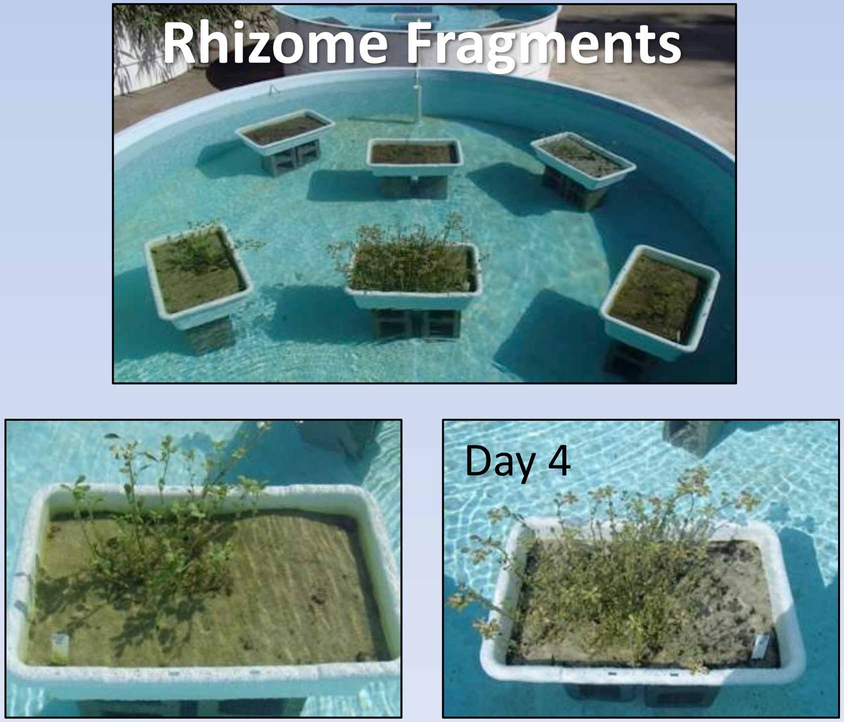
Phase 2: What about role of woody rhizome fragments—resprouting from bud bank?

Rhizome fragmentation: spurs sprouting of dormant buds in many clonal species, including *Ludwigia*

Rhizome fragments generated via disturbance (bank erosion, wild pig rooting, excavation etc.

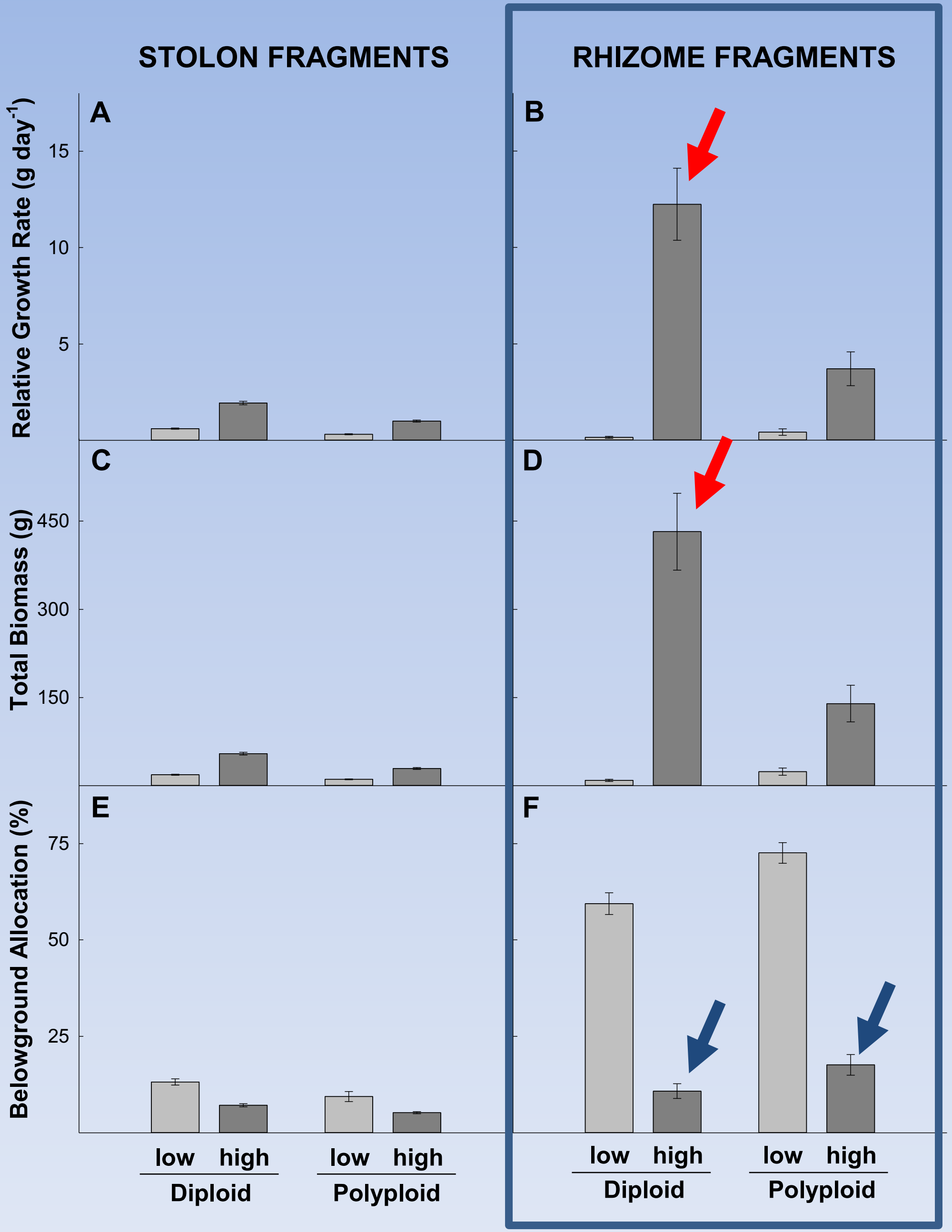
Phase 2: Response of shoot and rhizome fragments to contrasting nutrients

Two consecutive, fully factorial experiments—manipulating cytotype and sediment nutrients



Growth patterns

1. Nutrient response greatest from **rhizomes**
2. **Diploid cytotype** grew faster and larger
3. Shift in **biomass allocation**

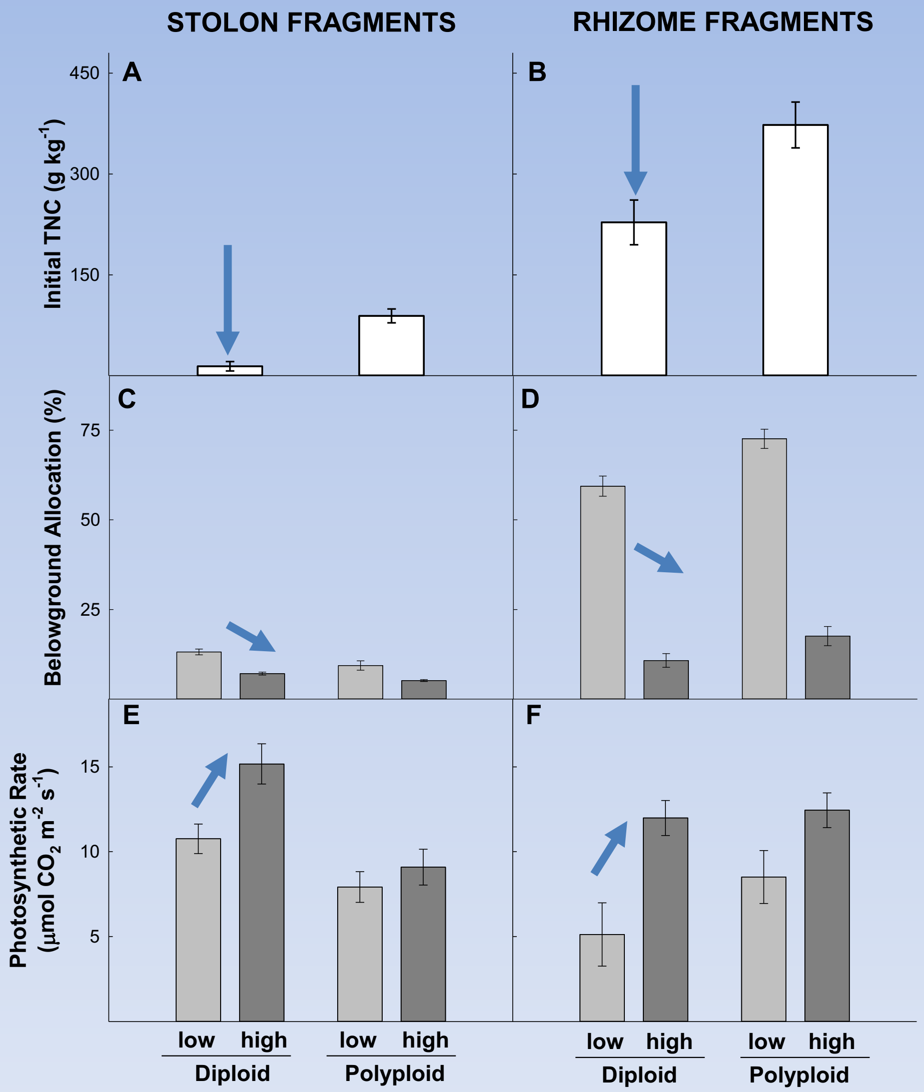


Means (± 1 S.E.)

Architectual Traits:
Polyploid: longer 1^0 Shoot
Diploid: more branching

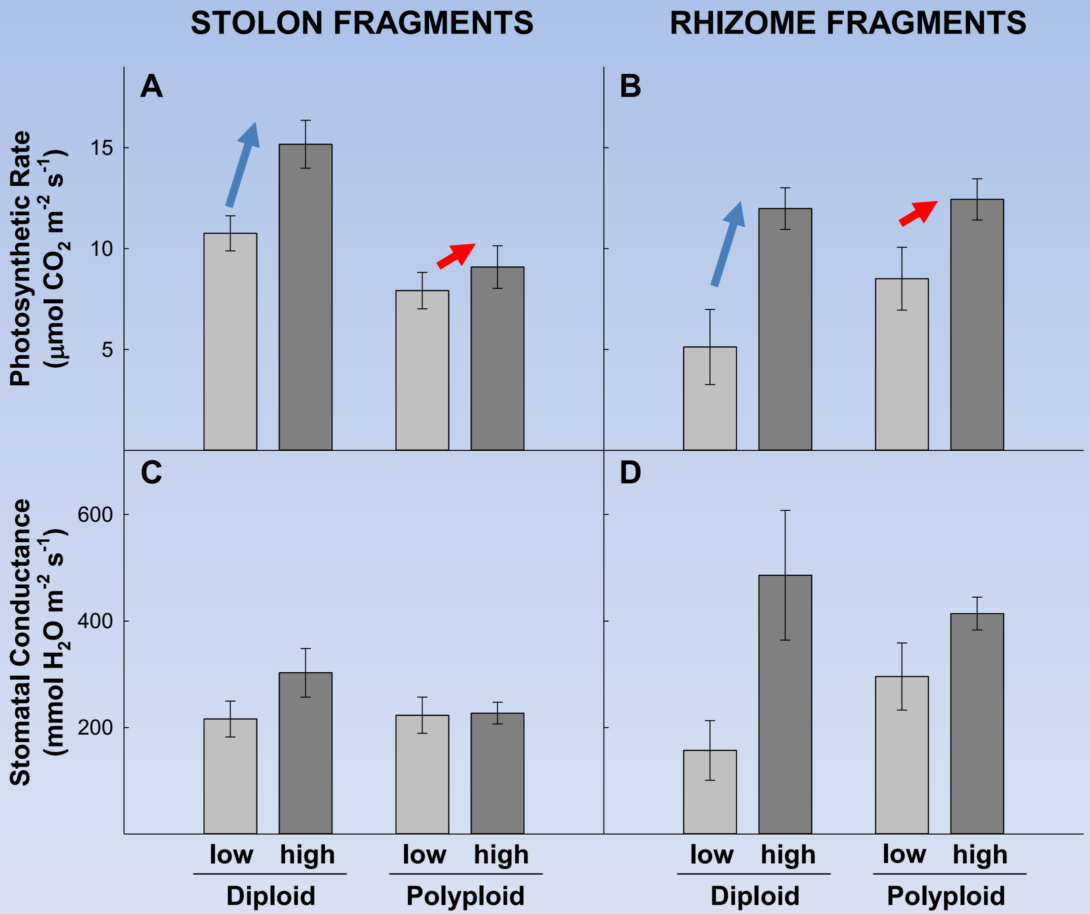
Which traits support this superior growth?

1. **Efficient use** of stored non-structural carbohydrates
2. **Shift** in biomass allocation
3. **Stronger increase** in C gain



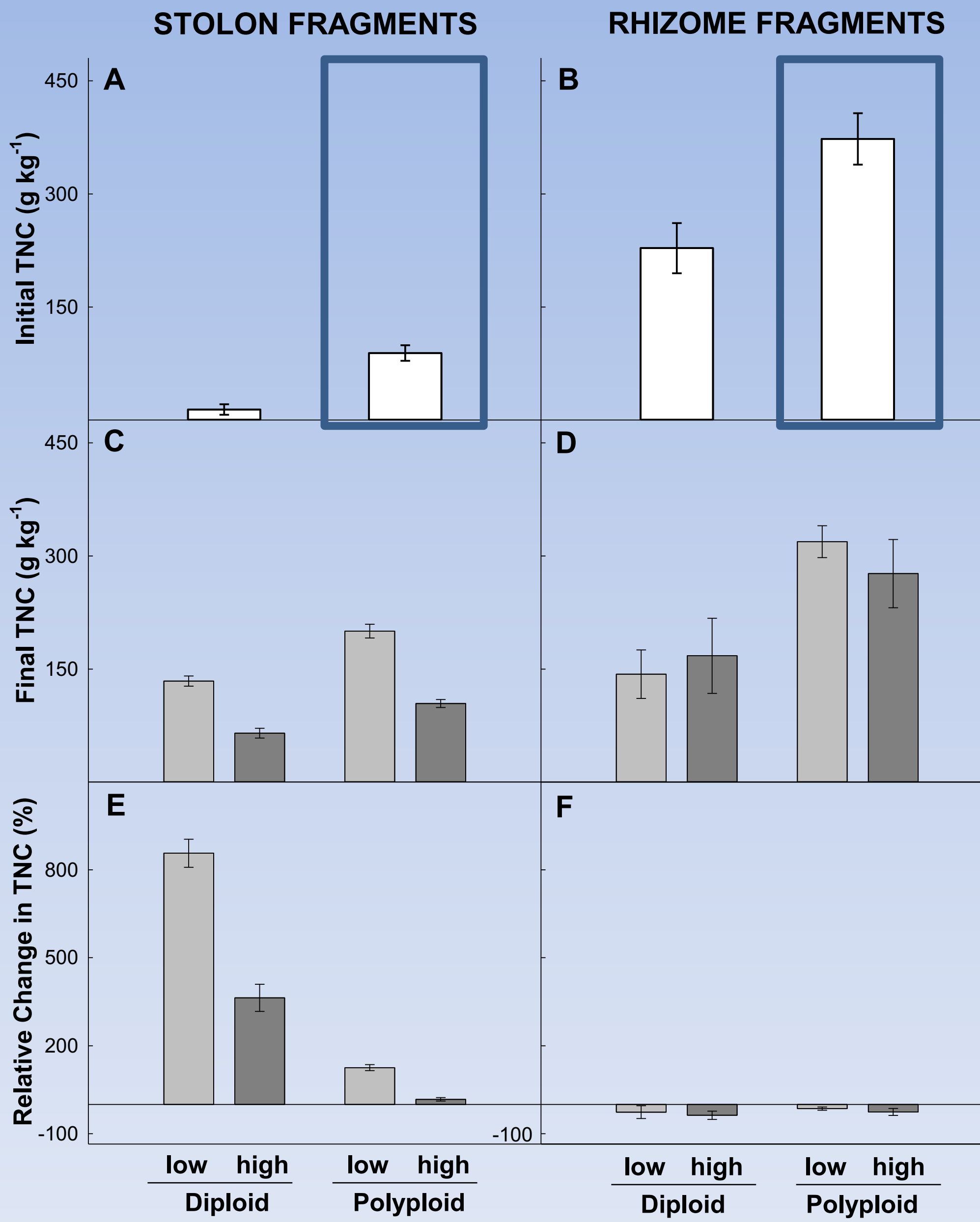
Physiology

1. Higher rates with **increased** sediment nutrients
2. Response greater in **diploid**



Carbon storage

1. Polyploids had **2X** initial TNC
2. BUT, **diploids more efficient** at using it for biomass production

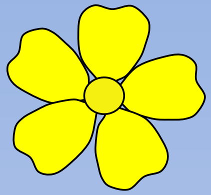


Conclusions

1. Expectation of polyploid superiority **not supported**
2. **Diploid** has greater ability to maximize resource uptake, use, & allocation across resource gradients at **colonizing phase**

Management implications

1. Prioritize **rapid response** to newly colonizing diploid invaders
2. Focus on **reducing nutrient loads**, particularly in areas with diploid congener



Seed Germination Of *Ludwigia* Species Under Changing Environmental Conditions

Freshwater aquatic ecosystems are at risk and vulnerable to biological invasions and climate change.

Many macrophytes rely on clonal reproduction to spread, but also allocate resources to seed production

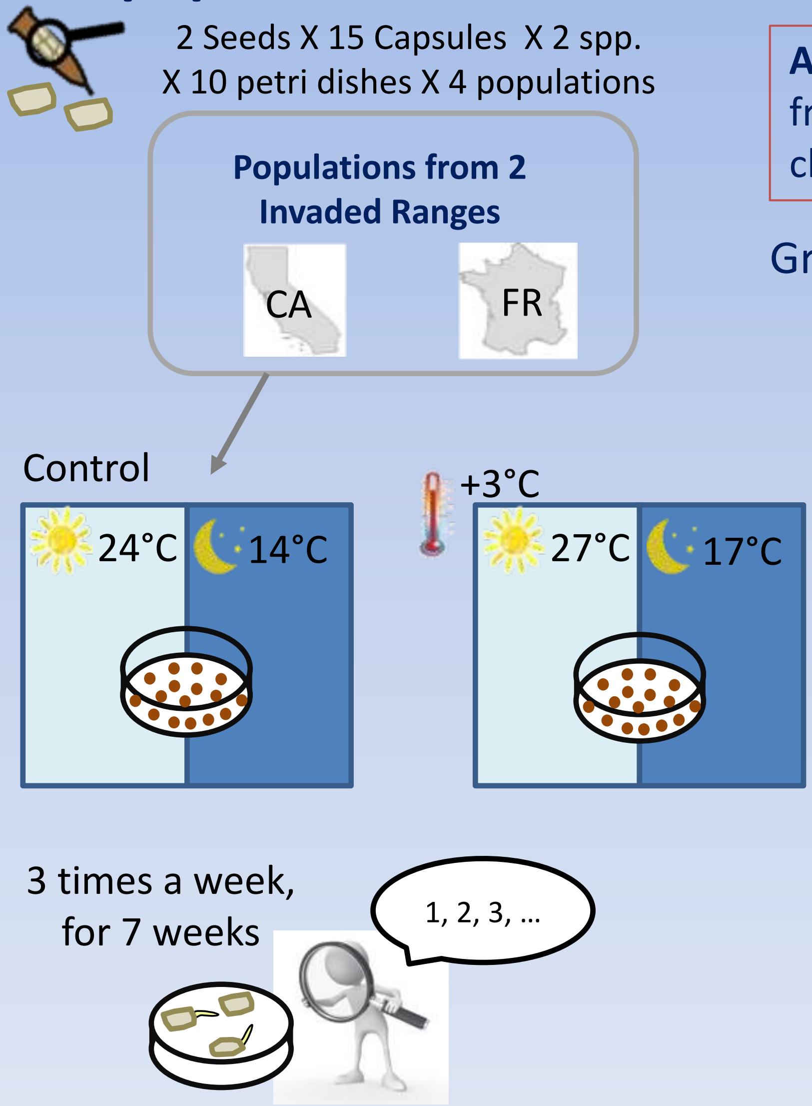
Recruitment via sexual reproduction is predicted to be increasingly more important with changing climate in riverine wetlands.

Understanding recruitment response to environmental temperature increase is needed
for management and restoration under changing conditions

Climate warming and water primroses: germination responses of populations from two invaded ranges.

Aim: test germination responses of *Ludwigia* populations from California and France to 3°C warming predicted in climate change models.

Growth Chamber Experiment, University of Rennes



2 Seeds X 15 Capsules X 2 spp.
X 10 petri dishes X 4 populations

Populations from 2 Invaded Ranges

Control

+3°C

3 times a week,
for 7 weeks

Key Results:

1. Sexual reproduction can contribute to invasiveness of *Ludwigia* spp.

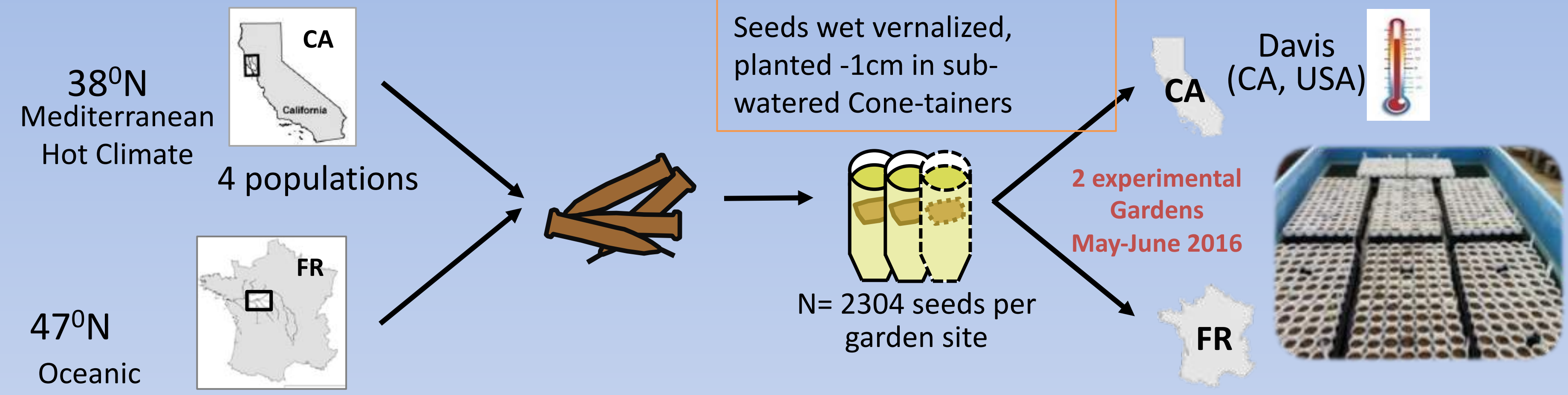
2. Regardless of temperature, germination rates were > 80% faster and higher for *L. hexapetala* from California, and for 2 populations of *L. peploides* from France

3. Germination capacity will be maintained with 3°C temperature rise

Gillard M, Grewell GJ , Deleu C, Thiébaut G. 2017. Aquatic Botany 136:155-163

Outdoor Seed Germination Experiments in Contrasting Climates

Aim: test the germination capacity and the seedling growth of ***Ludwigia hexapetala*** and ***L. peploides* ssp. *montevidensis***, invasive in USA and Europe



38^{0}N
Mediterranean
Hot Climate

4 populations

47^{0}N
Oceanic
Climate

4 populations

Seeds wet vernalized, planted -1cm in sub-watered Cone-tainers

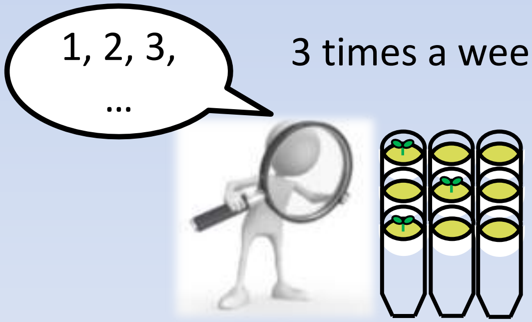
N= 2304 seeds per garden site

2 experimental Gardens May-June 2016

Davis
(CA, USA)

Rennes
(France)

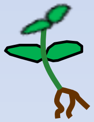
2 taxa X 4 populations X 2 climates



3 times a week

Seedling emergence assessed

After 47 days:- Test of embryo viability for ungerm



Seedlings shoots and roots
- length
- dry biomass

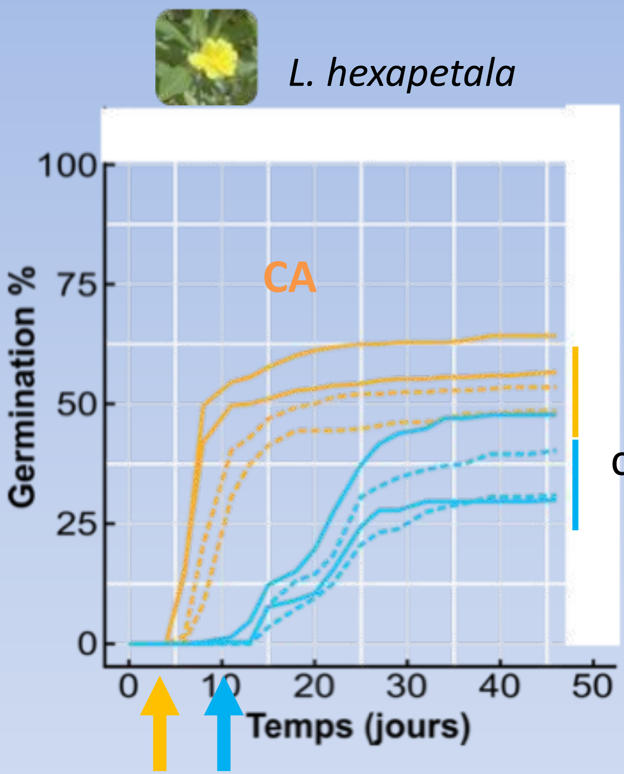
Cumulative germination percentage, survival and growth of seedlings

Reciprocal transplant of seeds of the two taxa from two invasive ranges into two experimental gardens characterized by Oceanic and Mediterranean-type climates.

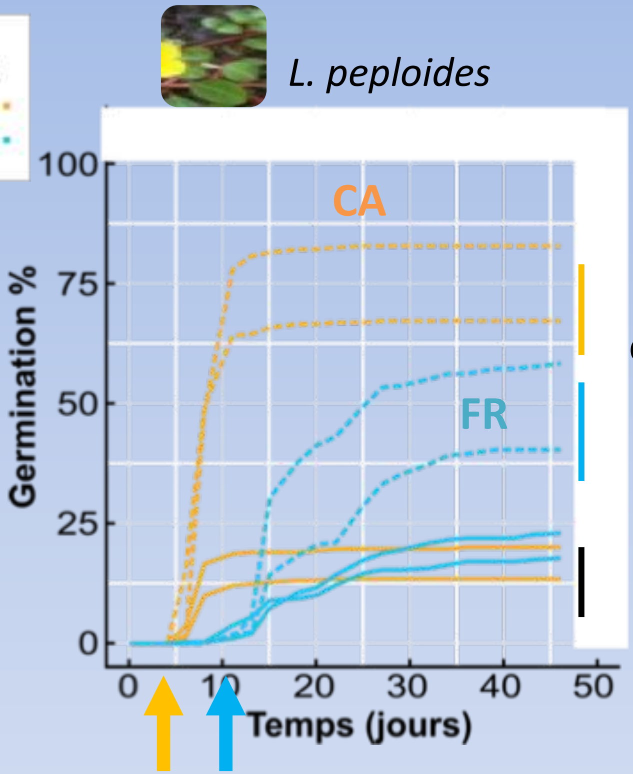
Speed and Germination Rate

Vitesse et taux de germination

The invasiveness of water primroses in ranges with temperate Oceanic climates may increase as global temperatures rise



effect climate



effect climate
+
effect provenance

Germination % of all pops. LUHE was highest in CA.

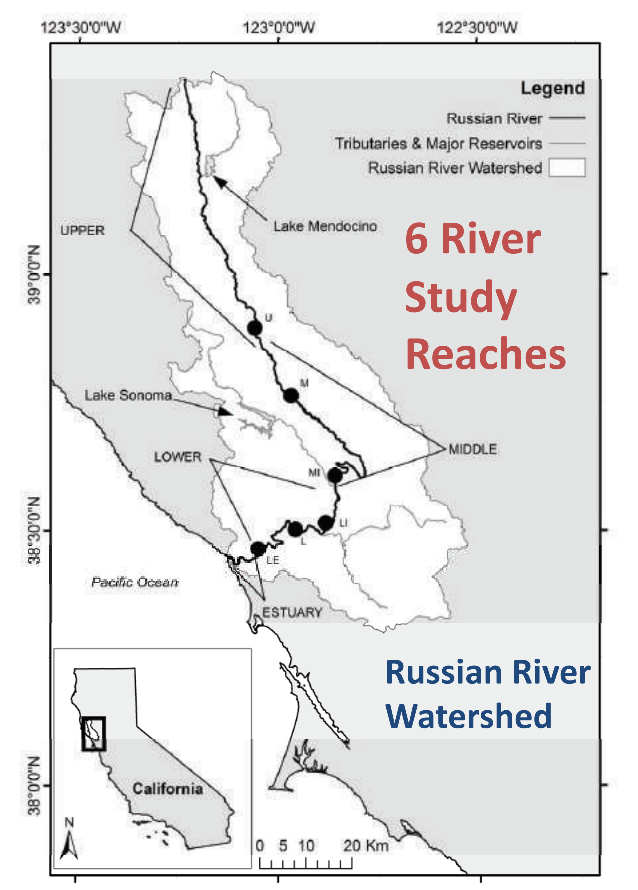
Higher temperatures increased or maintained germination %s and velocity, but…

Seedling survivorship decreased at higher temperature. However, surviving seedlings produced greater biomass at higher air temperatures

Population origin of the seed had low impact on *L. hexapetala* responses to temperature, but greatly influenced germination of *L. peploides,* with 3.3-fold higher germination of seeds from France under higher air temperatures.

Gillard et al. 2017. *Frontiers in Plant Science* 8:Article 1677. Open Access

Dynamics of Ludwigia hexapetala invasion at three spatial scales in a regulated river



6 River Study Reaches

Russian River Watershed

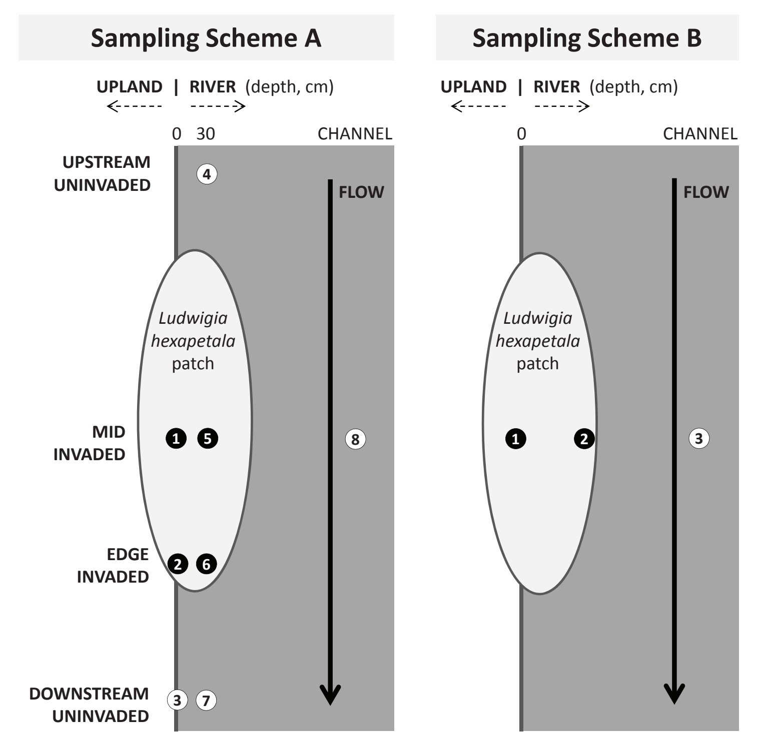
Russian River, California

- Managed river with two major reservoirs & several seasonal instream impoundments
- *L. hexapetala*
  - Earliest record in CA – 1940
  - Earliest record in Russian River watershed – 1970
  - Invasion now from upper river into estuary.

Clonal fragments disperse by hydrochory, but factors associated with expansion of established population patches are unknown

WHAT DRIVES INVASION? DRIVERS MAY VARY WITH SPATIAL SCALE OF STUDY

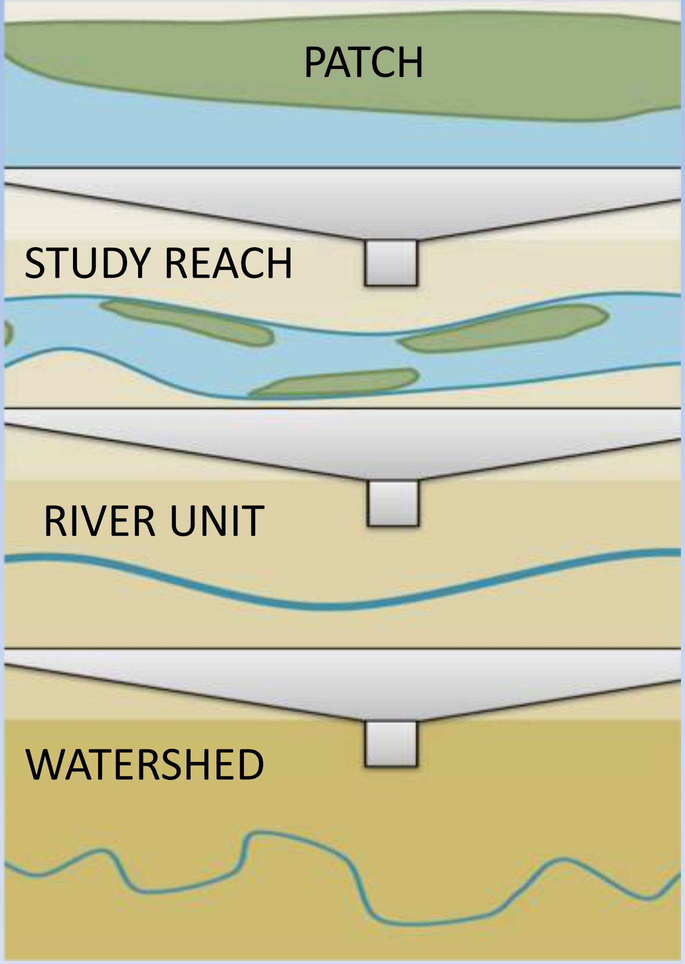
Study region



Sample locations: **A)** patch-scale, **B)** reach scale studies evaluating *L. hexapetala* distribution & abundance relative to hydrology, env variables.

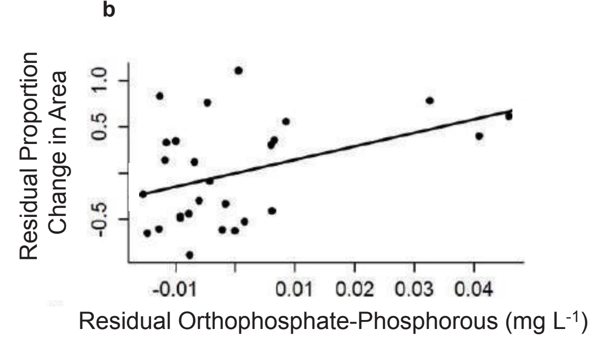
Invaded (filled circles) and uninvaded (open circles) sample locations relative to a *L. hexapetala* patch; water depth increases to right

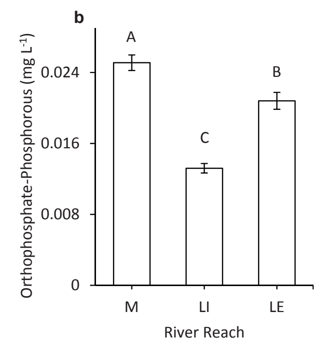
4 yr Field Study, 3 Spatial Scales
Population Patch, River Reach, Watershed

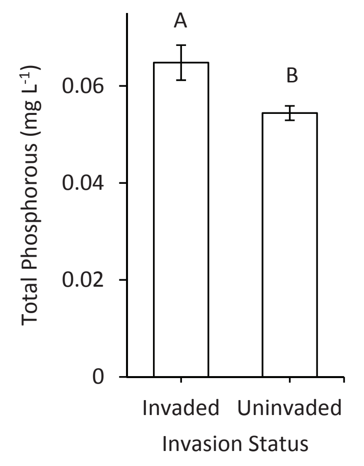


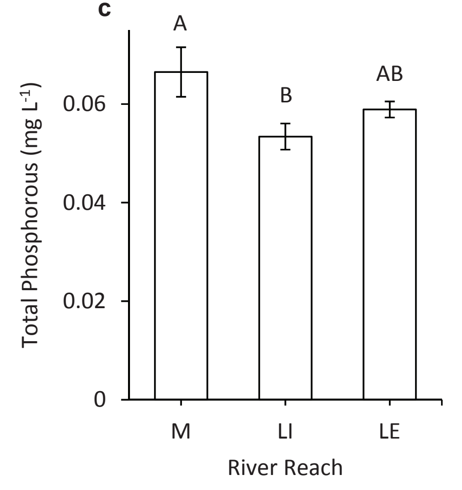
WHAT DRIVES INVASION?
DRIVERS MAY VARY WITH SPATIAL SCALE OF STUDY

Results: Spatial Dynamics of *Ludwigia hexapetala* Invasion Russian River

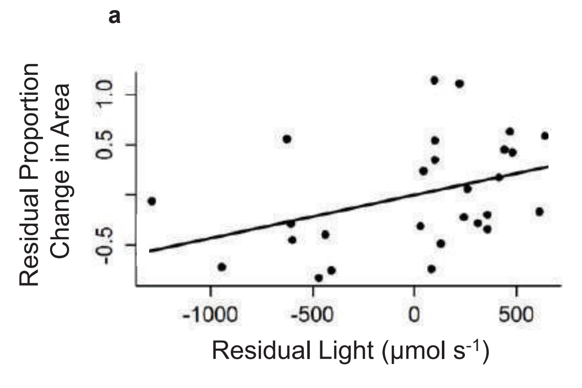


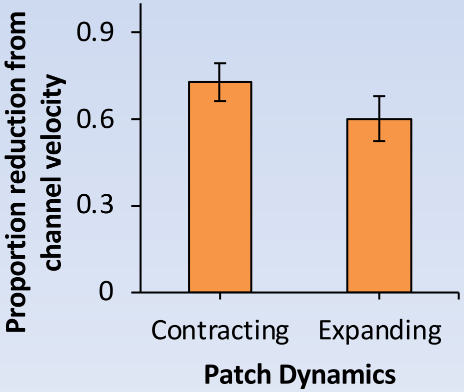






Patch Scale: NOT ALL PATCHES ARE EXPANDING. *L hexapetala* population patches expanded where available light and aqueous phosphorus were elevated relative to uninvaded areas.



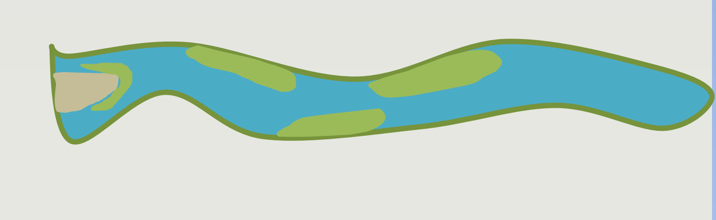


PATCH SCALE

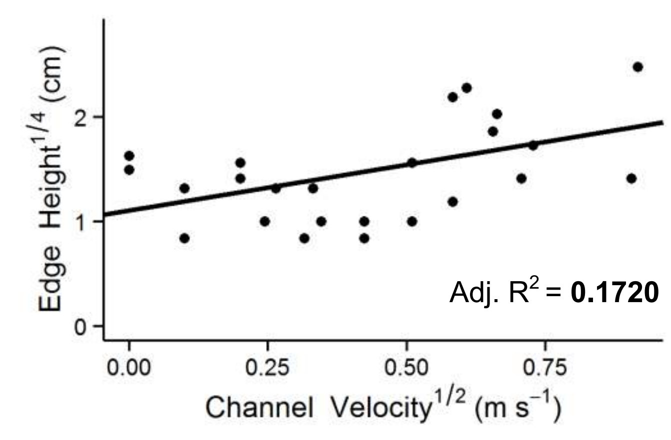
Water velocity reduced in invaded patches, Greater velocity reduction in contracting patches

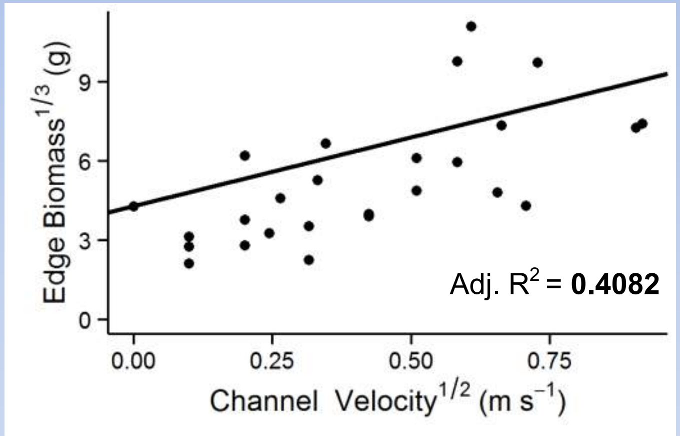
GLM p=0.0367

Results - Reach

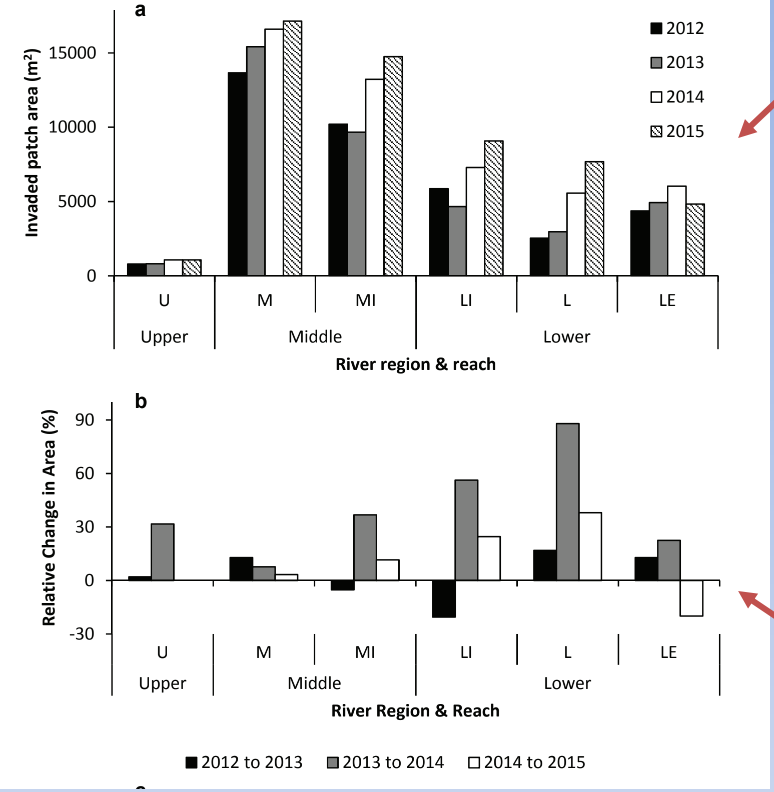


Altered architecture & growth due to high water velocity

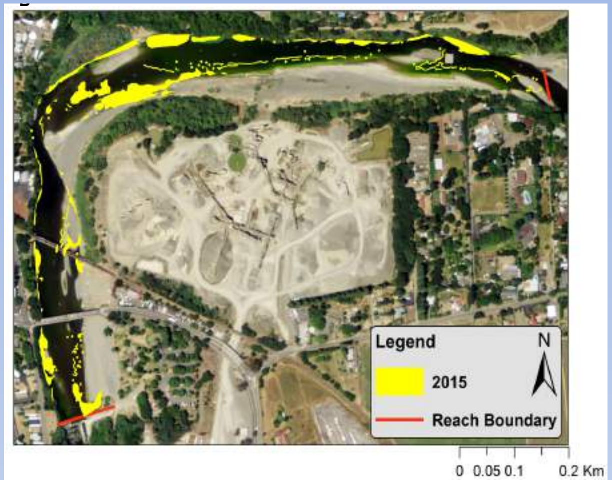




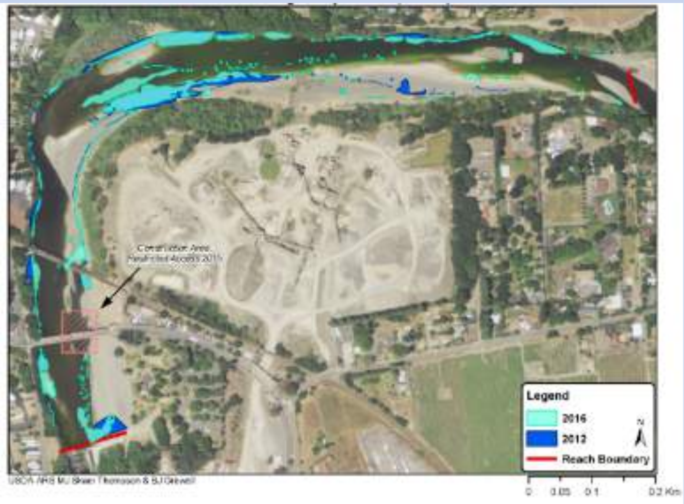
"hedgerow"
-like growth
form



A. Absolute invaded area of L. hexapetala over 4 yrs in Russian River by river region and reach



B. Relative change in invaded area of *L. hexapetala* over 4 yrs in Russian River by river region and study reach

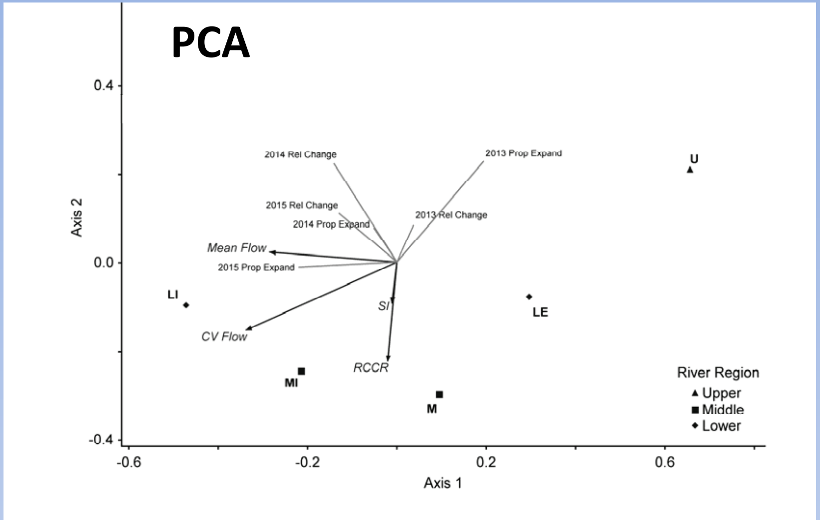


Invaded patches did not expand unabated; **greatest expansion** occurred in the middle river (up to 37%) and lower river (up to 88%).

In contrast, **up to 20% contraction** of invasive patches occurred locally above seasonal instream impoundments.

Results - Watershed

- Upper river
  - Less variation in mean daily flow, high ‘constancy’
  - Low total invaded area
  - High proportion of expanding patches
- Middle river
  - Greater variation in mean daily flow
  - Greater frequency of high flow events
  - High total invaded area, High Expansion
- Lower river
  - Greater flow (cfs)
  - More sinuous
  - Invasion more patchy
  - Contracting patches in seasonally impounded reaches



First 2 axes of **PCA on correlation coefficients of 6 variables describing change spatial extent of *L. hexapetala* over 4 yrs in the Russian River**

Vectors indicate Pearson correlation coefficients (r) of hydrologic and channel morphology variables: Sinuosity Index (SI); mean daily flow, 4 yr period (MeanFlow); coefficient of variation (CV Flow) in mean daily flow for the 4 yr study period.

PCA analysis revealed key patterns in 4 yr distribution & abundance: **increasing invasion in middle river away from summer impoundments, patches contracting behind summer impoundments** in lower river

Hydrology:
major driver of invasion dynamics across **three spatial scales**

At reach and watershed scales, **increasing variability in hydrologic parameters** correlated with patch structure and spatial dynamics of the invasion.

***L. hexape*tala was most abundant in areas with high relative variation in flow**

These findings provide the foundation for development of spatially-prioritized integrated hydrologic and invasive plant management strategies that could improve ecological restoration outcomes in the Russian River.

Spatially-targeted weed management

- most critical in middle and upper river
- lower risk of spread from reaches influenced by summer dams: invasion impeded, propagule pressure reduced
- reducing recreational disturbances, weed biomass, propagule transport from middle river could reduce allofragmentation and downstream dispersal and colonization

Management of summer impoundments:

If planning removal or restriction of summer dams to improve fish habitat, prioritize upstream control of *L. hexapetala* to achieve optimum restoration outcomes

THANK YOU !

This research was supported by USDA-ARS and
US Army Corps, Engineer Research and Development Center,
Aquatic Plant Control Research Program, Vicksburg, MS

RESEARCH TEAM


Brenda Grewell
USDA-ARS


Mike Netherland
APCRP and University of Florida


Russian River
Watershed


Rebecca Drenovsky
John Carroll University


John Gaskin
USDA ARS NPMR


Gabrielle Thiébaut
University of Rennes 1


Morgane Gillard
University of Rennes
PhD 2017, now Post
Doc UCD Grewell Lab


Joy Futrell, M.S.
USDA ARS Davis


Meghan Skaer
Thomason, PhD 2016
UCD/USDA-ARS Davis