

AN ABSTRACT OF THE THESIS OF

Jennifer C. Rowe for the degree of Master of Science in Wildlife Science presented on July 17, 2013.

Title: Patterns in Responses of an Amphibian Assemblage to Wetland Restoration

Abstract approved:

Tiffany S. Garcia

Wetland restoration success in attaining wildlife conservation goals can be confounded by the presence of multiple biological invaders. Wetland management activities typically target invasive plants, but bottom-up responses of higher trophic levels in novel communities are difficult to predict. We surveyed plant and amphibian assemblages at 26 sites enrolled in the Wetlands Reserve Program (WRP) in the Willamette Valley, Oregon to determine the relative importance of plant management, non-native species presence, and surrounding landscape for amphibians across multiple life history stages. Explanatory variables negatively associated with native anuran counts included percent invasive plant cover, invasive bullfrog counts, non-native fish presence, and area of urban land cover. In addition, native anurans were positively associated with WRP site age, suggesting that the benefits of restored wetlands may increase over time. Although invasive plant management provided indirect benefits to native amphibians, the most effective way to enhance native amphibian populations may be through eliminating the strong top-down forces exerted by non-native vertebrates. We also explored the impact of restoration activities on predator/prey dynamics by analyzing bullfrog diet contents. We sampled vegetation and analyzed bullfrog diet contents from 10 WRP wetlands

categorized by management intensity and hydrology to determine whether prey consumption patterns (abundance, richness, and % large prey), diet breadth, and dietary community (based on taxonomic and prey size composition) differed among wetlands categorized by management regime and hydroperiod. We found disparities in the diet breadth with respect to wetland categories, with bullfrogs consuming a disproportionate abundance of few prey orders at actively managed sites. Diet breadth could have been influenced by frequent applications of restoration treatments, as invertebrate species richness and abundance is often negatively linked to habitat disturbance. Dietary taxonomic composition also differed between wetland groups, but prey size composition did not. Instead, prey size was strongly influenced by site-level vegetation covariates; large-bodied prey consumption was positively associated with plant species richness. However, diet dry mass was highest in wetlands with high invasive plant cover, suggesting that other factors, such as foraging activity rates, should be considered to fully understand how bullfrogs meet energy demands in managed wetlands. Variation in native and invasive amphibian responses to wetland restoration efforts may provide important information on how habitat structure and composition influence trophic dynamics. Given that the outcomes of plant control on wildlife may not follow planned trajectories in invasive-dominated systems, this research addresses the need for a community approach to assessments of restoration success.

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Patterns in Responses of an Amphibian Assemblage to Wetland Restoration

by
Jennifer C. Rowe

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Jennifer C. Rowe, Author

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CONTRIBUTION OF AUTHORS

Dr. Tiffany Garcia contributed to all aspects of this thesis, from the development of the ecological questions to the interpretation of results to the editing of draft manuscripts. Her role in stimulating scientific discussions and critical thought proved invaluable for the formation of the project.

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DEDICATION

To my family, my friends, and the wild

“In nature nothing exists alone.”

— *Rachel Carson*

Value of Restored Wetlands to Native and Invasive Amphibians in the Willamette Valley, Oregon

Chapter 1:

GENERAL INTRODUCTION

Biological invasions are a driving force behind ecosystem transformations worldwide and are deemed one of the primary threats to global biodiversity (Wilcove et al. 1998). The ecological and economic impacts of invasive species are profound, making them a significant component of human-caused global environmental change (Vitousek et al. 1997). Approximately 42% of at-risk species are foremost threatened by biological invaders (Pimentel et al. 2005), with freshwater ecosystems being among the most vulnerable to invasion (Cox and Lima 2006).

Freshwater habitats are utilized by amphibians, a taxonomic group experiencing unprecedented global declines (Blaustein and Kiesecker 2002); forty-one percent of the world's amphibians are threatened with extinction (Richter-Boix et al. 2012). Members of this group possess a complex lifecycle and semi-permeable skin which enhances sensitivity to a suite of natural and anthropogenic stressors, including habitat loss (Lehtinen et al. 1999), climate change (Kiesecker et al. 2001), ultraviolet-B radiation (Blaustein et al. 2001), chemical contaminants (Blaustein et al. 2003), emerging infectious disease (Garner et al. 2006), and biological invasions (Pimentel et al. 2005). Potential synergisms between these stressors can be exacerbated by human disturbance, which is a dominant factor determining biological invasion success (Didham et al. 2007). Amphibians are a worldwide conservation priority, thus sparking considerable interest in understanding the impacts of invasive species on this taxon in light of other threats.

In habitats that experience disturbance across spatiotemporal scales, it is especially difficult to quantify the effects of invasive species on ecosystem function and biodiversity. Restoration treatments to control invasive plant species can paradoxically create disturbance that disrupts community dynamics (i.e., causes increased variability in resource utilization patterns) and opens niches to invaders of multiple trophic levels (*fluctuating resource hypothesis*; Davis et al. 2000) (Shea and Chesson 2002, Hobbs et al. 2006). Invaders tolerant to human-modified habitats are favored during initial phases of wetland creation or restoration, thus priority effects in colonization can greatly determine the trajectory of response rules (Hulme 2006, Firn et al. 2010). Restoration actions that do not incorporate the current functional state of a system may have unintended consequences on native wildlife (Suding et al. 2004, Firn et al. 2010). For example, after the introduction of prairie willow (*Salix humilis*), efforts to restore southern Illinois sand barren prairie through fire were unsuccessful because invasive *Salix* and other woody species were fire tolerant (Anderson et al. 2000). Corresponding to the increasing prevalence of novel invasive-dominated systems like these, there is an emerging paradigm shift from successional models to adaptive alternative states models in restoration ecology. Although there are predicted behavioral and physiological correlates of invasion success, a stronger understanding of invasion potential within a broader context of community composition, habitat degradation, and evolutionary relationships is needed (MacDougall and Turkington 2005, Cohen et al. 2012).

Research on biological invasion has overwhelmingly focused on single-species invaders and has largely ignored the net impacts of multiple invaders (Preston et al. 2012, Kuebbing et al. 2013). Relationships among co-occurring invasive species are complex,

thus it is essential to analyze community impacts within a multivariate context (Preston et al. 2012, Gaertner et al. 2012). Interaction outcomes may be facilitative (i.e., invasional meltdown; Simberloff and Von Holle 1999), neutral (Kuebbing et al. 2013), or buffering (i.e., competition; Kuebbing et al. 2013). For example, the invasive South African grass *Ehrharta calycina* escapes herbivory pressure (*enemy release hypothesis*; Keane and Crawley 2002) by associating with other exotic plant species (Cushman et al. 2011). Contrastingly, exploitative competition between two invasive eastern hemlock insect herbivores, *Adelges tsugae* and *Fiorinia externa*, lessens host damage in co-occurring infestations by reducing densities of the more harmful *Adelges tsugae* (Preisser and Elkinton 2008). Multiple invaders can intensify net negative impacts on native amphibians by eliciting costly predator avoidance behaviors (Sih et al. 2010). Kiesecker and Blaustein (1998) discovered that trait-mediated indirect interactions with bullfrogs caused native *Rana aurora* larvae to reduce activity levels and spend more time in deep water, leading to lower survival and mass at metamorphosis and increased predation by non-native centrarchid fish. These cases elucidate the importance of adaptive management of invasives which targets the facilitator or the most aggressive invader (Gaertner et al. 2012, Kuebbing et al. 2013).

Native community dynamics may be transformed whenever a novel species is introduced to the system, particularly if it is a predator (Ricciardi and MacIsaac 2011) or a habitat-forming species (Gribben et al. 2009, Watling et al. 2011). In addition to the obvious direct effects of predation, predator invasions can have indirect cascading impacts on food webs (Estes et al. 2001), and these impacts are particularly strong in freshwater ecosystems (Cox and Lima 2006, Sih et al. 2010). Further, bottom-up

(resource-driven) effects of invasive plants can have wide-ranging community-level implications. Invasive plants can affect the quality of native amphibian breeding habitat by modifying temperature and UV-B penetration (Garcia et al. 2004), producing harmful chemical compounds (Watling et al. 2011), reducing macroinvertebrate communities (Kappes et al. 2007), and by increasing densities of physical structures (Davis et al. 2012) and potential refuge (Janssen et al. 2007, Hartel et al. 2007, Watling et al. 2011). Since the physical and chemical composition of emergent vegetation can have substantial impacts on biotic interactions (i.e., competition and predation), a decrease in microhabitat complexity elicits profound effects on amphibians, particularly in the larval stage (Harvey and Fortuna 2012, Davis et al. 2012).

The loss and degradation of wetlands in the western United States has reduced the availability and quality of breeding habitat for native species while aiding highly tolerant non-native species (Kentula et al. 1992). Only recently are the benefits of restoring wetland systems being fully recognized. In addition to providing habitat for obligate species, wetlands provide vital ecosystem services (e.g., flood abatement, drought control, groundwater recharge and purification, and erosion control) that are maximized when the abiotic and biotic components of the system are synchronized (Mitsch and Gosselink 2000). The Wetlands Reserve Program (WRP), first authorized under the 1992 U.S. Department of Agriculture (USDA) Farm Bill, is a voluntary program enacted to mitigate wetland loss on private lands (NRCS 2012). The program offers landowners easement or cost-share incentives to restore wetlands in agricultural landscapes (NRCS 2012). Depending on the enrollment option, NRCS will subsidize 75-100% of the restoration costs, while the landowner retains the title to the property (NRCS 2012). Not

only does the program make tangible contributions to the restoration of wetland functions on working lands, its benefits extend to regional watershed health and the economies of surrounding communities (NRCS 2012).

Although one goal of the WRP is to restore wetland-dependent fauna, our understanding of how wetland restoration activities impact higher-order consumers, such as amphibians, is surprisingly limited (Rewa 2005, but see Pechmann et al. 2001, Pearl et al. 2005, Petranka et al. 2007, Waddle et al. 2010, Shulse et al. 2010). Since the presence of multiple invaders in a system may further confound the benefits of invasive plant removal for native wildlife, we posit the need for restoration assessments in a community context. Further, we consider the influence of stressors encountered at different spatial scales and developmental stages. Biotic and abiotic characteristics of both the wetland and surrounding landscape can play a role in long-term amphibian population viability (Wilson 1992, Leibold et al. 2004) and regulate densities differently over ontogeny (Wilbur 1980, Sztatecsny et al. 2004, Van Buskirk 2005, Resetarits 2005). Lentic-breeding amphibians are an excellent focal assemblage to monitor the success of wetland restoration projects because they can reach high abundances, they utilize both aquatic and terrestrial habitats, and they are frequently cited as indicators of environmental quality. We were interested in determining the relative importance of local and regional factors on native amphibians in restored wetland communities consisting of the following regionally dominant invaders: the American bullfrog (*Lithobates catesbeianus*), reed canarygrass (*Phalaris arundinacea*), and non-native fish species (western mosquitofish [*Gambusia affinis*] and eastern warmwater centrarchid fish).

Reed canarygrass – Reed canarygrass is a cool season, rhizomatous perennial plant (Naglich 1994) commonly found in dense monotypic stands in wetlands and riparian habitats throughout the United States. Reed canarygrass has several qualities that make it an aggressive and widespread invader, such as high productivity and the ability to reproduce both sexually and vegetatively (Naglich 1994). It is a management priority because it outcompetes native plants and alters habitat function by promoting silt deposition and erosion, inhibiting wetland drainage, and increasing evapotranspiration rates (Naglich 1994). Reed canarygrass often grows too densely to provide suitable cover for wildlife, and it has been suggested that it could impede amphibian movements or limit suitable oviposition substrate (Naglich 1994). Recent studies have also investigated the potential for reed canarygrass to affect amphibian larvae directly through the production of toxic alkaloids or indirectly through the alteration of decomposed plant matter which modifies prey communities (e.g., Rittenhouse 2011).

American bullfrog – The American bullfrog is a widespread invasive anuran species that is highly tolerant of human-modified habitats and commonly present in Pacific Northwest WRP sites (Pearl et al. 2005, Adams and Pearl 2007). Outside its native range, the bullfrog plays a central role in displacing native anurans via interference competition and predation (Kiesecker et al. 2001). In the Willamette Valley, Oregon, the bullfrog has been cited as a major factor in the population declines of several native amphibian species, including the extirpated Oregon spotted frog (*Rana pretiosa*) and the threatened northern red-legged frog (*Rana aurora*), and it may negatively impact other pond-breeding amphibians native to the region (Nussbaum et al. 1983; Table 1.1). Due to interspecific differences in phenology, late-stage bullfrog larvae have a competitive

advantage over smaller, early-stage native amphibian larvae in obtaining limited food resources (Blaustein and Kiesecker 2002; Table 1.1), and adult bullfrogs are gape-limited generalist predators that often prey on other amphibians (Blaustein and Kiesecker 2002). Research to guide management strategies to prevent further bullfrog range expansion is a priority for amphibian conservation, especially as it is increasingly common for bullfrogs and native amphibians to occur syntopically in both ephemeral and permanent habitats (Ficetola et al. 2007, Cook et al. 2013).

Non-native fish – The intentional introduction of non-native warm water fishes for sportfishing has had dramatic effects on pond ecosystems in the Willamette River basin (Sanderson et al. 2009). Non-native fish are capable of influencing amphibian assemblages through direct (e.g., predation) and indirect interactions (e.g., inducing predator avoidance behaviors that reduce larval foraging opportunities) (Kats et al. 1988, Werner and McPeck 1994). Native pond-breeding amphibians in the Willamette Valley of Oregon generally evolved under fishless conditions (Hayes and Jennings 1986), but conversion of shallow, ephemeral ponds to deeper, permanent ponds has facilitated the spread of non-native fish (Adams et al. 2003). Many of these introduced species are eastern centrarchid sunfish which have coevolved with bullfrogs in their native range (Adams et al. 2003).

This thesis explored how invasive plant removal influences dynamics at higher trophic levels (e.g., between native and invasive amphibians and non-native fish) in WRP sites in the Willamette Valley, Oregon. The objective of Chapter 2 was to assess the abundance (counts) and diversity of invasive and native amphibian and plant assemblages

as a function of management intensity, local abiotic and biotic variables, and regional land use. For the three most abundant anuran species detected in surveys, we used an information theoretic approach to identify the model within the candidate set that was best supported by the data. Non-parametric multivariate statistics were employed to investigate community interactions and habitat associations for all life stages of the six amphibian species encountered. Analyses considered the influence of both local and regional covariates in order to determine if stage-specific response patterns existed.

Chapter 3 examined the effects of wetland restoration on the habitat value to predatory bullfrogs. We quantified diet characteristics (diet mass, diversity, and composition) of bullfrogs occupying wetlands under different wetland management regimes to determine if diet differs as a function of management intensity. Diet analysis is an important indicator of how habitat differences influence predator/prey dynamics in restored wetlands. This information, in turn, may predict bullfrog invasion potential at WRP sites in the Willamette Valley. Using an information theoretic approach to model selection allowed us to test competing hypotheses of the important management and habitat covariates influencing dietary differences. We also utilized Modified Costello Representations (Amundsen et al. 1996) and multivariate statistical analyses to characterize the dietary profile of bullfrogs in wetlands receiving different management regimes.

The final chapter (Chapter 4) synthesized the findings from Chapter 2 and 3 and emphasized the broader implications of this work. We also discussed future considerations for research evaluating the success of WRP management in providing suitable habitat for lentic-breeding amphibians. Given the paucity of studies tracking the

impact of restored wetlands on amphibians, and considering complex species interactions make restoration outcomes on wildlife difficult to predict, it is important to incorporate multi-trophic relationships into assessments of restoration success.

TABLES AND FIGURES

Table 1.1. – Amphibian population status and breeding ecology in the Willamette Valley in Oregon.

Species Common Name	Scientific Name	Oregon Status	Metamorphosis	Breeding
American bullfrog	<i>Lithobates catesbeianus</i>	Common	1-3 years	June-Aug
Long-toed salamander	<i>Ambystoma macrodactylum</i>	Common	3-5 months	Jan-Feb
Northwestern salamander	<i>Ambystoma gracile</i>	Unknown	5-16 months	Feb-April
Oregon spotted frog	<i>Rana pretiosa</i>	Extinct in valley	3-5 months	Feb-March
Pacific chorus frog	<i>Pseudacris regilla</i>	Common	3-5 months	Feb-March
Northern red-legged frog	<i>Rana aurora</i>	Sensitive	3-5 months	Jan-March
Rough-skinned newt	<i>Taricha granulosa</i>	Common	3-5 months	Feb-May

Adapted from USGS (2004)

Chapter 2:

**IMPACTS OF WETLAND RESTORATION EFFORTS ON AMPHIBIAN
ASSEMBLAGES IN MULTI-INVADER COMMUNITIES**

Jennifer C. Rowe and Tiffany S. Garcia

ABSTRACT

The success of restoration in attaining wildlife conservation goals can be strongly dependent on both site-scale and landscape-scale habitat characteristics, particularly for species with complex life cycles. Wetland management activities typically target plant communities, thus bottom-up responses in higher trophic levels may be dependent on spatially explicit habitat use. We surveyed plant and amphibian assemblages at 26 sites enrolled in the Wetlands Reserve Program (WRP) in the Willamette Valley, Oregon to determine the relative influence of plant management, non-native species, and surrounding landscape on amphibian counts across multiple life history stages. Explanatory variables negatively associated with native anuran counts included percent invasive plant cover, non-native fish presence, invasive bullfrog count, and area of urban land cover. In addition, native anurans were positively associated with WRP site age, suggesting that the benefits of restored wetlands may increase over time. The influence of regional urban land cover on amphibian counts varied among species and life stages, reflecting differences in dispersal capabilities, habitat requirements, and tolerances toward human-modified landscapes. This study emphasized the importance of adaptive approaches to maintaining diverse communities in restored habitats by considering impacts of synergistic stressors in a multi-invader context. Although invasive plant management provided indirect benefits to native amphibians, the most effective way to enhance native amphibian populations may be through eliminating the strong top-down forces exerted by non-native vertebrates.

INTRODUCTION

Wetland habitats in the United States have incurred significant losses in total area through agricultural and urban development, as well as hydrological modifications (Dahl 1990). Consequently, a disproportionately high number of obligate wetland species are listed as threatened or endangered (46%; Boylan and MacLean 1997 in Whigham 1999), contributing to simplified community structure and compromised ecosystem function (Gibbs 2001). A renewed appreciation of the ecological benefits provided by wetlands has prompted federal and state administrative policies to direct funding toward wetland creation, preservation, and restoration programs (Vottler and Muir 1996, Dahl 2006). The Wetlands Reserve Program (WRP), administered through the United States Department of Agriculture's Natural Resource Conservation Service (NRCS), is a voluntary project aimed at mitigating wetland loss by providing technical and financial support to landowners wishing to restore wetlands on agricultural land (NRCS 2011). Over 2.6 million acres of land are enrolled in the WRP with the objective of enhancing wetland function and restoring vitality of agricultural lands (NRCS 2012).

One goal of the WRP is to provide habitat for wetland-dependent fauna (NRCS 2012). Restoration success in achieving wildlife conservation goals is typically evaluated using hydrologic and vegetative criteria, with the assumption that faunal establishment is linked to floral establishment (Petranka et al. 2003, Gray and Teels 2006). The WRP prioritizes benefits for migratory birds (Gray and Teels 2006), and it follows that considerable information is available about the program's contribution to waterfowl conservation (King et al. 2006). However, relatively few studies have quantified the effects of plant management on other wetland-associated vertebrates (Petranka et al.

2003, King et al. 2006). Lentic-breeding amphibian species are experiencing global declines and are an excellent focal group in which to study the effects of wetland restoration. Amphibians are frequently cited as indicators of environmental quality as they possess a unique life history characterized by dispersal between aquatic breeding sites and upland hibernacula (Blaustein and Kiesecker 2002, Waddle et al. 2012).

The ability of amphibians to successfully establish and persist in restored wetlands is influenced by wetland-specific (site-scale) and landscape-scale habitat variables. Within breeding ponds, amphibians face both abiotic stressors (e.g., wetland desiccation, chemical contaminants, habitat disturbance) and biotic stressors (e.g., native and invasive competitors and predators) (Blaustein and Kiesecker 2002). They are also particularly sensitive to surrounding landscape composition because of annual breeding migrations (Semlitsch 1998). Isolated breeding ponds imbedded in fragmented landscapes can become population sinks if dispersers experience high mortality (Rothermel 2004). Both breeding pond quality and regional land use are potentially strong predictors of long-term amphibian diversity and abundance at restored WRP sites. Further, the relative importance of local and regional factors may depend on ontogeny, with survival in the aquatic life stages (eggs and larvae) regulated by within-pond processes and survival in the terrestrial life stages (juveniles and adults) regulated by both within-pond and surrounding landscape processes (Wilbur 1980, Sztatecsny et al. 2004, Van Buskirk 2005).

Invasive species can directly and indirectly reduce amphibian abundance and diversity at restored wetlands (Ricciardi and MacIsaac 2011). Many studies have examined the effects of a single invader on native amphibians, but it is exceedingly

challenging to disentangle community-level impacts (Preston et al. 2012). The presence of multiple invaders can produce additive effects on native communities when interactions between invaders are facilitative (i.e., invasional meltdown; Simberloff and Von Holle 1999). However, the effects of invasive species on amphibians may be mediated by the physical structure of emergent vegetation (Kiesecker et al. 2001, Porej and Hetherington 2005). Invasive plants can reduce the quality of amphibian breeding habitats (Brown et al. 2006, Davis et al. 2012), but may also provide refuge to diffuse antagonistic interactions (Janssen et al. 2007, Hartel et al. 2007, Watling et al. 2011). Thus, management of invasive plants can potentially have unintended negative impacts on native amphibian communities. Further, habitat disturbance caused by restoration actions may initially enhance invasion potential for exotic vertebrate competitors and predators of native amphibians (Shea and Chesson 2002). Exotic species that commonly co-occur in lentic, freshwater WRP habitats in the western United States are reed canarygrass (*Phalaris arundinacea*), the American bullfrog (*Lithobates catesbeianus*), and non-native fish species (western mosquitofish [*Gambusia affinis*] and eastern warm water centrarchids). These taxa are capable of reducing native amphibian abundance and diversity by altering ecosystem function and/or dynamics of biotic interactions, and their impacts may be mediated directly or indirectly via restoration management.

Our study objective was to determine whether native amphibian diversity and abundance (counts) at WRP sites in the Willamette Valley, OR was predicted by invasive plant management, the presence of non-native vertebrates (American bullfrogs and fish), and regional landscape quality. We hypothesized that active management would extend positive benefits to the plant community via reduced invasive cover which in turn would

translate to greater counts of native amphibians and reduced counts of invasive bullfrogs. However, we expected biotic interactions with non-native fish and bullfrogs to be the strongest determinants of native amphibian counts, with site-scale variables having a greater effect on the response for premetamorphic stages and landscape-scale variables having a greater effect on the response for postmetamorphic stages. To address these hypotheses, we explored (1) whether active management is effective at reducing unwanted plant species and increasing plant diversity, (2) whether invasive plant cover predicts amphibian counts and diversity given other habitat covariates, and (3) the relative importance of site-scale and landscape-scale variables in predicting life stage-specific amphibian counts. These aims highlighted how current invasive plant management strategies applied within the WRP contribute to the program's wildlife habitat restoration goals for amphibians in the presence of complex trophic interactions.

MATERIALS AND METHODS

This research was conducted in Oregon's Willamette Valley where approximately 43% of upland habitat has been converted for agriculture (Baker et al. 2004) and 57% of emergent wetlands have been lost within the last century (Morlan 2000). These losses parallel the Oregon state listing of 24% of wetland-dependent amphibians as *imperiled* in conservation status rank (Morlan 2000).

Survey Design

We selected 26 WRP sites between Portland (ca. 45° 28' 56.81"; elevation 47 m MSL) and Eugene (ca. 44° 11' 4.69"; elevation 100 m MSL) containing freshwater lentic

wetlands lacking permanent fluvial inputs based on (1) landowner permission to access site, (2) hydroperiod (both seasonal and permanent wetlands likely to remain inundated until the end of the study period in August), and (3) spatial independence (Fig. 2.1). At sites where multiple wetlands were present, a single water body was randomly selected. All study sites were separated by a distance of at least 2.5 km to limit potential for dispersal by individual amphibians between populations (Petranka et al. 2007). Sites ranged in age from 5 to 15 yrs ($\bar{x} = 9.81 \pm 3.36$ yrs) since enrollment in the WRP, and wetlands retained in the study ranged in size from 0.08 ha to 14.7 ha ($\bar{x} = 2.8 \pm 0.6$ ha) prior to any natural or mechanical drawdown. We categorized each wetland based on management intensity as *passively* managed ($N = 8$; received no management or only minimal intervention through hydraulic modifications) or *actively* managed ($N = 18$; intensive management activities were applied to >50% of the wetland area at least twice in the past 3 yrs) based on information from landowners and NRCS restoration technicians (Kross et al. 2008, Evans-Peters et al. 2012). In addition to management intensity (MGMT; passive or active), additional information obtained through landowner and NRCS communications included WRP age since enrollment (AGE; yrs) and wetland hydroperiod (HYDRO; seasonal or permanent). Seasonal wetlands were typically dry by late summer, while permanent wetlands could experience minimal drawdown but never dried fully.

Each site was visited on three occasions in 2011 (March-May, May-July, and July-August). Amphibian count data were collected during each sampling period while data on plant species composition were collected only during the May-July sampling period. During each site visit, we recorded two site-scale physical habitat characteristics:

water temperature (WATEMP; °C at 10 cm depth 1 m from waterline, averaged over 3 sampling periods) and log-transformed wetland area (AREA; calculated in MapSource and Google Earth version 6.2 from on-the-ground waterline delineation with a Garmin GPS unit). We also recorded information on non-native fish presence (FISH; absent or present, verified via landowner/NRCS communications and minnow trapping during each sampling period).

We incorporated six landscape-scale coverage variables using data layers developed by the USGS Gap Analysis Program (USGS 2011) and The Wetlands Conservancy (TWC 2009) in a Geographic Information System (GIS; ESRI ArcMap version 10.0). We created 1000 m buffers (Lehtinen 1999) around the 26 wetland study sites and calculated area (m²) of forest cover (FOR1000), urban land (URB1000), and wetlands (WET1000) from converted polygons within the buffer.

Plant community sampling – Vegetation surveys were conducted at each site once during peak growing season (May 12 – July 13, 2011). We demarcated 30 sampling points (1 m² quadrats) distributed evenly among 10 transect belts (5 m long) spaced at equal intervals around the entire wetland perimeter (modified from Mueller-Dombois and Ellenberg 1974). The three sampling quadrats in each belt were located in three habitat zones: shore (within 3 m upland of waterline), waterline, and shallow water (< 1 m water depth) zone. For each zone, we estimated % cover of plants, bare ground, and open water to the nearest 5% (Baines et al. 1994). Plant species were identified to the lowest taxonomic group and assigned to the categories of *invasive* or *native* to Oregon in order to produce a variable for mean site-level percent invasive plant cover (INVCOV).

Amphibian community sampling – Native and invasive amphibian species count data were estimated at each site during the three sampling periods. Sampling periods were defined to maximize detection of all life stages of amphibians between the breeding and emergence periods. We conducted 30 min time-restricted amphibian searches following standard breeding pond visual encounter survey (VES) protocol along a curvilinear wetland perimeter transect (Crump and Scott 1994, Olson et al. 1997). Starting from a random point, the observer walked clockwise along the waterline and systematically searched within 1 m of either side of the path. The observer spent an equal proportion of time searching the waterline, the shallow water zone (1 m out from waterline), and the shore zone (1 m upland from waterline) (Crump and Scott 1994). Counts of amphibian species by life-stage were recorded for all individuals encountered. VESs were supplemented with D-frame dipnetting for species/life stages unobservable at the surface (e.g., salamander larvae). Dipnet sweeps were taken at 5 min intervals and standardized to cover a length of 1 m in roughly 1 cm of substrate in the shallow water zone (Crump and Scott 1994). Catch per dipnet sweep was added to the visual encounter survey count data for each site visit since dipnet sweeps yielded detections of species and life stages that were not detected in VES.

Statistical Analysis

Plant community – Plant diversity was characterized using species richness (Whittaker 1972), Simpson's diversity (Simpson 1949), and gamma diversity (Whittaker 1972). Simpson's diversity (D) is the sum of the relative abundance (count) of each species following:

$$D = \sum p_i^2$$

where p_i equals the proportion of individuals of the i th species for an open community.

Gamma diversity describes the number of unique species present at a study site compared to all study sites combined (Magurran 2004).

We used linear models (*R* version 2.15.2; *R* Core Team 2012) to identify management and/or habitat variables that significantly influenced plant diversity in the wetland basin unit. Response variables for the analysis included plant species richness (log-transformed), Simpson's diversity (cube-transformed), and mean percent ($\bar{x}\%$) invasive plant cover. Our model set (for each response) contained all combinations of explanatory variables MGMT, AGE, and HYDRO without interaction terms, as well as the null intercept-only model. These three predictors were selected based on *a priori* hypotheses about their roles in regulating the plant community (Table 2.1). We ranked competing models in the set using Akaike's information criterion corrected for small sample sizes in *R* packages *bbmle* and *AICcmodavg* (AIC_C ; Akaike 1973, Hurvich and Tsai 1989). Models with a $\Delta AIC_C < 2.0$ from the top-ranked model were considered competitive (Burnham and Anderson 2002). Model weights (w_i) represented the relative support for the model given the data, and parameter estimates with confidence intervals were used to determine the direction and strength of the effects.

Anuran counts –Amphibian count data were analyzed using generalized linear models (GLMs) in *R* packages *bbmle* and *AICcmodavg* for the three most common anuran species encountered: Pacific chorus frog (*Pseudacris regilla*), northern red-legged frog (*Rana aurora*), and American bullfrog. Species count data were recorded as the highest encounter during any one sampling period for each life stage (egg, larvae,

juvenile, and adult) to limit the potential for multiple counts of the same individual over the survey season (Denton and Richter 2013). Since the biphasic, aquatic-terrestrial life cycle of amphibians may expose them to different stressors over ontogeny, we analyzed premetamorphic (eggs and larvae) and postmetamorphic (juveniles and adults) counts separately.

Independent variables considered in GLMs were bullfrog count (LICA; log-transformed and averaged over three sampling periods), AGE, INVCOV, FISH, HYDRO, and URB1000 (log-transformed). Pairwise combinations of these predictor variables were assessed for multicollinearity, and since Pearson coefficients were $r < 0.70$ (Shulse et al. 2010), all predictors were retained in the initial pool of variables. For each anuran response variable, we developed a set of 16 empirical candidate models based on *a priori* hypotheses of important ecological interactions informed through a literature review (Table 2.2, Appendix 2.1). These candidate models were limited to fewer than two predictors to prevent overfitting. Residuals indicated overdispersion (Hoef and Boveng 2007), thus GLMs were fit using a negative binomial error distribution with log link function:

$$\log(\varphi) = \beta_0 + \beta_1 x_1$$

Candidate models were compared using QAIC_C values, model weights (w_i), and maximum log-likelihood ratio statistics (LL) (Johnson and Omland 2004). Since the overdispersion coefficient (\hat{c}) was greater than 1, we ranked models with QAIC_C (as opposed to AIC_C) following:

$$\text{QAIC}_C = \frac{-2\ln(L)}{\hat{c}} + 2k + \frac{2k(k+1)}{n-k-1}$$

where L is the maximum likelihood estimate for the model and k is the number of fitted parameters (Symonds and Moussalli 2010). All models with $\Delta\text{QAIC}_C < 2.0$ were considered competitive and were retained following examination of diagnostic plots for fit (Burnham and Anderson 2002). However, if an additional parameter with minimal explanatory power was added to a model within the $\Delta\text{QAIC}_C < 2.0$ set and did not improve the model's maximum log-likelihood, the model with the additional parameter was considered uninformative (Burnham and Anderson 2002). We assessed the relative strength of the variables included in these models from parameter estimates and 95% confidence intervals.

Amphibian community analysis – Statistical analyses for the entire amphibian assemblage, including rare species, were non-parametric. Response variables included counts of eggs, larvae, juveniles, and adults (averaged over three sampling periods) for all amphibian species/life stages detected in surveys. Site-scale covariates included AGE, INVCOV, FISH, WATEMP, HYDRO, and AREA (log-transformed), while landscape-scale covariates (coverage within a 1000 m radius from the site; log-transformed) included FOR1000, WET1000, and URB1000.

Non-metric multidimensional scaling (NMS; Mather 1976) was performed in the *vegan* package in *R* to describe important patterns in species composition by ordinating the 26 sample units in amphibian species space (average count for each species and life stage for each site). The ordination was overlain with a joint plot to display the strongest correlations between the environmental variables and the ordination axes based on the Pearson's r statistic. The r^2 values represented the correlation between the ordination distance and the distance in original space. NMS was conducted using a random starting

configuration with the Sørensen (Bray-Curtis) distance measure. Amphibian count data were relativized by species maximum to rescale and equalize the influence of disproportionately abundant species and life stages. To facilitate detection of relevant relationships between community composition and habitat variables with minimal accumulation of noise, we considered removing rare species. Six species/life stage combinations never occurred in the matrix, and upon comparing the cumulative variance of column (species) sums prior to the adjustment and after the adjustment, we determined that it was appropriate to remove these rare individuals from subsequent analyses. NMS was followed by a multi-response permutation procedure (MRPP; Mielke 1984) executed in *R* to compare differences in amphibian species composition between categorical grouping variables (FISH, HYDRO, and MGMT). MRPP is a nonparametric procedure for testing whether there is a significant difference between two or more groups of sample units by comparing within-group and between-group Sørensen (Bray-Curtis) dissimilarity matrices, weighted by group size (n) (Mielke 1984).

RESULTS

Plant community – A total of 96 plant species/genera were present at the 26 wetland sites sampled (87 at actively managed wetlands and 42 at passively managed wetlands), with a mean plant species richness of 11.8 (95% confidence interval [CI] = 9.22 to 14.32) per site (Table 2.3). Native plant species having the highest mean percent coverage (\bar{x} %) were spikerush (*Eleocharis spp.*; \bar{x} % = 11.0, 95% CI = 7.15 to 14.87), cattail (*Typha latifolia*; \bar{x} % = 4.9, 95% CI = 1.92 to 7.84), and American water plantain (*Alisma subcordatum*; \bar{x} % = 2.4, 95% CI = 0.37 to 4.51), and invasive species having the

highest coverage were reed canarygrass (*Phalaris arundinacea*; \bar{x} % = 15.6, 95% CI = 7.78 to 23.32), meadow foxtail (*Alopecurus pratensis*; \bar{x} % = 3.4, 95% CI = 0.50 to 6.36), and water smartweed (*Polygonum amphibium*; \bar{x} % = 2.0, 95% CI = -0.46 to 4.35). Total invasive cover and reed canarygrass cover at the sites were highly correlated ($r = 0.704$, $p < 0.001$), and invasive cover at the study sites was highly dominated by reed canarygrass.

Plant species richness, Simpson's diversity, and gamma diversity were higher at actively managed sites (Table 2.3). The best models ranked by AIC_C indicated that management intensity most adequately explained the variation for Simpson's plant diversity and % invasive cover (Table 2.4). HYDRO was included along with MGMT in a competitive model predicting Simpson's plant diversity, but its parameter estimate did not have a significant effect on the slope of the response (Table 2.4). Simpson's diversity was higher ($\beta_{\text{MGMT}} = 0.56$, 95% CI = 0.21 to 0.70) and % invasive cover was lower ($\beta_{\text{MGMT}} = -16.21$, 95% CI = -30.03 to -2.39) at actively managed sites (Table 2.4). Percent vegetative cover (Welch's two-sample t-test; $t(22.78) = 1.45$, $p = 0.16$) and % bare ground ($t(20.79) = 0.87$, $p = 0.40$) were not significantly different between actively and passively managed sites.

Amphibian community – All six amphibian species inhabiting the Willamette Valley were encountered during surveys: Pacific chorus frog, northern red-legged frog, rough-skinned newt (*Taricha granulosa*), northwestern salamander (*Ambystoma gracile*), long-toed salamander (*Ambystoma macrodactylum*), and American bullfrog. Amphibian diversity was similar between actively and passively managed sites under multiple metrics (Table 2.3). Chorus frogs were the most common species, occupying all 26

survey sites, followed by invasive bullfrogs, which occurred at 20 (76.9%) sites (Fig. 2.2a). Northwestern salamanders and long-toed salamanders were rarely detected, each occurring at only 3 (11.5%) sites (Fig. 2.2a). The northern red-legged frog, a threatened species, was present at 13 (50.0%) sites and occurred most frequently at seasonal, fishless wetlands (Fig 2.2b). Native amphibians occupied fishless sites more often than sites containing non-native fish, except for rough-skinned newts which were most common at fish-bearing permanent sites (Fig. 2.2b). The invasive bullfrog also occurred most frequently at sites with non-native fish (Fig. 2.2b). Most amphibians were more common at actively managed wetlands as opposed to passively managed wetlands, apart from long-toed salamanders which were detected at 2(25.0%) of passively managed sites and 1 (5.6%) actively managed site (Fig. 2.2c).

WRP management characteristics such as AGE, INVCOV, and HYDRO commonly influenced anuran counts. AGE was included in the top-ranked models for premetamorphic life stages of Pacific chorus frog and postmetamorphic life stages of northern red-legged frog, having a positive effect on the slope of the response given habitat covariates (Table 2.5, Fig. 2.3). The best model for postmetamorphic red-legged frogs and competitive models for postmetamorphic chorus frogs indicated a negative association between counts and INVCOV (Table 2.5, Fig. 2.3). Non-native species consistently appeared in the highest-ranked models for native anurans. Postmetamorphic chorus frogs and red-legged frogs were negatively associated with LICA given covariates (Table 2.5, Fig. 2.3). Non-native fish presence (FISH) had a stronger parameter effect on the slope of the native species' responses than bullfrog counts (LICA) when included in models, and FISH was the only informative variable included in top models for

premetamorphic red-legged frogs (Table 2.5, Fig. 2.3). Native anuran counts were consistently lower whereas bullfrog counts were higher when non-native fish were present (Table 2.5, Fig. 2.3). The area of urban cover surrounding a wetland (URB1000) had a negative effect on postmetamorphic chorus frog counts and occurred in models alongside site-level covariates of LICA or FISH (Table 2.5). No models were informative for premetamorphic nor postmetamorphic bullfrogs, since the null models were competitive in the set and all parameter effects had 95% confidence intervals crossing zero.

The NMS ordination converged after 20 iterations at a 2-dimensional solution to represent the relationship between species counts and wetland sample units, with final stress of 0.202 and instability of 0.00 ($p = 0.020$, $R^2 = 0.748$). The strongest ($p < 0.05$) quantitative habitat vectors related to amphibian species composition were HYDRO ($r^2 = 0.377$, $p = 0.005$) and FISH ($r^2 = 0.317$, $p = 0.014$). Bullfrogs were associated with FISH and HYDRO along Axis 1 (egg: $r = 0.942$, $r^2 = 0.299$, $p = 0.013$; juvenile: $r = 0.612$, $r^2 = 0.336$, $p = 0.006$; adult: $r = 0.844$, $r^2 = 0.287$, $p = 0.018$), whereas native amphibians were negatively associated (Fig. 2.4). Chorus frog larvae (Axis 1 $r = -0.999$, $r^2 = 0.348$, $p = 0.002$) and long-toed salamander larvae (Axis 1 $r = -0.976$, $r^2 = 0.272$, $p = 0.001$) were strongly negatively associated with FISH and HYDRO, while other native species and life stages were not significantly correlated to the ordination axes (all $p > 0.05$) (Fig. 2.4). Multi-response permutation procedure (MRPP) results indicated that there were significant differences in amphibian species composition between sample units categorized by FISH (non-native fish absent [$N = 12$] vs. non-native fish present [$N = 14$]; $A = 0.020$, $p = 0.041$) and HYDRO (permanent [$N = 10$] vs. seasonal [$N = 16$]; $A =$

0.033, $p = 0.002$), but not MGMT (active [$N = 18$] vs. passive [$N = 8$]; $A = -0.006$, $p = 0.77$).

DISCUSSION

Active vegetation management in Wetlands Reserve Program sites in the Willamette Valley, Oregon is effective at reducing unwanted invasive plant species and increasing plant diversity (also see Evans-Peters et al. 2012). Although amphibian diversity and community composition did not differ between actively and passively managed WRP sites, the impact of management on the wetland plant community indirectly transcended to the native amphibians through the effect on invasive plant cover. Postmetamorphic life stages of Pacific chorus frog and northern red-legged frogs were negatively associated with percent invasive plant cover, and active management reduces the cover of invasive wetland plants. Conversely, neither premetamorphic nor postmetamorphic stages of bullfrogs showed a relationship with percent invasive cover. Reed canarygrass, which dominated the invasive plant community, may provide unsuitable egg deposition substrate for native amphibians because of its thick culm (Watson et al. 2000). Larval amphibian mortality may increase in wetlands choked by dense reed canarygrass cover due to the accumulation of toxic alkaloids and excessive organic input resulting in anoxic conditions (Rittenhouse 2011). These negative effects on early life stages may be reflected in postmetamorphic life stages as decreased adult recruitment. Further, movements of postmetamorphic stages of relatively small-bodied native anurans (as opposed to heavier, large-bodied bullfrogs) may be impeded by the dense above ground biomass and tangled rhizomatous mats formed by reed canarygrass.

Results of our models indicate that bullfrogs may be more tolerant of invasive vegetation at WRP sites. Thus, management actions that reduce the cover of invasive reed canarygrass (and other invasive plants) could improve habitat quality for native amphibians, especially for postmetamorphic red-legged frogs and chorus frogs.

Premetamorphic chorus frogs and postmetamorphic stages of red-legged frogs were positively associated with the additional management variable of WRP site age. The relationship between WRP age and native anuran counts suggests the potential for temporally-explicit recolonization following habitat alteration. The benefits of restored wetlands for native amphibians may increase over time corresponding to vegetative succession and system stability. Bullfrog counts were not associated with WRP age, possibly because bullfrogs often readily colonize and are fairly tolerant of bare-ground habitats characteristic of initial phases of restoration (Porej and Hetherington 2005). Antagonistic encounters between bullfrogs and native species may be influenced by priority effects in timing of colonization and occur more frequently in newly created or restored wetlands, leading to the exclusion of natives. Many studies have found that interactions between bullfrogs or non-native fish and native amphibians are highly context-dependent and mediated by habitat quality (Hayes and Jennings 1986, Adams 1999, Pearl et al. 2005, Adams et al. 2011). For this reason, dense emergent vegetation occurring at later stages of restoration could provide important refuge for native amphibian species (e.g., Kiesecker et al. 2001).

Species-specific and ontogeny-specific differences in native anuran associations with invasive vertebrates were detected. Bullfrog count was a negative predictor of postmetamorphic chorus frog and red-legged frog counts, however non-native fish had

the strongest negative relationship with native anuran counts and occurred in top models for all life stages. While bullfrogs have been implicated in the decline of red-legged frogs in the Willamette Valley (Nussbaum et al. 1983), several studies describe behaviors in native anurans that suggest they are adapting to the presence of bullfrogs. In fact, Hayes and Jennings (1986) reasoned that non-native fish – not bullfrogs – are the strongest contributing factor to rapid frog declines. Kiesecker and Blaustein (1997) found that red-legged frog larvae from populations syntopic with bullfrogs exhibited antipredator behaviors (e.g., reduced activity levels and increased refuge use) when exposed to bullfrog chemical cues. However, experimental studies have documented reduced red-legged frog and chorus frog activity levels, development rates, and survivorship due to exploitative competition from bullfrogs (Kupferberg 1997, Kiesecker et al. 2001). Our study only detected negative effects of bullfrogs on postmetamorphic phases, and this is likely due to asymmetric phenologies of native anurans and invasive bullfrogs. The majority of the chorus frog and red-legged frog postmetamorphic detections were of juveniles, with emergence events corresponding with bullfrog breeding season and an elevated likelihood of encounter. Further information is needed on the long-term dynamics of coexisting native and invasive populations, especially as bullfrogs are increasingly common in seasonal ponds throughout the Willamette Valley (Cook 2011, Cook et al. 2013).

The strongest structuring components of amphibian community composition were the presence of non-native fish and wetland hydrology; management regime did not directly influence the species composition of the amphibian assemblage. Ordination of amphibian communities with respect to hydroperiod and fish revealed a contrast between

native amphibian and bullfrog associations. Native amphibians – especially aquatic larval stages of long-toed salamanders (rarely detected), red-legged frogs, and chorus frogs – were negatively associated with non-native fish and permanent hydroperiods while most life stages of bullfrogs were positively associated. Invasive bullfrogs have a larval period that typically extends beyond one year, so it follows that permanent water bodies will enhance successful development to metamorphosis and subsequent natal pond returns (Boone et al. 2004). Long-toed salamanders, chorus frogs, and red-legged frogs, however, commonly metamorphose within one season in the Pacific Northwest (Jones et al. 2005) and larvae may be afforded greater protection from vertebrate predators in seasonal wetlands (Skelly 1996). NMS allowed us to explore stage-specific responses to habitat and management variables that were not detected through modeling since we did not incorporate HYDRO into *a priori* GLMs.

Consistent with previous studies (e.g., Pearl et al. 2005), we found reduced native amphibian occurrence (especially for rarely-detected long-toed salamanders and northwestern salamanders) but increased invasive bullfrog occurrence at sites inhabited by non-native fish. The rough-skinned newt, however, was an anomaly among native amphibian species, occurring most commonly at sites with non-native fish. This species is highly toxic and unpalatable to many predators (Brodie 1968), offering an explanation for its association with fish. The most frequently encountered species of fish in this study were largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) which coevolved with bullfrogs in their native eastern range (Adams et al. 2003). Amphibians native to the Willamette Valley evolved in the absence of these novel predators; as such, they may not possess innate or learned antipredator behaviors to

respond appropriately to risk (Pearl et al. 2005, Garcia et al. 2012). Conversely, bullfrogs in their invasive western range are facilitated by bluegill sunfish that reduce densities of aeshnid dragonflies that commonly prey on bullfrog larvae (Werner and McPeck 1994, Adams et al. 2003). *Invasional meltdown* (Simberloff and Von Holle 1999) between fish and bullfrogs is known to produce intensified direct and indirect impacts on native amphibians (e.g., Kiesecker and Blaustein 1998).

Urban land cover was negatively associated with counts of postmetamorphic chorus frogs, but was not associated with patterns of community composition which included rare species and life stages. The relative importance of urban land cover varied among species and life stages; this may reflect differences in habitat requirements/specialization, dispersal distance, reproductive potential, and physical tolerances (Cushman 2006). Postmetamorphic chorus frogs are dependent on upland terrestrial habitats and migrate to aquatic breeding sites once winter rains begin (Nussbaum et al. 1983, Bulger et al. 2003). Thus, high quality, connected matrix habitat consisting of forest buffers, wetlands, and minimal human disturbance is expected to facilitate chorus frog dispersal. In contrast to native chorus frogs, red-legged frogs and bullfrogs were unaffected by urban cover. These species have relatively large dispersal distances compared to the chorus frog (Smith and Green 2005), and thus may be less constrained by landscape composition immediately surrounding a central wetland. Also, bullfrogs are tolerant of human modified habitats and chemical contaminants (Smith et al. 2004, Boone et al. 2007), and may even experience competitive release in urban landscapes which are sub-optimal for native amphibians. An additional variable

describing regional agricultural land would have provided further insight into the anthropogenic pressures faced by native amphibians at these sites.

Our results emphasize the importance of accounting for both site-scale and landscape-scale conditions in conservation planning, especially for species that utilize aquatic and terrestrial habitats throughout their annual cycle. By including inter-patch landscape structure as a scoring criteria for WRP wetland locations, practitioners can better provide for the requirements of native amphibians, increase the persistence of viable breeding populations (Lehtinen et al. 1999), and minimize the risk of creating population sinks or ecological traps (Shulse et al. 2010). At the site-scale, invasive species, especially fish, strongly influenced amphibian assemblages. In the Willamette Valley, where flooding events allow for widespread movement of aquatic organisms, it is imperative that managers incorporate landscape-scale dynamics into adaptive strategies. Efforts to restore local and regional habitat quality (e.g., removing invasive plant species, creating vegetative buffers, and reducing human impacts) may benefit native amphibians by indirectly contributing to the resistance of vertebrate invasions (Adams and Pearl 2007). However, the most effective way to enhance native amphibian populations may be through focusing wetland creation in habitats resilient to or removed from non-native vertebrates.

In multi-trophic invaded systems, complex species interactions make management outcomes on wildlife difficult to predict. This study illustrates that strong top-down forces exerted by non-native vertebrate species can be primary regulators of native amphibian abundance and diversity. Restoration ecology currently focuses on bottom-up

effects of invasive plant management on biodiversity, presenting the need for a paradigm shift which also considers higher-order interactions within novel systems.

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TABLES AND FIGURES

Table 2.1 – Variables selected for inclusion in multiple regression models predicting plant species richness, Simpson’s plant diversity, and % invasive plant cover (with direction of predicted effect), based on *a priori* hypotheses informed by literature sources.

Parameter	Identifier	Source	Predicted Effect on Response
WRP active management regime; active or passive	MGMT	Evans-Peters et al. 2012	Active: plant richness (+), Simpson’s plant diversity (+), % invasive plant cover (-)
WRP site age	AGE	Rejmánek 2000; Larson et al. 2001	Plant richness (+), Simpson’s plant diversity (+), % invasive plant cover (-)
Wetland hydroperiod; seasonal or permanent	HYDRO	Miller and Zedler 2003; Boers et al. 2007	Permanent: plant richness (-), Simpson’s plant diversity (-), % invasive plant cover (-)

Table 2.2 – Variables selected for inclusion in multiple regression models for counts of each anuran species (with direction of predicted effect), based on *a priori* hypotheses informed by literature sources.

Parameter	Identifier	Source	Predicted Effect on Response
WRP site age	AGE	Summers 2010	<i>Pseudacris regilla</i> (+), <i>Rana aurora</i> (+), <i>Lithobates catesbeianus</i> (+)
\bar{x} % invasive cover	INVCOV	Rittenhouse 2011	<i>Pseudacris regilla</i> (-), <i>Rana aurora</i> (-), <i>Lithobates catesbeianus</i> (-)
Bullfrog count (log)	LICA	Kiesecker et al. 2001	<i>Pseudacris regilla</i> (-), <i>Rana aurora</i> (-)
Non-native fish presence	FISH	Adams et al. 2003; Werner and McPeck 1994	<i>Pseudacris regilla</i> (-), <i>Rana aurora</i> (-), <i>Lithobates catesbeianus</i> (+)
Wetland hydroperiod	HYDRO	Babbitt 2005	<i>Lithobates catesbeianus</i> (-)
Urban land area within 1000 m	URB1000	Riley et al. 2005	<i>Pseudacris regilla</i> (+), <i>Rana aurora</i> (+), <i>Lithobates catesbeianus</i> (+)

Table 2.3 – Summary of plant and amphibian species diversity at 26 Willamette Valley WRP sites. Richness = average number of species per wetland group (with 95% confidence interval [CI]). Simpson’s index = a measure of diversity that takes into account the number of species present as well as the relative abundance (count) of each species. Gamma diversity = the total number of unique species in the study area. Amphibian species diversity reflects the detection of any life stage, bringing maximum possible site richness to 6.

	<i>N</i>	Plant Diversity			Amphibian Diversity		
		Richness (95% CI)	Simpson’s Index	Gamma	Richness (95% CI)	Simpson’s Index	Gamma
Management Regime:							
Active	18	13.1 (9.77 to 16.45)	0.94	87	3.0 (2.52 to 3.48)	0.76	6
Passive	8	8.8 (5.24 to 12.26)	0.87	42	2.6 (1.86 to 3.39)	0.73	5
Total	26	11.8 (9.22 to 14.32)	0.93	96	2.9 (2.50 to 3.27)	0.75	6

Table 2.4 – Results of model selection using AIC_C to rank competing candidate models for WRP vegetation characteristics which included all combinations of variables of management regime (MGMT), WRP site age since enrollment (AGE), and wetland hydroperiod (HYDRO). The relative support for each model within the candidate set was determined from its AIC_C weight (w_i) ranging from 0 (no support) to 1 (full support). R^2 values represent the amount of variation explained by the model. Only models with $\Delta AIC_C < 2.0$ are reported.

Model	k	ΔAIC_C	w_i	R^2	Estimated slope parameters (95% CI)
Simpson's plant diversity					
MGMT + HYDRO	4	0.0	0.348	0.267	$\beta_{MGMT} = 0.557$ (0.238 to 0.692) $\beta_{HYDRO} = 0.229$ (-0.273 to 0.655)
MGMT	3	0.6	0.261	0.164	$\beta_{MGMT} = 0.558$ (0.205 to 0.698)
Vegetation species richness					
MGMT	3	0.0	0.312	0.111	$\beta_{MGMT} = 1.480$ (0.926 to 2.366)
null (intercept only)	2	0.5	0.246		$\beta_{null} = 10.216$ (8.166 to 12.794)
% invasive plant cover					
MGMT	3	0.0	0.531	0.196	$\beta_{MGMT} = -16.213$ (-30.033 to -2.393)

Table 2.5 – Results of QAIC_C model selection comparing best fit models predicting counts (premetamorph and postmetamorph) of three anuran species as a function of Willamette Valley WRP habitat variables. Only models with $\Delta\text{QAIC}_C < 2.0$ are reported in the table. Each model is accompanied by its QAIC_C value, number of parameters (k), model weight (w_i), and maximum log-likelihood ratio statistic (LL). Where parameter estimates have a 95% confidence interval (CI) that crosses zero, the predictor is considered uninformative. Refer to Table 2.2 for variable codes and Appendix 2.1 for variables considered in each candidate model set.

Model	k	ΔQAIC_C	w_i	LL	Estimated slope parameters (95% CI)
Pacific chorus frog (<i>Pseudacris regilla</i>) – Premetamorph					
PA1: FISH + AGE	4	0.0	0.579	-139.07	$\beta_{\text{FISH}} = -2.061$ (-2.943 to -1.198) $\beta_{\text{AGE}} = 0.176$ (0.037 to 0.314)
Pacific chorus frog – Postmetamorph					
PT1: LICA + INVCOV	4	0.0	0.263	-75.69	$\beta_{\text{LICA}} = -1.310$ (-1.979 to -0.648) $\beta_{\text{INVCOV}} = -0.048$ (-0.090 to -0.008)
PT2: LICA + URB1000	4	1.1	0.149	-76.26	$\beta_{\text{LICA}} = -1.193$ (-1.924 to -0.497) $\beta_{\text{URB1000}} = -2.564$ (-4.308 to -0.042)
PT3: FISH + URB1000	4	1.2	0.147	-76.27	$\beta_{\text{FISH}} = -2.571$ (-4.172 to -1.059) $\beta_{\text{URB1000}} = -2.653$ (-4.388 to -0.177)
PT4: LICA	3	1.7	0.114	-78.08	$\beta_{\text{LICA}} = -0.693$ (-1.006 to -0.384)
Northern red-legged frog (<i>Rana aurora</i>) – Premetamorph					
RA1: FISH + AGE	4	0.0	0.274	-54.21	$\beta_{\text{FISH}} = -5.962$ (-10.582 to -2.857) $\beta_{\text{AGE}} = 0.385$ (-0.036 to 0.967)
RA2: FISH	3	0.0	0.271	-55.77	$\beta_{\text{FISH}} = -3.883$ (-6.282 to -1.605)
RA3: FISH + URB1000	4	1.2	0.152	-54.80	$\beta_{\text{FISH}} = -3.381$ (-5.613 to -1.254) $\beta_{\text{URB1000}} = -2.389$ (-4.747 to 1.203)
RA4: FISH + INVCOV	4	1.9	0.104	-55.18	$\beta_{\text{FISH}} = -3.728$ (-6.045 to -1.541)

					$\beta_{\text{INVCOV}} = -0.047$ (-0.139 to 0.035)
Northern red-legged frog – Postmetamorph					
RT1: FISH + AGE	4	0.0	0.185	-26.79	$\beta_{\text{FISH}} = -3.936$ (-8.026 to -0.912) $\beta_{\text{AGE}} = 0.653$ (0.127 to 1.306)
RT2: LICA + AGE	4	0.3	0.157	-26.95	$\beta_{\text{LICA}} = -1.912$ (-4.207 to -0.304) $\beta_{\text{AGE}} = 0.625$ (0.066 to 1.353)
RT3: INVCOV	3	0.9	0.116	-28.80	$\beta_{\text{INVCOV}} = -0.061$ (-0.128 to -0.002)
RT4: null	2	1.4	0.091	-30.46	$\beta_{\text{null}} = 0.785$ (-0.393 to 2.630)
RT5: LICA	3	1.9	0.070	-29.30	$\beta_{\text{LICA}} = -0.461$ (-1.083 to 0.111)
American bullfrog (<i>Lithobates catesbeianus</i>) – Premetamorph					
LA1: null	2	0.0	0.239	-97.40	$\beta_{\text{null}} = 4.194$ (3.271 to 5.520)
LA2: HYDRO	3	0.5	0.189	-96.23	$\beta_{\text{HYDRO}} = 1.683$ (-0.441 to 4.182)
LA3: URB1000	3	1.9	0.092	-96.94	$\beta_{\text{URB1000}} = 1.370$ (-1.643 to 3.991)
LA4: AGE	3	2.0	0.089	-96.98	$\beta_{\text{AGE}} = -0.126$ (-0.409 to 0.155)
American bullfrog – Postmetamorph					
LT1: FISH	3	0.0	0.265	-101.03	$\beta_{\text{FISH}} = 1.635$ (-0.025 to 3.231)
LT2: null	2	0.7	0.190	-99.29	$\beta_{\text{null}} = 3.670$ (2.941 to 4.628)

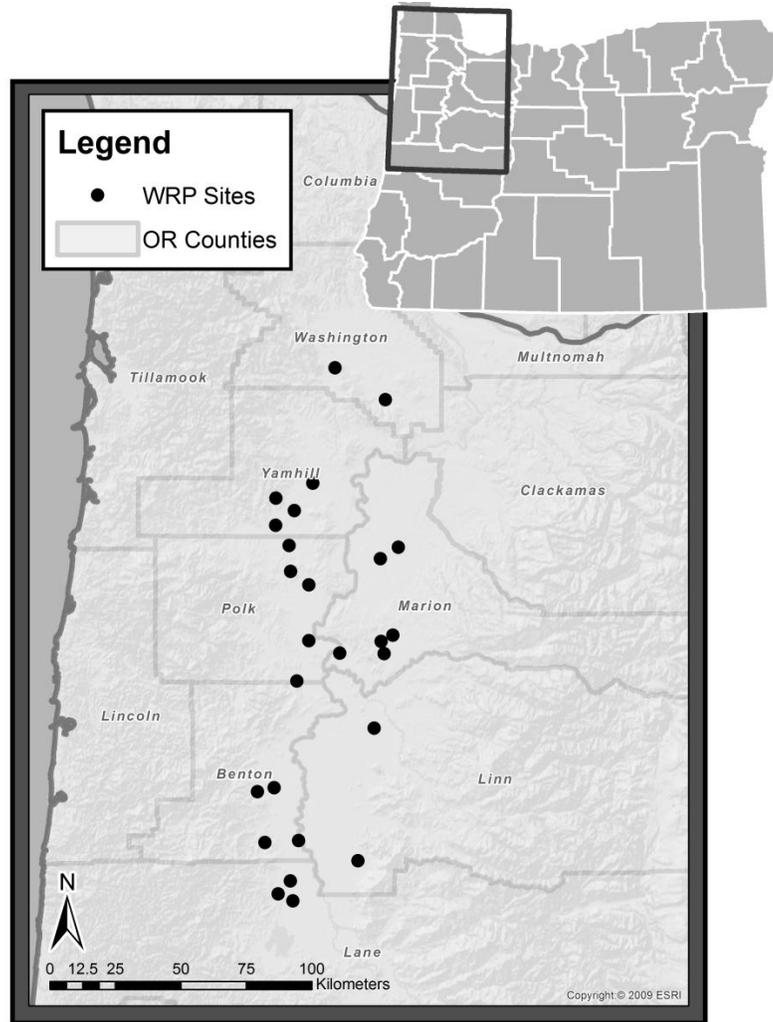


Figure 2.1 – Locations of 26 Wetlands Reserve Program (WRP) wetlands sampled for plants and amphibians in the Willamette Valley, Oregon.

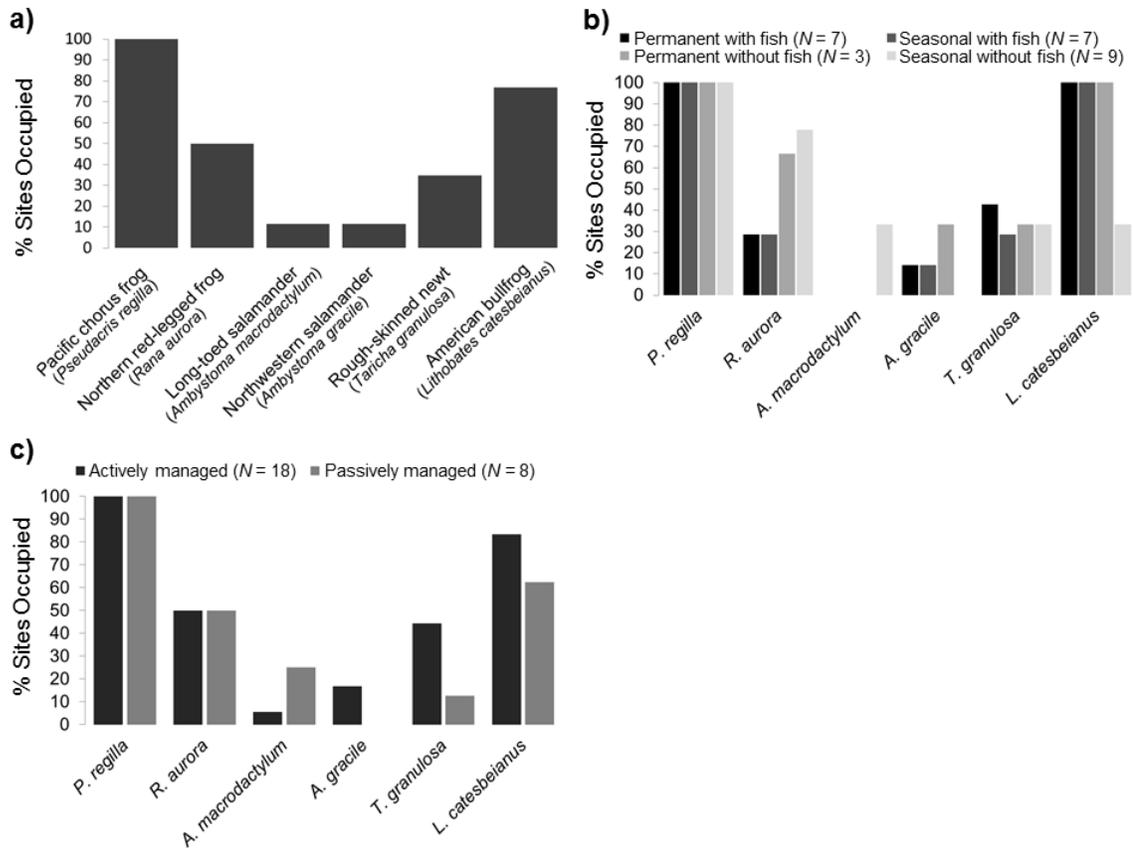


Figure 2.2 – Bar plots depicting the percentage of sites occupied for all six amphibian species detected from visual encounter surveys and dipnet sampling: a) the percentage of all 26 sites occupied by amphibians, b) the percentage of sites occupied, categorized by fish presence and hydroperiod, and c) the percentage of sites occupied, categorized by management regime.

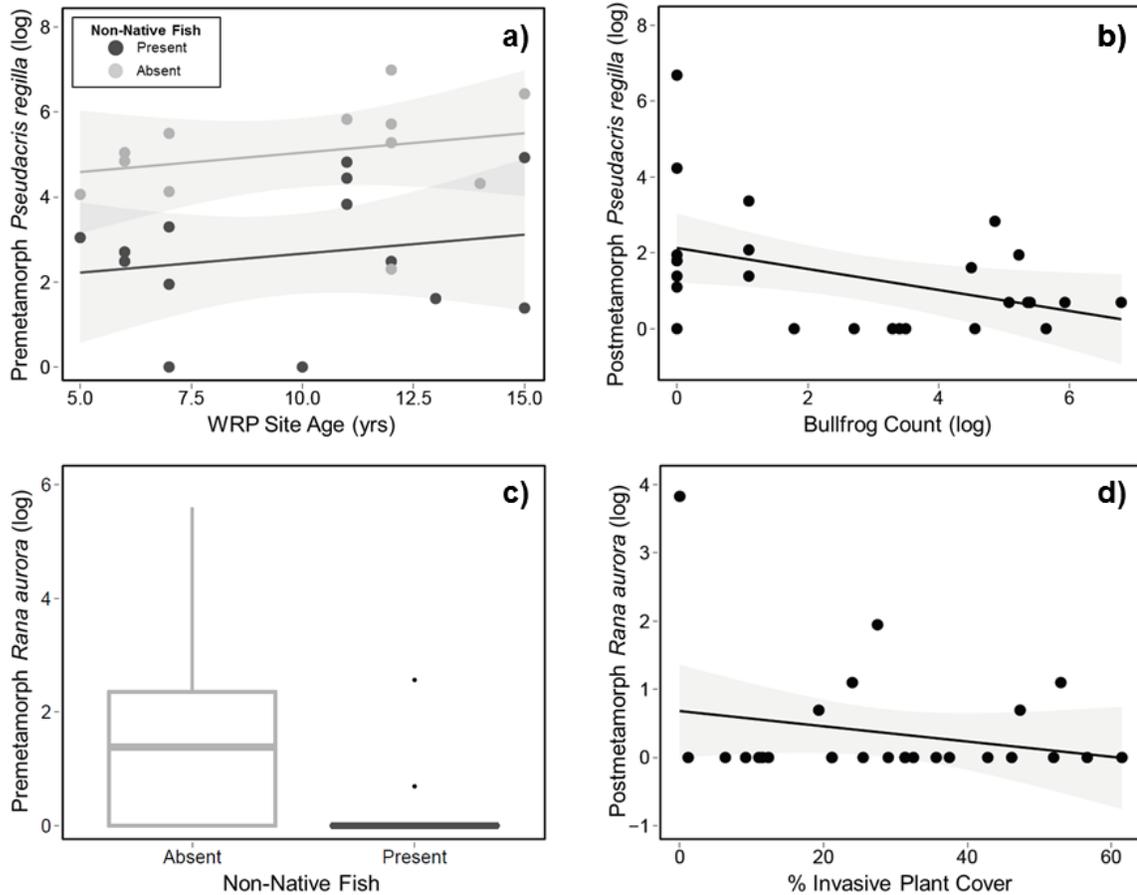


Figure 2.3 – Relationships between predictors included in ‘best’ generalized linear models (selected based on ΔQAIC_C value, number of parameters, and maximum log-likelihood ratio statistic) and log-transformed anuran counts for *Pseudacris regilla* a) premetamorphs (eggs and larvae) and b) postmetamorphs (juveniles and adults), and *Rana aurora* c) premetamorphs and d) postmetamorphs. For a, b, and d, lines represent best fit of means with shaded 95% confidence intervals. Figures representing the best model for premetamorph and postmetamorph *L. catesbeianus* are not shown because no parameters were informative. Refer to Table 2.5 for model selection results.

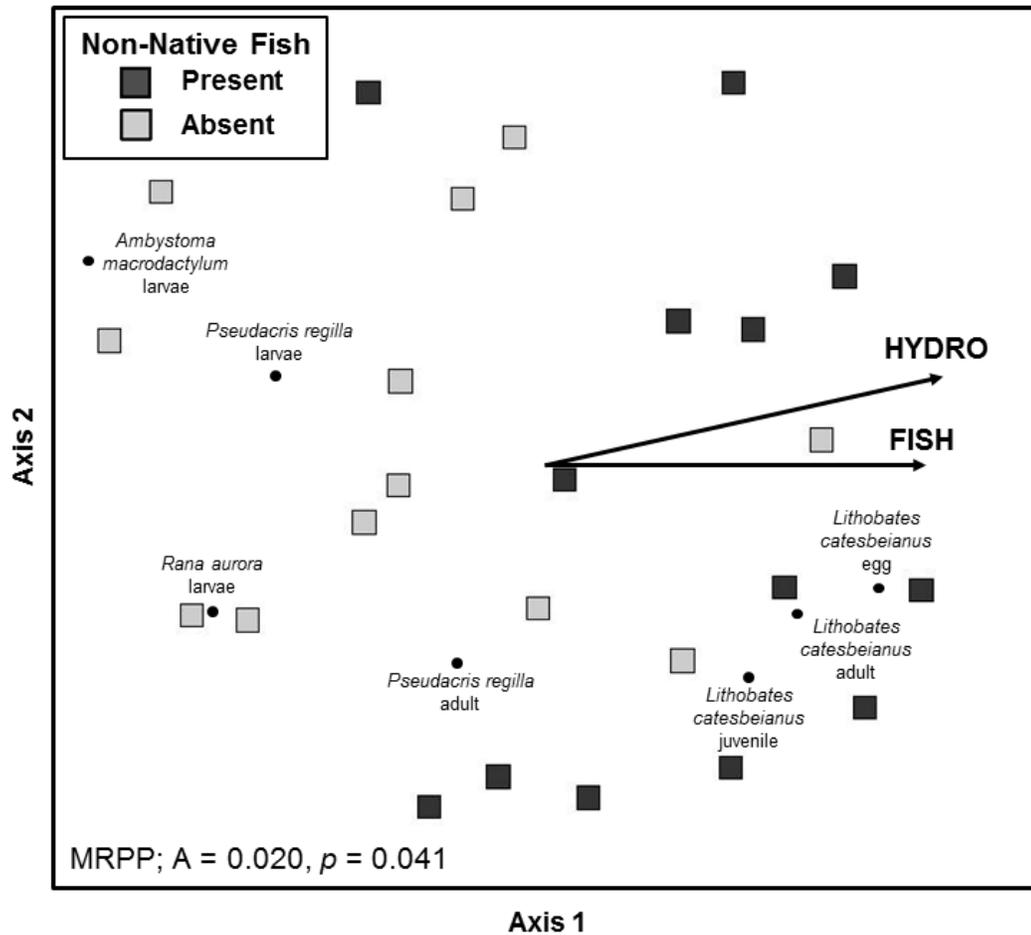


Figure 2.4 – Non-metric multidimensional scaling (NMS) plot of the ordination of sample units in species space overlain with a joint plot showing relationships with the strongest environmental gradients along axes 1 and 2. Only species and environmental vectors that are strongly ($p < 0.05$) correlated to the ordination axes are shown. Sites that are clustered near each other have lower Sørensen distances and thus more similar species composition. Species/life stage relationships are denoted by labeled black dots. Shaded boxes distinguish presence/absence of non-native fish at a wetland site. Significant differences in species composition occurred between wetlands categorized by fish presence (MRPP; $A = 0.020$, $p = 0.041$).

Chapter 3:

**A COMMUNITY ASSESSMENT OF RESTORATION IMPACTS ON
AMERICAN BULLFROG (*LITHOBATES CATESBEIANUS*) DIET**

Jennifer C. Rowe and Tiffany S. Garcia

ABSTRACT

Understanding the dynamics of predator-prey interactions following habitat restoration is challenging in invasive-dominated ecosystems. Prolific invasive predators, such as the American bullfrog (*Lithobates catesbeianus*), can exert strong top-down control of prey, with foraging influenced by vegetation structure regulated through management activities. We sampled vegetation and analyzed bullfrog diet contents from 10 restored wetlands categorized by management intensity and hydrology to determine whether prey consumption patterns (abundance, richness, % large-bodied prey), diet breadth, and dietary community (based on taxonomic and prey size composition) differed among wetland categories. Dietary abundance, richness, and % large prey did not depend on wetland category. However, we found disparities in the diet breadth with respect to wetland categories; bullfrogs consumed a disproportionate abundance of few prey items at actively managed sites. Diet breadth could have been influenced by frequent applications of restoration treatments which reduce invertebrate species richness and abundance. Large-bodied prey consumption was correlated with plant species richness, however diet mass was greatest in wetlands with high invasive cover, suggesting that factors such as foraging activity rates should be considered to fully understand how bullfrogs meet energy demands in managed wetlands. Variation in bullfrog diet as a function of management regime may inform invasion potential and provide an important indicator of how habitat influences trophic dynamics.

INTRODUCTION

The prevailing paradigm in restoration ecology is to return the vegetative community to historical conditions by altering abiotic characteristics and disturbance regimes (Dobson 1997). However, spontaneous community assembly during succession can shift systems toward unanticipated alternative stable states (Suding et al. 2004, Firn et al. 2010). It is difficult to predict how restoration actions will influence colonizing species composition (i.e., response rules) even if the desired plant community and functional characteristics are achieved (Firn et al. 2010). Hence there is a pressing need for research on how higher-order trophic levels respond to alterations in wetland structure and function following restoration efforts (Able et al. 2008). Studies that consider higher trophic levels when evaluating restoration success often use species occupancy or diversity metrics (Ruiz-Jaen and Aide 2005). Rarely is restoration success assessed via interspecific interactions within the existing community, such as predator-prey dynamics or competitive relationships (James-Pirri et al. 2001). These interactions can have significant direct and indirect effects on wetland community structure, and analysis of foraging behaviors through diet contents can capture cascading impacts of restoration efforts across trophic levels (Pomara et al. 2003).

Understanding the dynamics of predator-prey interactions following restoration is challenging in invasive-dominated ecosystems (Firn et al. 2010). Invasive predators can have extensive impacts on community structure and ecosystem function by introducing strong top-down effects (D'Antonio and Vitousek 1992, Mack et al. 2000, Estes et al. 2001, Sih et al. 2010). Generalists are often highly successful biological invaders because a wide diet breadth allows for opportunistic prey switching in response to

resource availability (Caut et al. 2008). Amphibian generalist predators have particularly wide-reaching trophic effects because they exhibit dietary changes paralleling life stage transitions (Fagan and Hurd 1991, Lima and Moreira 1993, Werner et al. 1995, Hirai and Matsui 1999) and transport energy between aquatic and terrestrial linkages (Korschgen and Moyle 1955, Jiang and Morin 2005). In the western United States, the American bullfrog (*Lithobates catesbeianus*) is a widespread, highly successful invasive generalist predator having dramatic impacts on wetland communities (Nussbaum et al. 1983). Although the diet primarily consists of aquatic insects, bullfrogs will opportunistically consume fish, crayfish, amphibians, reptiles, small mammals, and birds (Nussbaum et al. 1983, Korschgen and Moyle 1955). The bullfrog is implicated in the declines of several co-occurring native amphibian species via competition and predation pressure (Nussbaum et al. 1983, Adams and Pearl 2007).

Since direct removal of established bullfrog populations is typically unfeasible, there is considerable interest in understanding the indirect role of habitat features in resisting or facilitating bullfrog invasion (Adams and Pearl 2007). Invasive plants frequently provide inferior resources for native wildlife relative to pre-existing plant species (Rogalski and Skelly 2012), and arthropod richness is often linked to plant richness (Root 1973, Siemann et al. 1999, Brose 2003). Thus, a more diverse prey base may be available for bullfrogs in habitats with diverse plant communities. Further, large-bodied prey may be supported by habitat features such as vegetative complexity (*enemies hypothesis*; Root 1973), high productivity (*species richness productivity relationship*; Jennings and Warr 2003, Šimová et al. 2013), and permanent hydroperiods (Skelly 1996). However, bullfrogs were negatively associated with densely vegetated littoral zones in

several studies (e.g., Pearl et al. 2004, Porej and Hetherington 2005), findings which are corroborated by evidence that dense plant cover reduces bullfrog prey search efficiency and encounter rates (*hunting efficiency hypothesis*; Babbitt and Tanner 1997, Brose 2003, Doubledee et al. 2003). Since invasive plants often reach very high biomass (Reinhardt Adams and Galatowitsch 2005), bullfrogs may forage less successfully at wetlands choked with monotypic stands.

Invasive bullfrogs have the capacity to reach high densities in restored wetlands, posing a challenge to wildlife conservation goals. Bullfrogs commonly occupy sites enrolled in the Wetlands Reserve Program (WRP), a voluntary conservation easement program (NRCS 2012), throughout their Pacific Northwest invasion range. A central objective of WRP wetland restoration is to rehabilitate degraded habitat function by recreating initial plant and hydrologic conditions (NRCS 2009). Consistent with this goal, invasive plant management is commonly applied, with efforts in the Pacific Northwest primarily targeting reed canarygrass (*Phalaris arundinacea*). WRP wetland communities are ideal systems in which to explore restoration impacts on predator-prey dynamics (Rewa 2005, Fleming et al. 2012) given the gradient of management regimes, which range from *passive* (receiving no invasive plant removal treatments within 3 years) to *active* (invasive plant removal treatments applied to >50% of the wetland area at least twice within the past 3 years) (Evans-Peters et al. 2012, Kross et al. 2008).

We posited that differences in plant management regimes would be reflected in bullfrog diets. The bullfrog's prominent ecological role as well as its abundance in Pacific Northwest wetlands makes it an ideal species for which to analyze the effects of restoration on higher-level consumers (James-Pirri et al. 2001). By quantifying diet

characteristics (diet mass, prey abundance, prey taxa richness, and taxonomic/prey size composition) as a function of wetland management regime, we can determine whether management intensity and site-level vegetation covariates influence the forage quality for bullfrogs (Joyce 2002). We expected that invasive plant cover would be lower and plant species richness higher at actively managed wetlands compared to passively managed wetlands. We hypothesized that bullfrog prey consumption would be associated with management and vegetative characteristics, with bullfrogs consuming more diverse (i.e., greater prey taxa richness and wider diet breadth) and larger prey items at actively managed sites. To investigate these predictions, we analyzed bullfrog diet and vegetation composition at WRP sites and determined (1) abiotic predictors of bullfrog diet characteristics, (2) diet mass as a function of management regime, and (3) taxonomic and prey size composition differences by management regime.

MATERIALS AND METHODS

Sampling Design

We selected 26 privately-owned wetlands enrolled in the Wetlands Reserve Program in the Willamette Valley, Oregon based on wetland hydroperiod and landowner permission to access. Bullfrogs were successfully sampled at 11 sites consisting of a mix of permanent ($N = 6$) and seasonal ($N = 5$) wetlands, with management varying in intensity from passive ($N = 4$) to active ($N = 7$) (Fig 3.1). Nine sites contained non-native fish including warmwater centrarchid species and western mosquitofish (*Gambusia affinis*), 1 site contained native threatened Oregon chub (*Oregonichthys crameri*), and 1

site was fishless. All wetlands were separated by a distance of at least 2.5 km to reduce the likelihood of movement of individuals among sites (Petranka et al. 2007).

Bullfrog collection – Wetlands occupied by bullfrogs ($N = 21$) were sampled during peak bullfrog breeding activity (15 July – 18 September, 2011 between 11:00 hrs and 16:00 hrs). Efforts were made to collect at least 5 post-metamorphic individuals per site via hand/net capture and/or hook and line sampling. Up to 3 repeat visits (over three days) were conducted if 5 individuals could not be collected. We immediately euthanized animals following standard protocol by submersing them in a solution of tricaine methanesulfonate (300 mg MS-222/L water; OSU IACUC ACUP 4144). We recorded weight (g), snout-vent length (mm), and sex for each animal and assigned a size class: adult (SVL > 90 mm) and juvenile (SVL < 90 mm) (Werner et al. 1995). Specimens were transported on ice to the laboratory at Nash Hall, Oregon State University, Corvallis, Oregon.

Vegetation community – Vegetation surveys were conducted during peak growing season (May 12 – July 13, 2011) to characterize wetland vegetation covariates. We demarcated 30 sampling points (1 m² quadrats) distributed evenly among 10 transect belts spaced at equal intervals around the wetland perimeter (modified from Mueller-Dombois and Ellenberg 1974). Each 5 m belt was arranged perpendicular to the wetland perimeter and contained three quadrats overlapping the shore zone, the waterline, and the shallow water (< 1 m depth) zone. We estimated % cover of native and invasive plants (identified to the lowest taxonomic group), bare ground, and water present within the quadrats to the nearest 5% (Palmer 1991).

Diet Analysis

We excised the alimentary canal at the anterior and posterior sphincters of the stomachs and fixed them in separate, labeled vials of 95% ethanol for 36 hrs prior to processing contents. Prey items were flushed with dechlorinated water and observed with a dissecting microscope (Wang et al. 2008). Each diet item was classified as small (<9 mm), medium (9-16 mm), or large-bodied (>16 mm) based on mean taxa size from literature sources (size categories following Poff et al. 2006). Three newly-metamorphosed bullfrogs displayed minimal tail vestiges and contained empty stomachs; we excluded these individuals from the final dataset because it was possible the empty stomachs were a function of an incomplete ontogenetic transition to a fully-functional predator morphology (Jancowski and Orchard 2013). Vegetative material was not included in analyses since interest was in quantifying predator-prey relationships, not incidental catch. Total dry mass for each stomach was obtained by drying contents for 24 hrs at 50°C, or until a constant dry mass was achieved (Dodd 2010).

A small percentage of diet items (10% of the entire dataset) were damaged to the point where identification was limited to the order-level. To address inconsistencies in the resolution to which stomach contents were identified, we followed a *remove parent, merge children* (RPMC) method outlined in Cuffney et al. (2007) for handling ambiguous taxa that conserved a high proportion of original taxa richness. We nested the child (lowest taxonomic identification) within the parent (course identification to the order-level) if the ambiguous parent's abundance exceeded that of the children. Otherwise, we removed the ambiguous parent taxa and kept the associated children.

We used these RPMC taxonomic data to describe dietary diversity consisting of prey item abundance (N), dietary richness, and percentage (\bar{x}) of large prey items. We also estimated the index of absolute importance by numbers (*IN*) for all prey items following:

$$IN = \sqrt{A_{Ni}} \times \sqrt{O_i}$$

where A_{Ni} is percentage of numerical abundance for prey i (% of total number of prey items) and O_i is the percentage of stomachs in which prey i occurs (Houlihan et al. 2008). This compound index was used because it provides a more balanced summary of dietary prey importance than a component index (e.g., A_{Ni} or O_i separately) (Liao et al. 2001).

Habitat predictors of dietary response variables – Analyses were carried out at the site scale (sites as replicates) to make inferences to management regime, thus the diet data of individual bullfrogs were averaged for the collection site. To reduce the number of comparisons and maintain adequate variation in predictor values, wetlands were nested within an assigned wetland type (WETTYPE) describing both management regime and hydroperiod (e.g., seasonal-passive, seasonal-active, permanent-passive, and permanent-active). Only one wetland could be categorized as seasonal-passive and only one bullfrog could be collected from this site (containing non-native fish), so we opted to remove this type from the analysis. We conducted one-way analysis of variance (ANOVA) with *post hoc* Tukey's pairwise comparisons to determine whether percentage (\bar{x}) invasive plant cover and vegetation species richness differed between wetland categories. We also regressed bullfrog snout-vent length (SVL) on diet mass and WETTYPE in *R* version 2.15.2 software (*R* Core Team 2012).

We used multiple linear regression analysis in *R* package *bbmle* to identify habitat covariates that significantly influenced bullfrog dietary response variables of average dietary abundance (log-transformed), average prey taxa richness (cube-transformed), and % of large prey items in the diet. To account for the potential influence of unequal stomach numbers collected at wetlands ($\bar{x} = 6.9 \pm 2.1$ stomachs), dietary richness was rarefied using *R*'s *vegan* package by subsampling 5 times without replacement over 1000 iterations. Rarefaction is a procedure used to standardize sampling effort (i.e., number of stomachs collected per wetland) by rescaling larger sample sizes and estimating diversity given a smaller number of samples (Colwell et al. 2004). The iterative process generates the expected number of species (S_n) in a small sample n drawn at random from a larger pool of N samples given by the equation:

$$E(S_n) = \sum_{i=1}^S \left[1 - \binom{N - N_i}{n} / \binom{N}{n} \right]$$

where S is the total number of species in the collection and N_i is the number of individuals of the i th species (Hulbert 1971). Explanatory habitat variables included WETTYPE, site-level mean % invasive plant cover (INVCOV), site-level plant species richness (PLANTRICH), water temperature at the time of bullfrog collection (WATEMP; taken at 10 cm depth 1 m from shore), maximum wetland depth (MAXDEP; deepest point of 10 haphazard samples), and WRP site age (WRPAGE). All pairwise combinations of these predictor variables were assessed for multicollinearity, and only predictors with Pearson coefficients of $r < 0.70$ (Shulse et al. 2010) were used in models. For each response variable, an initial set of five candidate models (including the null intercept-only model) was determined *a priori* based on hypotheses of important

ecological factors (Table 3.1). These candidate models were limited to one explanatory variable to prevent overfitting. Each set of candidate models was ranked according to Akaike's information criterion corrected for small sample sizes (AIC_C ; Akaike 1973) and model weights (w_i). We produced a final confidence set of parsimonious models with lowest AIC_C that adequately explained the variation in the dependent variable. Models with a $\Delta AIC_C < 2.0$ were empirically well supported by the data and considered competitive following examination of residuals and diagnostic plots (Burnham and Anderson 2002).

Diet community analysis – Modified Costello Representations (Amundsen et al. 1996) were produced to qualitatively compare the relationship between prey occurrence and abundance for animals collected across wetland types (Fig. 3.2). The arrangement of diet items in the modified Costello figure provides information about the collective (population-level) diet breadth which can be used to infer the feeding strategy (degree of specialization or generalization).

Non-metric multidimensional scaling (NMS; Mather 1976) was executed in *R* to visually display relationships among diet composition and habitat. Two separate ordinations were performed to visualize differences in the dietary community with respect to wetland categories and habitat variables. Each ordination was overlain with a joint plot to display the strongest correlations between the environmental variables and the ordination axes based on the Pearson's r statistic. The r^2 values represented the correlation between the ordination distance and the distance in original space. The first ordination arranged sites in dietary taxonomic space with respect to the strongest taxa associations with the site. The taxonomic species matrix was comprised of the site

average abundance of prey items at the order-level, with rows representing sites and columns representing taxonomic orders. Columns were relativized by taxon maximum to equalize the influence of disproportionately abundant diet orders (McCune and Mefford 1999). The second ordination arranged sites in functional trait space with respect to habitat variables. The trait (prey size) matrix contained the abundance of dietary items classified as small (<9 mm), medium (9-16 mm), or large-bodied (>16 mm) for each bullfrog averaged to the site-level. The ordination was overlain with habitat variables in the environmental matrix, which included PLANTRICH, INVCOV, WRPAGE, MAXDEP, and WATEMP. Both ordinations were run from a random starting configuration in diet space using the Sørensen (Bray Curtis) distance measure. A non-parametric multi-response permutation procedure (MRPP; Mielke 1984) corresponding to each NMS was performed using the *vegan* package in *R* to quantitatively determine whether there was a difference in community composition (with respect to taxa and prey size) among wetland types using the mean between-site dissimilarity in Sørensen (Bray Curtis) city-block distance. MRPP is a nonparametric procedure (compatible with unbalanced designs) for testing whether there is a significant difference in community distances between two or more groups of sample units, based on environmental characteristics. The resulting *p*-value is associated with the test of the null hypothesis of greater within-group dissimilarity than between-group dissimilarity, using a within-class weighting measure of $n(n-1)$.

RESULTS

The 66 bullfrogs (31 adults, 35 juveniles) collected from 10 wetlands retained for final analyses produced a dataset of 504 diet items (average number per stomach: 7.5 ± 8.4). The percentage invasive plant cover differed significantly between wetland categories of seasonal-active, permanent-active, and permanent-passive (ANOVA; $F_{2,7} = 9.608$, $p = 0.010$). Seasonal-active wetlands contained significantly less invasive plant cover compared to permanent-active wetlands (Tukey's HSD; $p = 0.008$), but all other pairwise comparisons were not significant (p -values > 0.12). Plant species richness ($F_{2,7} = 0.049$, $p = 0.952$) was the same across all wetland groups.

Habitat predictors of diet – AIC_C model selection indicated that no models containing one-or-two variable combinations of WETTYPE and habitat covariates of INVCOV, PLANTRICH, WRPAGE, WATEMP, and MAXDEP adequately explained the variation in the dietary response variables of diet abundance and richness (Table 3.1, Table 3.2). Null models were selected as the best models for these response variables, and although models N2 and R2 satisfied the $\Delta AIC_C < 2$ condition for a competitive model, parameter estimates indicated minimal contribution to the variation in each response (Table 3.2). However, the single best model for the % of large-bodied prey items in the diet contained the predictor PLANTRICH (Table 3.2). There was a significant positive relationship between plant richness and % large prey items ($\beta_{PLANTRICH} = 0.141$, 95% CI: 0.026 to 0.256).

Linear regressions illustrated a significant relationship between diet mass and snout-vent length (SVL) for animals from seasonal-active ($R^2 = 0.236$, d.f. = 8, $p = 0.008$), permanent-active ($R^2 = 0.516$, d.f. = 8, $p = 0.001$), and permanent-passive ($R^2 =$

0.188, d.f. = 8, $p = 0.056$) ponds (Fig. 3.3). Diet mass was significantly greater relative to SVL for animals from permanent-active wetlands ($m = 1.575$) compared to animals from permanent passive ($m = 0.119$, $p = 0.000$) and seasonal-active ($m = 0.537$, $p = 0.002$) wetlands (Fig. 3.3).

Diet community analysis – The modified Costello representation indicated that the degree of dietary specialization differed between wetland types (Fig. 3.4). Although bullfrogs from all wetlands had a wide diet breadth suggesting a generalist feeding strategy, individuals collected from the seasonal-active wetlands utilized two prey orders in disproportionate dominance: Coleoptera ($IN = 47.55$) and Diptera ($IN = 41.98$). Bullfrogs collected from permanent-active wetlands showed dietary specialization on Odonata ($IN = 38.30$). Additionally, bullfrogs consumed a greater abundance of vertebrates in the form of fish, anurans, and caudates in the permanent-active ($IN = 16.64$) and permanent-passive ($IN = 16.22$) wetlands compared to seasonal-active ($IN = 2.11$) wetlands.

There were significant differences in the dietary community composition between wetland categories (MRPP; $A = 0.080$, $p = 0.029$) (Fig. 3.5). Non-metric multidimensional scaling (NMS) ordination of the bullfrog collection sites in taxonomic (order-level) space converged after 20 iterations at a 2-dimensional solution with a minimal stress value of 0.094 ($R^2 = 0.956$, $p = 0.020$). The taxonomic orders most strongly associated with the ordination included Gasteriosteiformes ($r^2 = 0.668$, $p = 0.001$), Gastropoda ($r^2 = 0.774$, $p = 0.004$), Hymenoptera ($r^2 = 0.755$, $p = 0.009$), and Isopoda ($r^2 = 0.755$, $p = 0.014$).

NMS ordination of bullfrog collection sites in functional trait (size) space allowed for the visualization of prey item size in relation to wetland categories and habitat variables (Fig. 3.6). Dietary prey size composition did not differ between wetland categories (MRPP; $A = 0.019$, $p = 0.36$). The ordination converged after 20 iterations at a 2-dimensional solution with a minimal stress value of 0.004 ($R^2 = 0.999$, $p = 0.020$). The strongest habitat associations ($r^2 > 0.330$) with the ordination included MAXDEP ($r^2 = 0.599$, $p = 0.040$), INVCOV ($r^2 = 0.421$, $p = 0.16$), and PLANTRICH ($r^2 = 0.421$, $p = 0.154$). A gradient between large ($r = -0.879$) and small ($r = 0.999$) prey items was observed along Axis 1, while medium prey formed a gradient in the negative direction along Axis 2 ($r = -0.959$). Large prey items were most strongly associated with PLANTRICH ($r = -0.984$).

DISCUSSION

Wetlands categorized by management and hydrologic characteristics differed in % invasive plant cover in a manner not consistent with our *a priori* hypothesis. Previous studies have found significantly less invasive plant cover at actively managed wetlands relative to passively managed wetlands (Evans-Peters et al. 2012, Rowe and Garcia, in review). Conversely, we found % invasive plant cover to be correlated with hydroperiod permanence, not management. Permanent-active wetlands had the highest % invasive plant cover in our study, with intermediate invasive cover at the permanent-passive wetlands, and the lowest invasive cover at seasonal-active wetlands. All wetland groups had similar plant species richness. This is likely a function of our sample size (seasonal-active $N = 4$, permanent-active $N = 3$, and permanent-passive $N = 3$) and may not

represent the typical vegetative community composition relative to management activities in Willamette Valley WRP wetlands.

We found differences in the diet breadth and dietary community composition with respect to wetland groups, but wetland groups did not predict the abundance, richness, or % large-bodied prey taxa in stomachs. Although dietary prey abundance was not influenced by wetland type, the diet mass was. Percentage of large-bodied prey in the diet also was not a function of wetland type, but instead was dependent upon plant species richness.

Bullfrogs from all wetlands exhibited a wide diet breadth, but bullfrogs from the actively-managed ponds consumed some prey items in a disproportionately high abundance. Habitat complexity may increase the diet breadth (generalization) by decreasing overall foraging efficiency (Werner and Hall 1974) and reducing the likelihood of specialization. However, since the actively-managed wetlands varied widely in % invasive plant cover (from low invasive cover in seasonally-active wetlands to high invasive cover in permanent-active wetlands), we cannot infer that foraging was a function of vegetation characteristics. Instead, diet breadth could have been influenced by habitat disturbance from frequent applications of restoration treatments. Invertebrate species richness and abundance is often negatively associated with abiotic change, possibly because of reduced opportunities for recolonization between disturbance events (Death and Winterbourn 1995). Greater biotic homogenization in the prey community may thus be reflected through the reduced diet breadth of bullfrog populations in actively managed sites (Devictor et al. 2008). In relatively stable environments, however, bullfrogs are expected to encounter prey at a rate proportionate to the degree of

overlapping microhabitat use. That consumption will match encounter rates is consistent with the bullfrog's sit and wait predator status and ectothermic physiological constraints which, in accordance with optimal foraging theory (Schoener 1971), may restrict active searching of highest quality food sources.

The prey size distribution did not significantly differ between wetland groups, but instead was strongly influenced by site-level vegetation covariates; large-bodied prey consumption was most strongly linked to plant species richness. Large-bodied organisms in the diet were mostly predatory and included caudates, anurans, fish, water bugs, water striders, and predacious diving beetles. This finding is consistent with the *enemies hypothesis* (Root 1973), which states that diverse plant communities support a greater abundance of herbivores which can be better exploited by large-bodied predators. In addition, the *species richness productivity relationship* links plant richness to system productivity, which influences the trophic chain length and supports larger body sizes (Jennings and Warr 2003, Šímová et al. 2013). Bullfrogs may have higher rates of encounter with large-bodied prey in habitats with high basal productivity and plant richness, resulting in the observed greater proportion of large prey in the diet. For example, bluegill sunfish (*Lepomis macrochirus*) consumed a greater proportion of *Daphnia* prey in the large size class as prey densities increased (Werner and Hall 1974). Our findings suggest that bullfrogs may encounter and consume large prey items more frequently at sites with high plant species richness.

Despite the association between high vegetation species richness and prey size, other factors including prey search efficiency and attack rate should be considered to fully understand how bullfrogs meet energy demands at differently managed wetlands.

Bullfrogs from permanent-active (high invasive cover) wetlands contained the greatest diet mass relative to snout-vent length compared to bullfrogs collected from permanent-passive (intermediate invasive cover) or seasonal-active (low invasive cover) wetlands. This finding suggests that bullfrogs at permanent-active wetlands may be facilitated by invasive plant cover, contrary to our initial hypothesis. Given that invasive bullfrogs are generally more tolerant to habitat alteration relative to native amphibians (D'Amore et al. 2009), it follows that diet may be positively influenced by the presence of invasive cover. For example, invasive common reed (*Phragmites australis*) increased larval bullfrog growth and survival in experimental enclosures (Rogalski and Skelly 2012). As prey themselves, adult bullfrogs may be able to forage for extended periods of time with reduced threat of predation under the cover of densely-growing invasive vegetation (e.g., reed canarygrass) (Rogalski and Skelly 2012). The benefit of increased foraging activity may serve to counteract any hindered search efficiency or attack rate (Brose 2003, Doubledee et al. 2003). Further, we characterized prey size based on the typical length of mature individuals from primary literature sources, not based on direct measurements of diet items. Thus, there may be a disparity between the size of prey available to bullfrogs and the size of prey actually utilized, contributing to the deviance between diet mass and prey size composition.

We found differences in the dietary taxonomic community relative to wetland types, with prey orders Gasterosteiformes, Gastropoda, and Isopoda most strongly associated with permanent-passive (intermediate invasive cover) wetlands and Hymenoptera most strongly associated with permanent-active (high invasive cover) wetlands. Taken along with information about abundance and functional role of prey

items within the habitat, it would be possible to speculate on the effect of bullfrog predation on trophic dynamics and ecosystem processes (Duffy et al. 2007). If bullfrogs consume a disproportionate abundance of a prey item relative to its availability in the habitat, predation may disrupt critical functional dynamics within the system (D'Antonio and Vitousek 1992, Gordon 1998, Cummins et al. 2005). For example, gastropods and isopods are scrapers that principally feed on periphytous algae, and their decline in systems with high primary productivity could result in eutrophication (Cummins and Klug 1979). Further, if bullfrogs consume preferred prey in proportion to environmental availability, information about habitat quality can be obtained from prey bioindicator status. For example, many Hymenopterans are strongly associated with plant richness and habitat complexity in the form of nesting structures such as downed woody debris (Tschardt et al. 1998). Gastropods are often considered bioindicators of heavy metal contamination (Mahmoud and Abu Taleb 2013).

Certain dietary items were of particular interest due to the novelty of their presence or previously undocumented nature. Three-spined sticklebacks (*Gasterosteus aculeatus*), which possess morphological predator defenses, were found in 2.4% of the total diet contents and 7.9% of stomachs. The finding that bullfrogs (juveniles in particular) were consuming this armored fish is consistent with Jancowski and Orchard (2013). Oregon chub (*Oregonichthys crameri*), a federally listed threatened minnow species endemic to the Willamette Valley, was consumed in relatively high abundance at a reintroduction site, comprising 10.0% of the dietary abundance and occurring in 33.3% of bullfrog stomachs. In addition, one stomach contained a very rare beetle (*Donaciella*

sp.). To our knowledge, no other study has documented the consumption of Oregon chub or *Donaciella* by bullfrogs.

An important limitation of this study is that we did not relate bullfrog dietary composition to prey availability. Although numerous studies have described the bullfrog as a generalist predator (e.g., Korschgen and Moyle 1955, Balfour and Morey 1999), we cannot validate that the bullfrog diet in our study reflects the relative availability of prey items as we did not sample the invertebrate community. Our interest was in relating habitat and management characteristics to diet and energy acquisition, irrespective of prey availability. Further, comparisons of diet contents to the prey base are inherently biased by the assumption that an animal samples its environment in the same way the researcher does (Begon et al. 1996). For example, Bouska (2004) found minimal evidence for a correlation between prey use and availability in bullfrogs occupying Willamette Valley wetlands. It is likely that the bullfrog diet varies greatly based on seasonal abundances of prey and position in the water column or terrestrial habitat. Regardless of the prey reserves present in the habitat, the success of bullfrogs in attaining nutritional and energetic requirements in restored wetlands can be directly assessed through dietary composition and mass/volume. As such, dietary information can be used to predict invasion success when long-term comparisons of food webs prior to and after invasion are not available (e.g., Zanden et al. 1999).

Diet contents of generalist invasive predators may be used as a proxy for understanding trophic interactions and resulting impact on ecosystem function over time. Diet analyses of invasive species can provide additional information about the foraging behavior and relative success of important predators in these managed habitats. Insight

into dietary overlap between invasives and their native congeners could prove especially useful for determining competitive relationships in restored wetland communities (Kupferberg 1997, Cohen and Bollens 2008). Invasive bullfrogs and native anurans can occupy similar dietary niches (e.g., Wu et al. 2005), so bullfrogs could significantly transform the resource base for native species (Carlsson et al. 2009). Interspecific differences in diet could reflect partitioned microhabitat use which in turn could increase the probability of co-occurrence (Werner et al. 1995). Multi-trophic assessments are useful for determining the functional impact of invasive predators on recipient systems. One aspect of restoration success may be the ability of a system to resist invasion, in which case habitat quality for an invader may be directly inferred from diet.

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TABLES AND FIGURES

Table 3.1 – Candidate models considering the effect of habitat covariates on dietary response variables (transformed) ranked using Akaike’s information criterion (AIC_c) and model weights (w_i).

Model	Response Variable	Factor	ΔAIC_c
N1	Average N	null	0.0
N2		WRPAGE	1.7
N3		INVCOV	3.0
N4		WATEMP	3.8
N5		WETTYPE	6.0
R1	Rarefied RPMC diet richness	null	0.0
R2		WATEMP	1.9
R3		PLANTRICH	3.5
R4		WETTYPE	3.7
R5		WRPAGE	3.8
L1	Dietary % large-bodied prey items	PLANTRICH	0.0
L3		null	3.0
L4		MAXDEP	5.5
L5		WATEMP	5.7
L6		WETTYPE	6.4

Table 3.2 – Results of AIC_C model selection to compare competing models predicting dietary response variables (transformed) of average N, richness, and % large prey items in the diet as a function of wetland-scale habitat variables. Competing models from an initial pool of *a priori* models (Table 3.1) were ranked by AIC_C and model weight (w_i). Only the best set of linear models ($\Delta AIC_C < 2.0$) are reported. Richness was rarefied to account for unequal sample sizes of stomachs nested at the wetland scale.

Model	k	AIC_C	ΔAIC_C	w_i	Parameter estimate (95% CI)
Average N					
N1: null	2	24.6	0.0	0.539	$\beta_{NULL} = 1.704$ (1.230 to 2.178)
N2: WRPAGE	3	26.3	1.7	0.235	$\beta_{WRPAGE} = 0.079$ (-0.049 to 0.207)
Rarefied RPMC diet richness					
R1: null	2	-3.4	0.0	0.534	$\beta_{NULL} = 1.224$ (1.107 to 1.340)
R2: WATEMP	3	-1.5	1.9	0.206	$\beta_{WATEMP} = 0.018$ (-0.013 to 0.049)
Dietary % large prey					
L1: PLANTRICH	3	26.5	0.0	0.655	$\beta_{PLANTRICH} = 0.141$ (0.026 to 0.256)

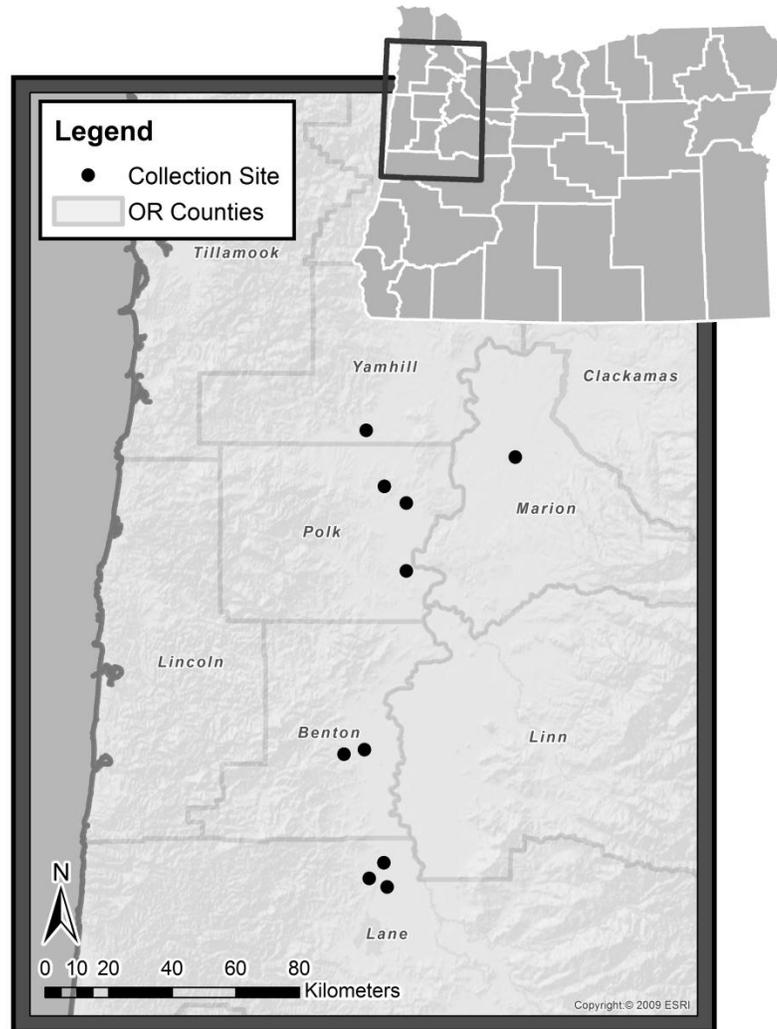
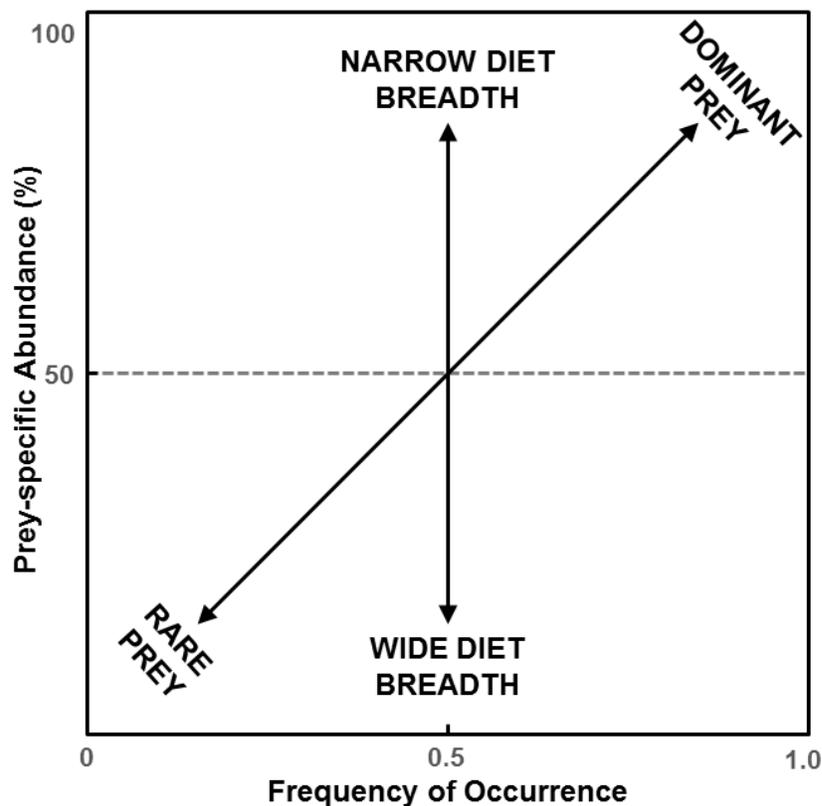


Figure 3.1 – Locations of the 10 Wetlands Reserve Program (WRP) sites in the Willamette Valley, Oregon (retained for analyses) from which bullfrogs were successfully collected.



Modified from Amundsen et al. 1996

Figure 3.2 – Explanation of diet breadth information received from plotting the relationship between the frequency of prey occurrence and prey-specific abundance (%) in a Modified Costello Representation. Items with high specific abundance and low frequency of occurrence reflect dietary specialization on a few individuals, whereas prey with low specific abundance and high occurrence are rarely eaten by most individuals. Prey importance in the diet increases diagonally from the lower left to the upper right of the figure. A wide diet breadth is characterized by the majority of diet items falling in the bottom half of the figure, whereas a narrow diet breadth is characterized by most items falling in the top half of the figure. Modified from Amundsen et al. 1996.

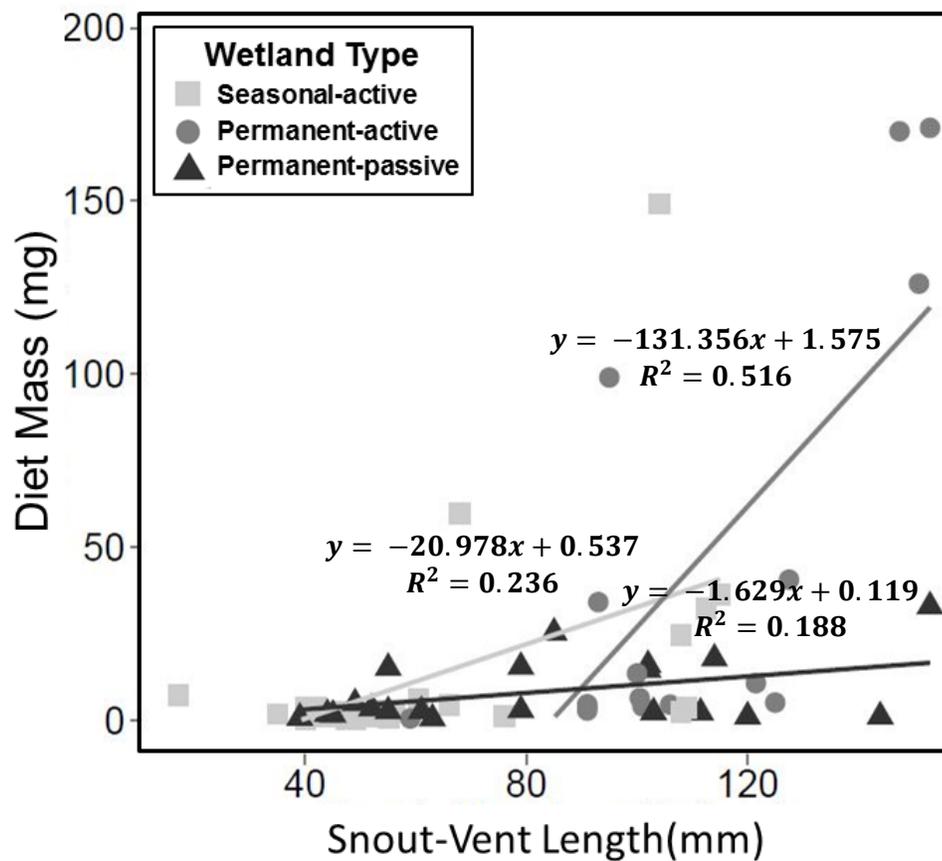


Figure 3.3 – Diet dry mass as a function of snout-vent length (SVL) for bullfrogs collected at seasonal-active wetlands ($n = 29$), permanent-active wetlands ($n = 17$), and permanent-passive wetlands ($n = 20$). Results of linear regressions showed a significant positive relationship between diet mass and SVL for animals from seasonal-active ($R^2 = 0.236$, $p = 0.008$), permanent-active ($R^2 = 0.516$, $p = 0.001$), and permanent-passive ($R^2 = 0.188$, $p = 0.056$) ponds. Bullfrogs from permanent-active showed a significantly greater increase in diet mass relative to SVL compared to bullfrogs from permanent-passive ($p < 0.001$) and seasonal-active ($p = 0.002$) wetlands. The relationship between SVL and diet mass did not differ significantly between bullfrogs collected from permanent-passive and seasonal-active wetlands ($p = 0.134$).

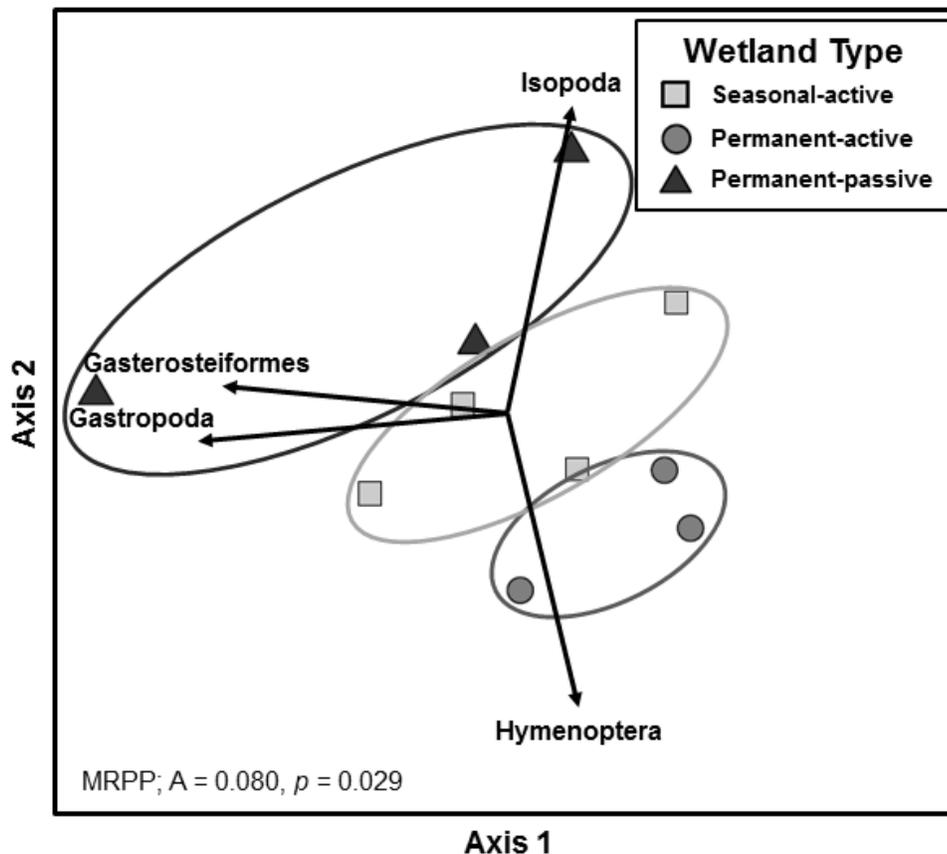


Figure 3.5 – Non-metric multidimensional scaling (NMS) ordination of the bullfrog collection sites in taxonomic (order-level) space, with strongest taxon associations ($p < 0.05$) overlaid as a joint plot. The taxon matrix contained the abundance of dietary items from each stomach averaged to the site-level. The ordination converged after 20 iterations at a 2-dimensional solution with a minimal stress value of 0.094 ($R^2 = 0.956$, $p = 0.020$). Sites are categorized by wetland type, with hulls grouping sites of the same classification. Based on the results of the multi-response permutation procedure (MRPP), there were significant differences in taxa composition between wetland categories ($A = 0.080$, $p = 0.029$).

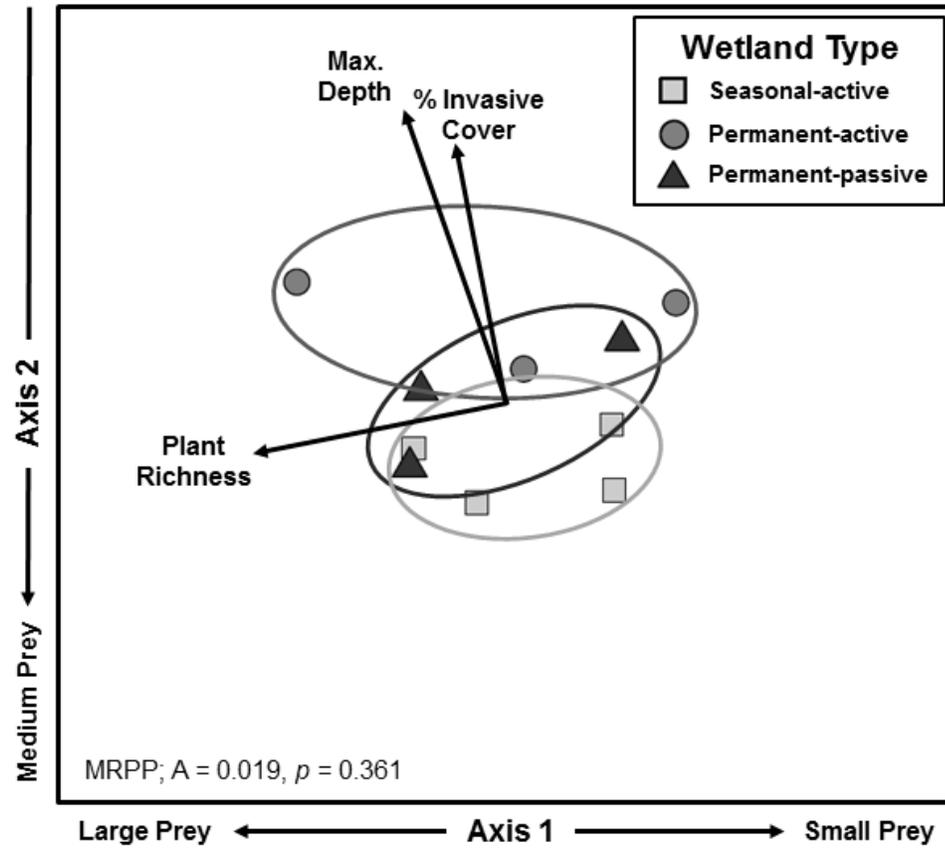


Figure 3.6 – Non-metric multidimensional scaling (NMS) ordination of the bullfrog collection sites in trait (prey size) space, with strongest habitat associations ($r^2 > 0.330$) overlaid as a joint plot. The functional trait matrix contained the abundance of dietary items classified as small (<9 mm), medium (9-16 mm), or large-bodied (>16 mm) for each bullfrog averaged at the site-level. The ordination converged after 20 iterations at a 2-dimensional solution with a minimal stress value of 0.004 ($R^2 = 0.999$, $p = 0.020$). Sites are categorized by wetland type, with hulls grouping sites of the same classification. A gradient between large prey items ($r = -0.879$) and small prey items ($r = 0.999$) was observed along Axis 1, while medium prey formed a gradient in the negative direction along Axis 2 ($r = -0.959$). Based on the results of the multi-response permutation procedure (MRPP), there were no significant differences in dietary size composition between wetland categories ($A = 0.019$, $p = 0.36$).

Chapter 4:

CONCLUSIONS

This thesis provided valuable insight into the factors contributing to the abundance (counts) and diversity of amphibian species in restored wetlands. Chapter 2 explored the relative effects of invasive plant management, biotic interactions with non-native species, and regional landscape quality on an amphibian assemblage while accounting for ontogenetic-specific differences. Chapter 3 presented an analysis of bullfrog dietary patterns relative to habitat characteristics at restored wetlands.

Each of these chapters was connected by our broader interest in understanding bullfrog invasion dynamics and the potential mechanisms that may allow them to persist in multi-invader restored wetland communities. In Chapter 2, we revealed differences between the habitat characteristics associated with high counts of native anurans and invasive bullfrogs. Bullfrogs, in general, were tolerant of other non-native species (especially fish), while native amphibians were negatively associated. In addition, there was no relationship between bullfrog abundance and Wetlands Reserve Program (WRP) site age, suggesting that bullfrogs are unaffected by habitat disturbance and temporary disruption of community dynamics during initial bare-ground phases of restoration. By quantifying relationships between bullfrog diet composition and habitat in Chapter 3, we gained insight into foraging behaviors that might promote bullfrog success in differentially managed wetlands. For example, bullfrogs had a greater diet mass relative to body size at sites containing greater % invasive plant cover, but they consumed a greater proportion of large-bodied prey items at sites with high plant species richness. This finding reflects a dichotomy between the resources available and what is actually

used, suggesting that bullfrogs may be able to better attain nutritional requirements at wetlands with high % invasive plant cover.

Since WRP active management primarily targets invasive plant species, we were particularly interested in understanding species-specific and ontogenetic-specific relationships with invasive plant cover (a proxy for management) in Chapter 2. We opted to use an observational approach to answer this question in an effort to conserve ecological realism for species occupying dynamic environments. However, this study would have greatly benefited from the addition of a controlled factorial experiment to test the effect of manipulated invasive plant and native plant densities on growth and survival of amphibians (native and invasive). Similarly, a field experiment consisting of replicated treatment plots could have also allowed for greater control over the conditions explaining amphibian associations while adding an element of ecological relevance. Logistical constraints in time and resources hindered the execution of these more rigorous designs. We feel as if our use of an observational study to explore our suite of hypotheses was appropriate given the desire to understand how WRP management translates to amphibians which face complex trophic interactions within a community context (Johnson and Omland 2004).

An inherent challenge to Chapter 2 was the inability to make robust comparisons of counts between all six amphibian species occupying the Willamette Valley due to differing life histories and breeding phenologies. Interpretation of multi-species abundance or occupancy models is often constrained by biased count data resulting from imperfect detection. This problem may be especially apparent in herpetological studies, since detection rates may differ as a function of habitat covariates and species may vary

in cryptic color patterns, activity levels, and breeding phenologies (Mazerolle et al. 2007). For these reasons, we use ‘count’ in place of ‘abundance’ to emphasize the estimate of a true population value. In this study, every effort was made to select sampling periods based on the most likely detection window for each species in the assemblage, but detection may have been biased toward late-season breeders (e.g., chorus frogs, red-legged frogs, rough-skinned newts, and bullfrogs) over early-season breeders (e.g., long-toed salamanders and northwestern salamanders). In addition, our survey methodology may have yielded higher detectability to conspicuous anurans (chorus frogs, red-legged frogs, and bullfrogs), breeding adult rough-skinned newts, and larvae of all species as opposed to adults of fossorial mole salamanders (long-toed salamanders and northwestern salamanders). Although the potential for detection bias is worth noting, we opted not to use occupancy models because binary presence-absence data provides less ecological information (Welsh et al. 2013). Ideally, we would have used abundance models accounting for detectability in open populations, however at present these models only exist for single-species analyses (e.g., Royle et al. 2007) or multi-species closed populations (e.g., Waddle et al. 2010). Since amphibian breeding seasons are inherently characterized by emigration and immigration events, the assumption of a closed population for which to estimate species-specific detectability through mark-recapture techniques would be unrealistic for our system. Further, recent simulation data suggests that occupancy models correcting for detection probabilities can have similar bias and even greater estimate variance compared to unadjusted models (Welsh et al. 2013).

We relied on community analysis to elucidate patterns in biotic communities relative to habitat characteristics. In Chapter 3, we examined bullfrog diet contents to

determine whether foraging patterns and prey item species and size (proxy for nutritional gain per unit effort) composition were a function of wetland management and habitat covariates. Initially, this chapter materialized from a question concerning whether bullfrog diet contained a disproportionately high abundance of native amphibians at actively managed or passively managed sites. However, limitations in the attainment of a large bullfrog sample size suitable for these comparisons required us to abandon this focus. Thus, no inference could be made into the potential for habitat mediation in antagonistic encounters between bullfrogs and native amphibians. This question would again need to be tested in an experimental setting to allow for the quantification of bullfrog prey attack rate as a function of direct manipulations of vegetation structure and composition. Still, we presented a novel approach to understanding impacts of restoration on an invader using diet contents, which could prove valuable in rapid assessments when multi-year studies of communities are not feasible.

Chapter 3 could have been supplemented with information on the macroinvertebrate prey community at the wetland sites. However, sampling would need to be compatible with the way bullfrogs sample their environment, otherwise comparisons between prey use and availability are not valid. Stable isotope analysis could have provided additional information on the food web structure as well as trophic position of prey consumed by bullfrogs long-term (Zanden et al. 2004). This approach could provide insight into the significance of bullfrog predation on energy flow within restored wetland system, with subsequent applications to ecosystem function.

A community-level approach to understanding bullfrog invasion allowed us to elucidate a multi-trophic picture of important players in invasion facilitation or resistance.

The results of this study shed light into the abiotic and biotic interactions at local and regional scales that support bullfrog populations and reduce native amphibian populations. The scope of this study included restored wetlands in the WRP in the Willamette Valley, OR, but results can be applied to other regions where bullfrog invasion threatens native biota. The development of management recommendations was not central to our objectives, but this thesis augments a gap in current understanding of the contributions the WRP is making to amphibian conservation. Results suggest that active invasive plant management could have positive impacts on native species, with the caveat that many questions still remain. However, the greatest benefit to native amphibians may come from habitat intervention which suppresses encounters with non-native fish. Native amphibians may greatly benefit from well-planned restoration projects which take an adaptive, holistic management approach and target resources on the most destructive factor contributing to population declines. Further studies are needed to understand the intricate dynamics present in multi-trophic invaded communities, especially as novel ecosystems become the norm and native populations face increasing risk of extinction.

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APPENDECES

Appendix 2.1 – Candidate model sets considered in multiple regression model selection to determine important predictors of abundance (summed highest encounters) over three sampling periods for both premetamorph (eggs and larvae) and postmetamorph (juveniles and adults) life stages of Pacific chorus frogs (*Pseudacris regilla*), northern red-legged frogs (*Rana aurora*), and invasive American bullfrogs (*Lithobates catesbeianus*). See Table 2.2 for descriptions of identifiers.

<i>Pseudacris regilla</i>					
Premetamorph			Postmetamorph		
Candidate			Candidate		
Model #	Ecological Model	ΔQAIC_C	Model #	Ecological Model	ΔQAIC_C
PA1	AGE + FISH	0.0	PT1	LICA + INVCOV	0.0
PA2	FISH	2.5	PT2	LICA + URB1000	1.1
PA3	FISH + LICA	3.6	PT3	FISH + URB1000	1.2
PA4	FISH + INVCOV	5.4	PT4	FISH + INVCOV	1.7
PA5	FISH + URB1000	5.5	PT5	LICA	2.0
PA6	LICA	5.5	PT6	FISH	2.0
PA7	LICA+ AGE	6.8	PT7	FISH + AGE	3.7
PA8	LICA + INVCOV	7.8	PT8	FISH + LICA	3.9
PA9	LICA + URB1000	8.4	PT9	LICA + AGE	4.2
PA10	AGE	12.3	PT10	INVCOV	7.5
PA11	null	13.0	PT11	INVCOV + URB1000	7.9
PA12	AGE + URB1000	14.7	PT12	URB1000	8.3
PA13	AGE + INVCOV	15.1	PT13	URB1000 + AGE	9.3
PA14	URB1000	15.3	PT14	AGE + INVCOV	11.1
PA15	INVCOV	15.8	PT15	null	11.5
PA16	INVCOV + URB1000	18.1	PT16	AGE	12.8

<i>Rana aurora</i>					
Premetamorph			Postmetamorph		
Candidate			Candidate		
Model #	Ecological Model	ΔQAIC_C	Model #	Ecological Model	ΔQAIC_C
RA1	AGE + FISH	0.0	RT1	FISH + AGE	0.0
RA2	FISH	0.0	RT2	LICA + AGE	0.3
RA3	FISH + URB1000	1.2	RT3	INVCOV	0.9
RA4	FISH + INVCOV	1.9	RT4	null	1.4
RA5	FISH + LICA	3.1	RT5	LICA	1.9
RA6	LICA	4.1	RT6	AGE + INVCOV	2.2
RA7	null	4.4	RT7	FISH	2.2
RA8	URB1000	5.4	RT8	AGE	2.3
RA9	INVCOV	6.1	RT9	LICA + INVCOV	2.7
RA10	INVCOV + LICA	6.5	RT10	FISH + INVCOV	2.8
RA11	LICA + AGE	6.8	RT11	URB1000	4.3
RA12	AGE	7.1	RT12	INVCOV + URB1000	4.4
RA13	LICA + URB1000	7.6	RT13	LICA + URB1000	4.5
RA14	AGE + INVCOV	8.5	RT14	URB1000 + FISH	4.7
RA15	URB1000 + INVCOV	8.7	RT15	FISH + LICA	5.0
RA16	URB1000 + AGE	9.0	RT16	URB1000 + AGE	5.2

<i>Lithobates catesbeianus</i>					
Premetamorph			Postmetamorph		
Candidate			Candidate		
Model #	Ecological Model	$\Delta QAIC_c$	Model #	Ecological Model	$\Delta QAIC_c$
LA1	null	0.0	LT1	FISH	0.0
LA2	HYDRO	0.5	LT2	null	0.7
LA3	URB1000	1.9	LT3	FISH + HYDRO	2.7
LA4	AGE	2.0	LT4	FISH + URB1000	2.7
LA5	FISH	2.6	LT5	AGE + FISH	2.8
LA6	HYDRO + URB1000	2.7	LT6	INVCOV	2.9
LA7	INVCOV	3.0	LT7	INVCOV + FISH	3.0
LA8	INVCOV + HYDRO	3.5	LT8	URB1000	3.0
LA9	AGE + HYDRO	3.6	LT9	AGE	3.3
LA10	FISH + HYDRO	4.6	LT10	HYDRO	3.3
LA11	AGE + URB1000	4.8	LT11	INVCOV + AGE	3.5
LA12	FISH + URB1000	5.0	LT12	INVCOV + HYDRO	5.5
LA13	INVCOV + URB1000	5.0	LT13	INVCOV + URB1000	5.6
LA14	INVCOV + AGE	5.0	LT14	AGE + URB1000	5.8
LA15	INVCOV + FISH	5.7	LT15	HYDRO + URB1000	6.4
LA16	AGE + FISH	5.9	LT16	HYDRO + AGE	6.4

Appendix 3.1 – Bullfrog diet note published in *Herpetological Review*

The American bullfrog (*Lithobates catesbeianus*) is an aggressive competitor and generalist predator that is profoundly impacting ecological communities throughout its invasive range in the western United States (Nussbaum et al. 1983. *Amphibians and Reptiles of the Pacific Northwest*. University of Idaho Press, Moscow. 336 pp.). In Oregon, the bullfrog is implicated in the declines of several native species of conservation concern, including the northern red-legged frog (*Rana aurora*), the Oregon spotted frog (*Rana pretiosa*), and the Western pond turtle (*Clemmys marmorata*) (Nussbaum et al. 1983). This is the first report that bullfrogs predate the Oregon chub (*Oregonichthys crameri*), a threatened minnow species endemic to the Willamette River basin of Oregon (Markle et al. 1991. *Copeia* 2:277-293). Prior to this account, only anecdotal evidence for a predator-prey interaction between bullfrogs and Oregon chub was available based on observations that the two species often occur syntopically in warm-water, off-channel habitats (Snyder 1908. *Bull. U.S. Bur. Fish.* 27:153-189). Scheerer (1999. *Fish Research Project, Annual Progress Report EF-91 VII-1*. Portland, Oregon. 24 pp.) and Scheerer and Apke (1998. *Fish Research Project E96970022, Annual Progress Report*. Corvallis, Oregon) previously noted that the largest chub populations were found at Willamette Valley basin sites devoid of bullfrogs, but subsequent diet analysis did not find evidence of bullfrog predation on chubs.

Our study site (44.4528°N, 123.3750°W) is a privately owned wetland located south of Corvallis, Oregon in the Beaver Creek drainage of the mainstem Willamette River. This wetland is part of a 30 acre restoration project initiated in 1992 consisting of

three permanent ponds which are seasonally connected through flooding. The site is also unique in that it serves as prominent Oregon chub reintroduction habitat under the Oregon Chub Recovery Plan (USFWS 1998. Recovery Plan for the Oregon Chub. Portland, OR. 86 pp.). A total of 573 chub from three source populations (DeHaan et al. 2010. United States Fish and Wildlife Service, Abernathy Fish Technology Center. Final Report) were introduced between 1997 and 1998 (USFWS 2010. Fed. Reg. 75:11010-11067). Also inhabiting this site are northern red-legged frogs (threatened), western pond turtles (sensitive species), and invasive American bullfrogs.

We collected a total of 6 adult bullfrogs on August, 9 2011 via hook and line sampling as part of a regional study on invasive species community dynamics. We euthanized animals following standard protocol and recorded measurements for weight, snout-vent length, and sex. Stomachs were excised and fixed in 95% ethanol for 36 hrs before contents were processed. Prey items were flushed with dechlorinated water and observed with a dissecting microscope to aid in identification to the lowest possible taxon. We aggregated contents to taxonomic order and assigned values of absolute and relative abundance (total count; N and N%) and frequency (number of stomachs containing item; F and F%), as well as occurrence (percentage of 6 stomachs containing item; O%) (Table 1).

Table 1. Diet of six bullfrogs collected from wetland in Corvallis, OR showing absolute and relative values of abundance (N and N%), frequency (F and F%), and occurrence (O%).

Prey Order	N	N%	F	F%	O%
Anura	3	10.0	2	8	33.3
Aranae	1	3.3	1	4	16.7
Caudata	2	6.7	2	8	33.3
Coleoptera	6	20.0	4	16	66.7
Cypriniformes	3	10.0	2	8	33.3
Diptera	5	16.7	4	16	66.7
Hemiptera	7	23.3	4	16	66.7
Hymenoptera	1	3.3	1	4	16.7
Odonata	2	6.7	2	8	33.3
Plant material	-	-	3	12	50.0
Total	30	100.0	25	-	-

A total of 30 prey items were found in the 6 stomachs, with orders Hemiptera and Coleoptera representing the greatest percentage of the diet (23.3% and 20%, respectively). Three Oregon chub (order Cypriniformes) were found in 2 out of 6 stomachs (33.3%). Although some digestive decomposition had occurred to the three specimens, enough tissue and distinguishing morphological features had been preserved for positive identification by Oregon State University ichthyologist, Doug Markle. Other vertebrates present in the diet included juvenile bullfrogs (order Anura), which were found in one-third of stomachs constituting 10% of the total diet, and northwestern salamanders (order Caudata; *Ambystoma gracile*), which were found in one-third of the stomachs and represented 6.7% of the total diet.

Since the Oregon chub's listing under the Endangered Species Act in 1993 (58 FR 53800; October 18, 1993), the species' conservation status has improved from *endangered* to *threatened* in part due to actions outlined in the Oregon Chub Recovery Plan published in 1998 (USFWS 1998). Recovery strategies were enacted to reverse the

decline of this species by protecting existing populations and designating critical habitat throughout its historic range (USFWS 1998; USFWS 2010b. Fed. Reg. 75: 21179-21189). At the time of Oregon chub reintroduction to our study site, threats to habitat integrity were identified as bullfrogs and agricultural runoff (USFWS 1998), although current management concerns are primarily over water quality (USFWS 2010). Our findings emphasize the importance of bullfrog removal and resistance strategies at chub reintroduction sites, especially since bullfrogs and chub have similar habitat requirements and frequently co-occur with other species of high conservation priority. Oregon chub continue to face synergistic pressures from habitat loss and invasive species, so carefully planned reintroductions and persistent bullfrog management will increase suitable habitat and aid in chub population recovery.

Stomach content specimens deposited in the laboratory in 174 Nash Hall, Corvallis, Oregon, 97331, USA.

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