Restoration Plan for Wocus (*Nuphar lutea polysepala*) in Upper Klamath and Agency Lakes, Oregon

Prepared for The Klamath Tribes

By

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Restoration Plan for Wocus (*Nuphar lutea polysepala*) in Upper Klamath Basin, Oregon

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Approved:____

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I. GLOSSARY

Nuphar lutea	Wocus parent species; other common names include Yellow Pond Lily and Yellow Water Lily
TKT	The Klamath Tribes
TNC	The Nature Conservancy
UKB	Upper Klamath Basin
UKAL	Upper Klamath and Agency Lakes
USFWS	U.S. Fish and Wildlife Service
Wocus	The Klamath language common name for *Nuphar lutea polysepala*
WRD	The delta of the Williamson River, a major tributary to Upper Klamath Lake; this term also refers to The Nature Conservancy's Williamson River Delta Preserve

II. ACKNOWLEDGMENTS

The restoration plan was prepared by Megan Skinner from The Klamath Tribes, with assistance from Christine Adelsberger (U.S. Fish and Wildlife Service) and Blake Eldridge and Heather Hendrixson of The Nature Conservancy. The Klamath Tribes gratefully acknowledge The Nature Conservancy's decade-long dedication to Wocus reintroduction in the Upper Klamath Basin. The Nature Conservancy's efforts have resulted in significant refinements in Wocus transplanting methods, which are of central importance to future reintroduction and research efforts in the basin. In particular, The Klamath Tribes acknowledge current The Nature Conservancy employees Heather Hendrixson, Blake Eldridge, Lori Nussbaum, and past employees Alix Lee, Natanya Hayden, Charlie Erdman, Melody Warner, Eli Berman, Carla Ross, Mel Van Scoyc, and Kellen Watson for their Wocus-related efforts. Additionally, assistance from former employees of The Klamath Tribes (Tony LaGreca) and U.S. Fish and Wildlife Service (Ruth Olson and John Reins) has been essential to this restoration process. Finally, Perry Chooktoot, director of The Klamath Tribes Culture and Heritage Department, provided valuable guidance and historical information about the significance of Wocus to indigenous communities in the Upper Klamath Basin.

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III. EXECUTIVE SUMMARY

Current species status

It is reasonable to assume that current Wocus populations in the lakes are only a fraction of what they once were given the extent of wetland reclamation during the 20th century. Currently, Wocus covers approximately 600 acres in UKAL and is largely confined to existing fringe wetlands, particularly those within the Upper Klamath Wildlife Refuge. It appears that current Wocus populations in the lakes are not sufficient to recolonize newly restored or emerging habitat without active reintroduction efforts.

Species habitat requirements and ecology

Preferred *Nuphar lutea* habitat includes organic mud and silt substrates in 2-8 feet deep eutrophic water protected from wind and wave action. Large *Nuphar lutea* colonies, sometimes many acres in size, occur where habitat is optimal. Isolated individuals may occur where conditions for establishment are hyperlocal in nature.

When compared with unvegetated areas of lakes, *Nuphar lutea* had a limited effect on water transparency, dissolved oxygen concentrations, and conductivity. However, several studies found significantly reduced sediment resuspension and light penetration in the water column during the growing season. The presence of *Nuphar lutea* also significantly increased sediment organic matter and sediment oxygen demand relative to open water habitats. It appears that *Nuphar lutea* rhizomes are capable of resorbing approximately 70% of nitrogen and phosphorus contained within leaves during senescence. Additionally, uptake of phosphorus through *Nuphar lutea* roots often decreases phosphorus sediment concentrations such that less is released into the water column when sediment is resuspended.

Macrophytes such as *Nuphar lutea* provide habitat for epibenthic, epiphytic, and mobile invertebrates. Specifically, Chironomidae, Ephermeroptera, and Ceratopogonidae densities were significantly higher in *Nuphar lutea* stands relative to unvegetated control plots. Additionally, *Daphnia* populations were significantly higher and individuals were significantly larger in high density *Nuphar lutea* patches relative to medium or low density patches, suggesting that high plant density limited *Daphnia* predation by Perch (*Perca fluviatilis*). It is reasonable to assume that *Nuphar lutea* beds also provide effective shelter for planktivorous or benthic-feeding fish seeking to avoid piscivorous predators.

Restoration strategy

Large, self-sustaining Wocus colonies are most common in the vicinity of functioning wetland habitat; because over half of the key Wocus habitat in UKAL has been drained since 1889, a key long-term Wocus restoration objective will be restoring functioning wetland ecosystems along the shoreline of UKAL.

A more immediate challenge to Wocus recolonization is a lack of large colonies in restored Wocus habitat within the UKB. Because optimal habitat exists in areas beyond the WRD, expanding reintroduction efforts to other areas of the lakes will allow restoration practitioners to facilitate natural Wocus spread. Additionally, continued reintroduction efforts in UKAL will be necessary for several more years given the many areas that could support Wocus, but that currently lack it; it seems a priority should be saturating available habitat before implementing further large-scale habitat restoration activities for the purpose of Wocus reintroduction.

Success criteria

Wocus populations will be considered restored to ecosystem carrying capacity when populations are both self-sustaining and capable of colonizing restored habitat without active reintroduction efforts.

Restoration actions

TNC will continue transplanting efforts in the WRD in the future, and USFWS has obtained funding to expand the reintroduction program to additional sites in other parts of the lakes. Reintroduction to areas beyond the WRD will begin in 2017. It is likely that TKT will play a supportive role in Wocus reintroduction in the future until working relationships are established with private landowners or funds are available for land acquisition.

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V. BACKGROUND

Overview

There is growing interest in the reintroduction of native wetland plants in the UKB of Oregon (Figure 1), specifically in wetland habitats associated with UKAL. The hypereutrophic lakes are subject to extensive cyanobacteria blooms and associated large diel fluctuations in oxygen and pH that create hostile conditions for native aquatic biota (Boyd et al. 2002). Additionally, the lakes are critical habitat for ESA-listed Shortnose (*Chasmistes brevirostris*) and Lost River (*Deltistes luxatus*) Suckers (USFWS 2012) and native Redband Rainbow Trout (*Oncorhynchus mykiss newberrii*). It is hypothesized that large-scale reintroductions of wetland plants, particularly Wocus, may provide habitat for native fish and their prey, and alter nutrient cycling such that fewer nutrients may be available for harmful cyanobacteria species. Wocus also has cultural value to TKT of Oregon (Colville 1897) and its reintroduction may therefore benefit both the aquatic ecosystem and tribal culture in the basin. See Appendix A for a detailed literature review about *Nuphar lutea* (generally) and Wocus (specifically) and their effects on wetland and lake ecology.

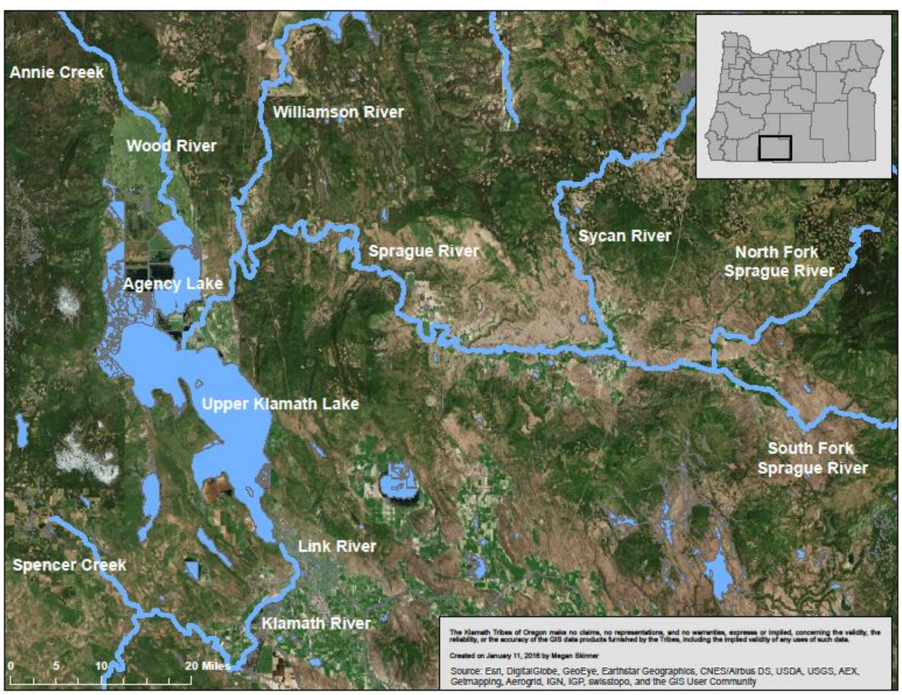

Figure 1. Upper Klamath Basin of Oregon.

General characteristics, distribution, and preferred habitat

Nuphar lutea (Yellow Water Lily, Yellow Pond Lily) is an aquatic perennial widely distributed throughout the Northern Hemisphere, occurring in North America, continental Europe, the British Isles, north-central Asia, Siberia, and occasionally in north Africa (Heslop-Harrison 1955); subspecies *Nuphar lutea polysepala* (Wocus) is native to western North America, occurring west of the Rocky Mountains from northern Mexico to the arctic.

Nuphar lutea spreads 3 to 6 feet on the water surface (Wennerberg 2004), and produces two types of leaves: submerged leaves with limited photosynthetic capacity, and floating leaves up to 15 inches in diameter (Heslop-Harrison 1955). Additionally, this species has large rhizomes up to 3 feet long and several inches in diameter; the rhizome and roots may constitute up to 80% of total biomass of the plant (Wetzel 2001). The nutrient storage capacity of the rhizome enables the plant to survive suboptimal conditions, including freezing temperatures, drought, or anoxic conditions for some time (Heslop-Harrison 1955, Borysiak et al. 2011).

Preferred *Nuphar lutea* habitat includes organic mud and silt substrates in 2-8 foot deep eutrophic water protected from wind and wave action (Heslop-Harrison 1955, Schroeder 2014). Large *Nuphar lutea* colonies, sometimes many acres in size, occur where habitat is optimal. Isolated individuals may occur where conditions for establishment are hyperlocal in nature (Heslop-Harrison 1955, Hart and Cox 1995).

Life History

Submerged leaves are produced in early spring, followed by floating leaves in April and May. Maximum root and rhizome growth occurs from May to August (Heslop-Harrison 1955). After reaching maturity in 2 to 3 years, *Nuphar lutea* produce up to 15 flowers per plant annually from mid-June to August (Heslop-Harrison 1955). In North America, *Nuphar lutea* can produce over one thousand small, olive green, oval-shaped seeds per flower (Hart and Cox 1995). Seeds ripen in the fall and may be dispersed by water currents either individually or within the fruit before sinking (Hart and Cox 1995); a mucilaginous coating allows the seeds to float for up to 72 hours (Hart and Cox 1995). Seed germination occurs in early winter. It is important to note that seed germination is erratic at best (Heslop-Harrison 1955) and seedlings are rarely observed in natural habitats despite rapid and wide dispersal of floating seeds (Hart and Cox 1995). Heslop-Harrison (1955) found that less than 20% of *Nuphar lutea* seeds germinated in a controlled environment, but determined that germination was most successful in diffuse light relative to low light conditions. Where seedlings have been observed, germination appeared to be most successful in 12 to 24 inches of water (Heslop-Harrison 1955).

There is some indication that sexual reproduction may be the major mechanism for long-distance dispersal (Fer and Hroudova 2008) and intersystem seed dispersal via endozoochory may be possible, although Smits et al. (1989) found that certain water fowl and fish species digest *Nuphar lutea* seeds completely, rendering them unviable. Regardless, low rates of seed germination suggest vegetative reproduction is likely the most probable dispersal and colonization method (Hart and Cox 1995, Barrat-Segretain 1996, Borysiak et al. 2011); local spread can be achieved through branching and growth of the rhizome, while wider dispersal (up to 50 miles in river corridors) is possible via fragmentation of the rhizome (Heslop-Harrison 1955, Fer and Hroudova 2008).

Effects of* Nuphar lutea *on nutrient cycling and water quality parameters

Phosphorus is removed from the water column of wetlands and shallow lakes via sorption to metal hydroxides-oxides, increases in biomass, and accretion in the sediments (Kadlec 1997). Sorption is typically the dominant process removing phosphorus in recently restored wetlands, while biomass tends to dominate during the first decade following restoration, and accretion is the principal mechanism for phosphorus removal over the long term (Kadlec 1997).

Nutrient uptake as a result of increases in aquatic macrophyte and algae biomass may result in short-term sequestration of nutrients in wetland and shallow lake systems. Perennial macrophytes also have the ability to store phosphorus obtained during the growing season in their root systems as above ground tissues senesce (Wetzel 2001). If macrophyte senescence does release substantial amounts of phosphorus and other nutrients into the water column, epiphyton and periphyton are fully capable of utilizing these released nutrients (Dodds 2003). Benthic or epiphytic algal communities can therefore function as effective phosphorus scavengers outside of the growing season (Wetzel 2001), particularly in areas with mild winters (Liboriussen and Jeppesen 2009). Indeed, as macrophytes, phytoplankton, and cyanobacteria senesce, a reduction in shading allows periphyton to photosynthesize (and take up nutrients) at levels similar to, or higher than, those observed during the growing season in some lakes despite cooling temperatures and reductions in incident radiation during this time period (Liboriussen and Jeppesen 2009). If periphyton growth is seasonal, phosphorus retention may be temporary with sloughing and senescence releasing the majority of phosphorus taken up during the growing season.

Accretion is the principal mechanism for phosphorus removal from wetlands and shallow lakes over the long term (Kadlec 1997). Long-term phosphorus sequestration results directly from phosphorus uptake during growth and incomplete decomposition and subsequent burial of organic matter following senescence (Juston et al. 2013). Wetlands dominated by macrophytes with more easily decomposed tissues, such as floating-leaved species, typically have reduced accretion rates relative to those with high densities of emergent vegetation (Graham et al. 2005).

When compared with unvegetated areas of lakes, *Nuphar lutea* had a limited effect on water transparency (Zbikowski et al. 2010), dissolved oxygen concentrations (Moss et al. 1998, Zbikowski et al. 2010, Holmroos et al. 2015), and conductivity (Zbikowski et al. 2010). These findings are not surprising given a lack of complex submerged vegetation in this species, allowing for the free exchange of water in and out of vegetated areas (Zbikowski et al. 2010). Interestingly, there is some disagreement regarding the effects on water temperature with one study (Holmroos et al. 2015) indicating water temperature increases in *Nuphar lutea* stands, while others (Moss et al. 1998, Zbikowski et al. 2010) suggest there is no significant difference relative to unvegetated areas. Despite simple submerged structures, *Nuphar lutea* has large floating leaves and an extensive rhizome system and therefore has the capacity to shade the water column and influence conditions in the sediment. Generally, macrophytes may inhibit the release of phosphorus from sediments and thereby reduce phosphorus internal loading in shallow systems by facilitating particle sedimentation and preventing or reducing sediment resuspension (Wetzel 2001, Jackson 2003). Indeed, several studies found significantly reduced sediment resuspension (Moss et al. 1998, Horppila and Nurminen 2005, Zbikowski et al. 2010) and light penetration in the water column (Zbikowski et al. 2010) during the growing season. The presence of *Nuphar lutea* also significantly increased sediment organic matter and sediment oxygen demand relative to open water habitats (Zbikowski et al. 2010).

Macrophyte senescence can release soluble nutrients to the water column and particulate organic matter to the sediments. It appears that *Nuphar lutea* rhizomes are capable of resorbing approximately 70% of nitrogen and phosphorus contained within leaves during senescence (Kok et al. 1990). Thus, *Nuphar lutea* leaf senescence releases only 30% of nutrients previously associated with leaf tissue into the water column and/or sediments (Kok et al. 1990). The capacity for *Nuphar lutea* to resorb these nutrients does not appear to vary between systems, suggesting that external environmental factors have little influence on the reallocation of nutrients as the plant ages (Kok et al. 1990). Tomaszewicz and Ciecierska (2009) found no increase in water column phosphorus concentrations during *Nuphar lutea* senescence. Additionally, uptake of phosphorus through *Nuphar lutea* roots often decreases phosphorus sediment concentrations such that less is released into the water column when sediment is resuspended (Nurminen and Horppila 2009).

Although Nuphar lutea may be capable of sequestering up to 70% of acquired nutrients in the rhizome, 30% of nutrients in leaf and stem tissue may still be released and utilized by other organisms upon *Nuphar lutea* senescence. This is a key consideration given that *Nuphar lutea* have soft structural tissues relative to emergent macrophytes such as Tule (*Schoenoplectus acutus*) and may therefore decompose more readily upon senescence. Indeed, phosphorus accretion rates are lower in restored wetlands with abundant *Nuphar lutea* relative to those dominated by Tule, indicating that the long-term phosphorus storage capacity for phosphorus may be reduced relative to that in Tule wetlands (Graham et al. 2005).

Effects of **Nuphar lutea** *on aquatic biota*

Macrophytes such as *Nuphar lutea* provide habitat for epibenthic, epiphytic, and mobile invertebrates (Moss et al. 1998, Zbikowski et al. 2010). Specifically, Chironomidae, Ephermeroptera, and Ceratopogonidae densities were significantly higher in *Nuphar lutea* stands relative to unvegetated control plots (Zbikowski et al. 2010). Additionally, *Daphnia* populations were significantly higher and individuals were significantly larger in high density *Nuphar lutea* patches relative to medium or low density patches, suggesting that high plant density limited *Daphnia* predation by Perch (*Perca fluviatilis*) (Moss et al. 1998). Larger populations of large *Daphnia* in high density *Nuphar lutea* beds were also correlated with a reduction in chlorophyll *a* concentrations as a result of *Daphnia* grazing on phytoplankton (Moss et al. 1998). It is reasonable to assume that *Nuphar lutea* beds also provide effective shelter for planktivorous or benthic-feeding fish seeking to avoid piscivorous predators (Moss et al. 1998).

Despite *Nuphar lutea* providing favorable conditions for aquatic animals, this species' submerged vegetative parts have relatively low structural heterogeneity; a lack of complex habitat structure, combined with heavy shading from floating leaves, creates conditions unfavorable to periphyton and epiphyton (Zbikowski et al. 2010). This is an especially important consideration given that attached algal communities may play a central role in cycling nutrients within the littoral community such that fewer are available for cyanobacteria and other undesirable organisms.

Wocus in the Upper Klamath Basin of Oregon

History

Wocus seed was historically one of the most important food sources for TKT. Members of TKT relocated to seasonal villages adjacent to prime seed harvesting areas where seeds were gathered from canoes (Figure 2) (Deur 2009). Harvest and processing took place between June and September with seed harvest occurring primarily in early summer before water levels dropped and wetlands began to dry (Deur 2009). Some harvest did occur in late summer with the help of specially designed footwear, similar to snowshoes, to facilitate travel over recently exposed wetland sediment (Deur 2009). Women typically harvested and processed seeds by parching, hulling, winnowing, and then storing seeds for later use (Deur 2009). Wocus harvest and processing was so central to the role of women in tribal societies of the UKB that the Wocus seed was a symbol of femininity in Klamath culture (Deur 2009).

Figure 2. A Klamath woman harvesting Wocus in Upper Klamath Lake. (E. Curtis 1923)

Wocus seeds varied in quality with those from fully ripe fruit (spokwas) being highest and seeds from under-ripe fruit (chiniakum) being lowest in quality (Colville 1897). Seeds could be processed into a variety of forms including shnaps (parched seed with a flavor similar to popcorn), shlotish (ground shnaps mixed with cold water), and shiwulinz (mush made from seeds of under-ripe Wocus fruit) (Colville 1897).

Tribal communities relied on Wocus to such an extent that they actively managed Wocus and wetland habitat to increase future yield. Oral accounts indicate that Klamath tribal members burned the edges of wetlands in the fall to facilitate regrowth of Wocus in areas that would otherwise be dominated by other wetland plants or upland vegetation (Deur 2009). There is also evidence that Klamath tribal members dammed lake outlets specifically to raise water level and increase Wocus habitat (Deur 2009). Tribal communities also had the foresight to manage harvest in such a way as to reduce localized overexploitation of the resource (Deur 2009).

Wocus populations likely began declining in UKAL with the onset of European settlement and conversion of fringe wetlands to agricultural land in the late 1880s. In the late 1800s and early 1900s, farmers constructed dikes and began draining the fringe wetlands to prevent flooding and to increase agricultural yield (Snyder and Morace 1997). In all, over half of the key Wocus habitat associated with Upper Klamath and Agency Lakes has been drained since 1889 (Snyder and Morace 1997), although some has since been restored.

Distribution

There is very limited information regarding historical Wocus distribution and population estimates in the UKB. Colville (1897) reported that Wocus covered over 10,000 acres of the Upper Klamath Marsh, but such historical accounts do not exist for UKAL. It is reasonable to assume that current Wocus populations in the lakes are only a fraction of what they once were given the extent of wetland reclamation during the 20th century. Currently, Wocus covers approximately 600 acres in UKAL and is largely confined to existing fringe wetlands, particularly those within the Upper Klamath Wildlife Refuge (Figure 3).

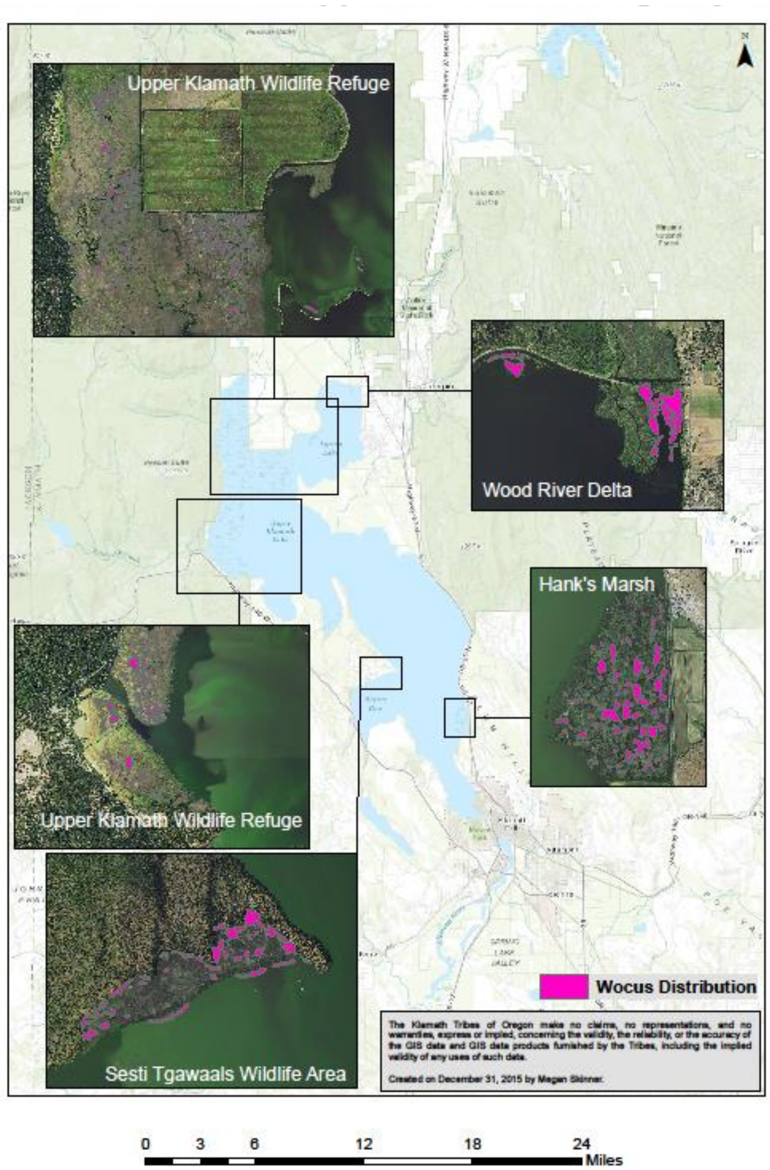

Figure 3. 2014 Wocus distribution in Upper Klamath and Agency Lakes.

Conclusion

Nuphar lutea has the potential to sequester a portion of acquired nutrients in the rhizome and sediments, may benefit zooplankton and benthic macroinvertebrate populations, and may provide habitat and cover for planktivorous and benthic-feeding fish. While the magnitude of these potential benefits in the UKB needs further research, this plant was likely a key component of the historic wetland ecosystem associated with UKAL and Wocus reintroduction may benefit this system in the future. Wocus was also a culturally important plant species and reintroduction will therefore benefit tribal communities in the basin.

VI. RESTORATION PROGRAM

Restoration strategy

Overview

Large, self-sustaining Wocus colonies are most common in the vicinity of functioning wetland habitat (Figure 3); because over half of the key Wocus habitat in UKAL has been drained since 1889 (Snyder and Morace 1997), a key long-term Wocus restoration objective will be restoring functioning wetland ecosystems along the shoreline of UKAL. While this is a lofty goal, large-scale restoration of Wocus habitat is also likely to address or mitigate other water quality issues in the lakes and should therefore be a priority. Similarly, restoration practitioners in the UKB should seek to limit any further loss of Wocus wetland habitat.

A more immediate challenge to Wocus recolonization is a lack of large colonies in restored Wocus habitat within the UKB. Asexual vegetative reproduction is likely the most probable dispersal and colonization method for Wocus (Hart and Cox 1995, Barrat-Segretain 1996, Borysiak et al. 2011). In systems with little or no hydrologic flow, this dispersal method is geographically limited (Heslop-Harrison 1955), meaning that widespread growth of Wocus beds in lakes requires many source populations from which rhizomes can spread and colonize. Currently, there is little recolonization of optimal habitat in restored wetlands (such as the WRD) because potential source colonies are so geographically distant. Reintroducing Wocus such that Wocus beds are numerous and evenly distributed geographically will be key in establishing self-sustaining beds that can contribute to further Wocus colonization. Given that optimal habitat exists in areas beyond the WRD, expanding reintroduction efforts to other areas of the lakes will allow restoration practitioners to facilitate Wocus spread. Additionally, continued reintroduction efforts in UKAL will be necessary for several more years given the many areas that could support Wocus, but that currently lack it; it seems a priority should be saturating available habitat before implementing further large-scale habitat restoration activities specifically for the purpose of Wocus reintroduction. We are unable to define goals and objectives in terms of specific numbers (total plant numbers, plant density, or acres of Wocus colonies) given a current lack of data regarding potential ecosystem carrying capacity, total acreage of suitable habitat, and numbers of plants necessary to create self-sustaining populations capable of recolonizing habitat without active reintroduction.

Restoration goal

The ultimate goal of the Wocus restoration program is to produce naturally self-sustaining populations that can effectively recolonize restored or emerging habitat without active reintroduction.

Restoration objectives

1. Increase Wocus populations in existing habitat through active reintroduction.
2. Protect existing populations and habitat from loss and degradation.
3. Restore or enhance Wocus wetland habitat in UKAL.

Success criteria

Wocus populations will be considered restored when populations are both self-sustaining and capable of colonizing restored habitat without active intervention. To determine if Wocus populations have met this recovery criteria, continued long-term monitoring of reintroduction efforts combined with additional surveys of emerging Wocus populations will be necessary. Additionally, because reintroduction efforts in the UKB are relatively young and early years were largely unsuccessful, further research and more long-term data will be required to help practitioners understand population thresholds at which these criteria can be met.

Restoration actions

Past restoration efforts in the Upper Klamath Basin

TNC has been working to reintroduce Wocus to their 7,509 acre WRD property since 2004. During this time, several transplanting methods have been utilized with varying results. Tested methods include burying rhizomes in the sediment and tying them to grade stakes; sinking rhizomes to the sediment in weighted burlap bags; burying rhizomes in sediment and tying them to rebar; burying rhizomes in soil above the water line and wiring to rebar stakes; planting small rhizome sections in containers filled with potting soil; propagation from seed at Western Native Plants (formerly Rock Bottom Nursery); burying nursery plants in the sediment and tying them to cinder blocks; attaching rhizomes to coir fabric and weighting with rocks; and pressing rhizomes into the sediment and staking them with bent rebar staples (Schroeder 2014). Early efforts were largely unsuccessful for a number of reasons: excessive wave action that uprooted plants, low water levels leading to desiccation, improper planting leading to rhizome rot, improper seasonal timing of transplanting (spring versus fall), high financial cost of nursery-raised plants, and failure of seeds to germinate. TNC has had greatest success planting large rhizome sections (Figure 4), securing with bent rebar stakes, targeting deeper-water areas, and planting in late spring or early summer. In the fall of 2014, over 66% of transplanted Wocus planted that spring/summer were still alive (Schroeder 2014) and a number of plants survived the winter. After ten years of transplanting efforts, TNC has concluded that whole Wocus plants with rhizomes and several leaves (Figure 4) should be planted in the spring in areas protected from wave action and with at least 3-4 feet of water and secured with bent rebar stakes (Schroeder 2014). See Appendix B for a description of additional Wocus monitoring and survival assessments conducted by TNC, TKT, and USFWS.

Figure 4. Example of a large Wocus rhizome ideal for successful transplanting. (E. Berman 2014)

To date, TKT has played a supporting role in Wocus reintroduction, assisting TNC in their WRD reintroduction project.

Current status of restoration efforts in the Upper Klamath Basin

In June 2016, TNC, TKT, and USFWS completed another round of Wocus transplanting in the WRD. In addition to the standard method of transplanting rhizomes, TNC and collaborators also transplanted Wocus contained in biodegradable rice-hull pots. These Wocus were propagated from seed at Western Native Plants in Bonanza, Oregon. Potted Wocus were transplanted alongside rhizomes to determine the ability of potted Wocus to establish, survive, and spread relative to rhizomes. Planting potted Wocus appears to be less labor intensive for restoration practitioners and is thus worth exploring. While TNC has tested potted plants in the past, the exact methods differed somewhat from those being tested in this investigation. See Appendix C for a detailed description of this planting study.

Future restoration actions

TNC will continue transplanting efforts in the WRD in the future, and other entities including USFWS, Trout Unlimited, and TKT are interested in collaborating and expanding the reintroduction program to other parts of the lakes. Indeed, USFWS recently received funding through their Partners for Fish and Wildlife restoration program to actively pursue reintroduction efforts on private waterfront property on the west shore of Upper Klamath Lake in collaboration with The Klamath Lake Land Trust, Trout Unlimited, and TKT (Figure 5). TKT and collaborators will also seek to involve tribal youth and tribal volunteers in the seed propagation, rhizome harvesting, transplanting, and monitoring events as the reintroduction effort expands. Overall, it is likely that TKT will play a supportive role in Wocus reintroduction in the future until working relationships are established with private landowners or funds are available for land acquisition.

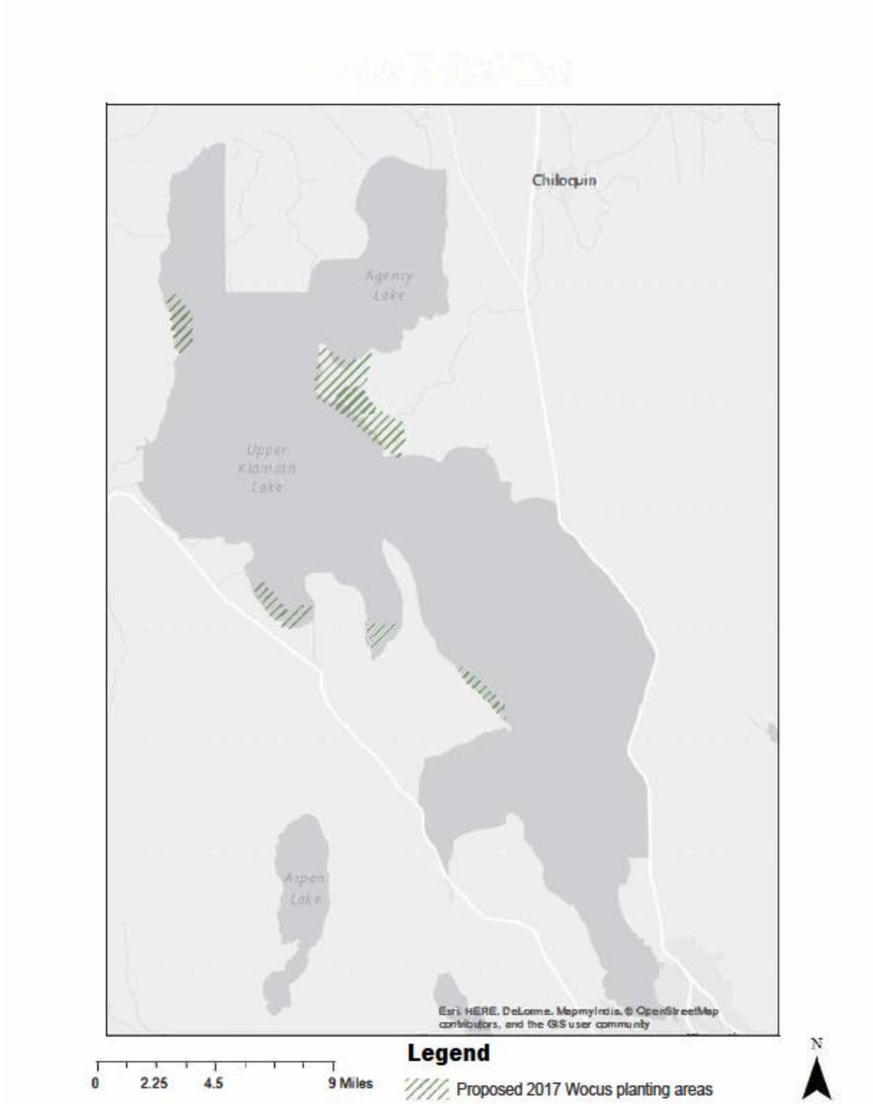

Figure 5. Proposed 2017 Wocus planting areas including areas beyond the Williamson River Delta. (C. Adelsberger 2016, USFWS)

In addition to transplanting efforts, continuing to monitor past transplants will be key in further refining site selection and planting methods to maximize transplanting success. See Appendix B for specific recommendations for future monitoring in the WRD and beyond.

Implementation schedule

The schedule outlined in this document is primarily focused on reintroducing Wocus to currently available habitat lacking self-sufficient and self-spreading populations. Future reintroduction effort will largely depend on the growing body of knowledge regarding Wocus reintroduction, survival, and spread in the Upper Klamath Basin. Actions in years beyond 2017 will rely on availability of funding, personnel, recruitment of private landowners, and the outcome of studies currently underway (continued monitoring and the planting study outlined in Appendix C), all of which are unknown or un-established at this time. If funding and personnel are available, we have access to private waterfront property with suitable Wocus habitat, and we find that the results of our planting study allow us to use transplanting methods requiring less effort, reintroduction activities will expand greatly. Activities planned for 2016 and 2017 will focus on acquiring these resources and knowledge and are therefore very important to the future of the restoration program. However, it is premature and speculative to plan beyond 2017 at this point given the many unknowns we are still presented with. The ability to adapt reintroduction and restoration efforts as we learn more is of central importance to a successful recovery program. As such, the following timeline does not include commitments for years beyond 2017, although current partners and collaborators are committed to continuing efforts beyond what is outlined here.

2016

- Complete spring transplanting in the WRD and collect data associated with the planting study (Appendix C).
- If necessary, collect seeds for Western Native Plants' propagation efforts
- Contact private landowners or entities working with private landowners to secure permission to transplant in areas beyond the WRD (Figure 5)
- Prepare for 2017 transplanting
    - Visit proposed transplanting areas to identify specific sites with appropriate exposure levels, substrate types, and water depth
    - Identify 2017 transplanting collaborators and partners

2017

- Conduct spring transplant monitoring in the WRD to determine how many transplants survived the winter
    - Monitor Wocus transplanted in 2014, 2015, and 2016

    - Collect data associated with the planting study (Appendix C)
- Analyze and interpret data, and write a final report for the planting study (Appendix C); determine if potted Wocus transplants were successful and reassess transplanting protocol as applicable
- Complete spring transplanting in the WRD and beyond
- If necessary, collect seeds for Western Native Plants' propagation efforts
- Contact private landowners or entities working with private landowners to identify additional transplanting sites beyond the WRD
- Prepare for 2018 transplanting
    - Visit proposed transplanting areas to identify specific sites with appropriate exposure levels, substrate types, and water depth
    - Identify 2018 transplanting collaborators and partners

VII. FUTURE RESEARCH NEEDS

Despite an emphasis on the role of periphyton in wetland and shallow lake nutrient cycling, it does not appear the relationship between periphyton and *Nuphar lutea* has received any attention. As discussed previously, *Nuphar lutea* rhizomes are capable of sequestering up to 70% of nutrients contained within leaves, but at least 30% of nutrients in senescing tissue will still be released to the water column or sediment. If robust periphyton populations are commonly associated with *Nuphar lutea*, periphyton could play a central role in utilizing any nutrients released during *Nuphar lutea* senescence and could contribute to a tight coupling of nutrients within the macrophtye/periphyton community. It may be that large *Nuphar lutea* leaves lead to shaded conditions unconducive to periphyton growth, but the relationship between these organisms should be studied nonetheless.

For the UKB, further research into the feasibility of seed propagation as a means for reintroduction would be beneficial. Specifically, a study assessing optimal germination conditions may assist restoration practitioners in planning seed plantings in UKAL. The time- and labor-intensive methods currently utilized limit the scope of Wocus reintroduction. Restoration via seed would increase efficiency and scope and reduce labor associated with reintroduction efforts. Additionally, further monitoring of transplanted Wocus is necessary to determine population thresholds at which recovery criteria can be met.

Finally, Wocus reintroduction has been offered as mitigation for nutrient loading in UKAL and pilot or laboratory studies assessing the capacity of Wocus to sequester nutrients would assist in restoration planning and implementation. Similarly, it is hypothesized that reintroduced Wocus may provide much needed habitat and cover for the endangered Sucker species and other native fish in UKAL, but further research is needed to test this hypothesis.

Also see Appendix A and B for additional recommendations for future research.

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APPENDIX A

Nuphar lutea in wetlands and shallow lakes with particular emphasis on the Upper Klamath Basin of Oregon

A literature review prepared for The Klamath Tribes

By

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March 2016

Overview

There is growing interest in the reintroduction of native wetland plants in the Upper Klamath Basin of Oregon (UKB) (Figure 1), specifically in wetland habitats associated with Upper Klamath and Agency Lakes (UKAL). The hypereutrophic lakes are subject to extensive cyanobacteria blooms and associated large diel fluctuations in oxygen and pH that create hostile conditions for native aquatic biota. Additionally, the lakes are critical habitat for ESA-listed Shortnose (*Chasmistes brevirostris*) and Lost River (*Deltistes luxatus*) Suckers and native Redband Rainbow Trout (*Oncorhynchus mykiss newberrii*). It is hypothesized that large-scale reintroductions of wetland plants, particularly Wocus (*Nuphar lutea polysepala*), may provide habitat for native fish and their prey, and alter nutrient cycling such that fewer nutrients may be available for harmful cyanobacteria species. Wocus also has cultural value to The Klamath Tribes of Oregon (TKT) and its reintroduction may therefore benefit both the aquatic ecosystem and tribal culture in the basin.

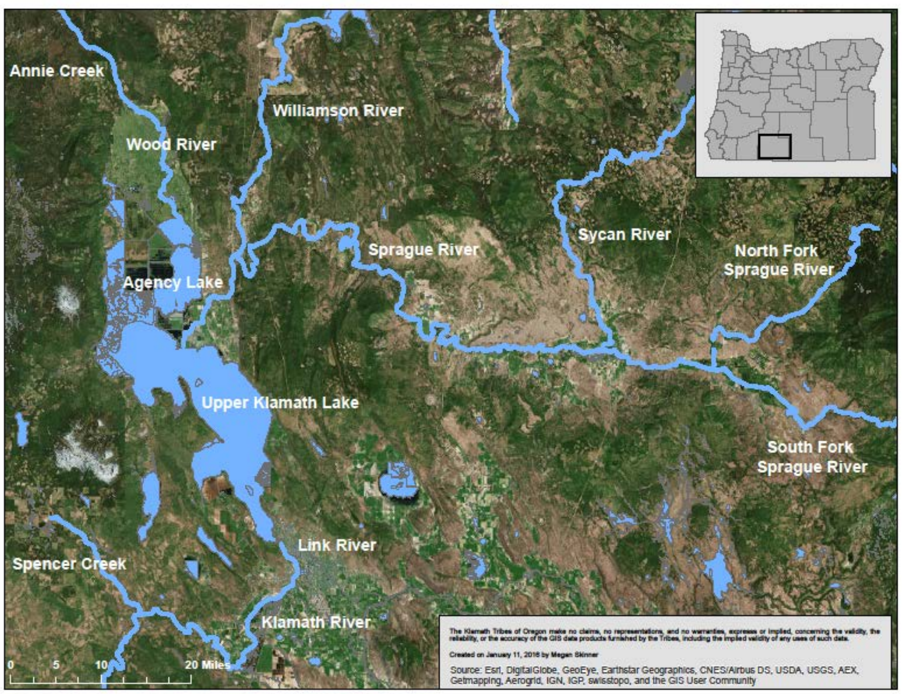

Figure 1. Upper Klamath Basin of Oregon.

The purpose of this literature review is to provide researchers and restoration practitioners in the UKB with technical information about *Nuphar lutea* (generally) and Wocus (specifically) and their effects on wetland and lake ecology. This literature review will assist in testing hypotheses associated with the perceived benefits of reintroduction and guide future Wocus reintroduction and restoration efforts.

General characteristics, distribution, and preferred habitat

Nuphar lutea (Yellow Water Lily, Yellow Pond Lily) is an aquatic perennial widely distributed throughout the Northern Hemisphere, occurring in North America, continental Europe, the British Isles, north-central Asia, Siberia, and occasionally in north Africa (Heslop-Harrison 1955). This species spreads 3 to 6 feet on the water surface (Wennerberg 2004), and produces two types of leaves: submerged leaves with limited photosynthetic capacity, and floating leaves up to 15 inches in diameter (Heslop-Harrison 1955). Additionally, this species has large rhizomes up to 3 feet long and several inches in diameter; the rhizome and roots may constitute up to 80% of total biomass of the plant (Wetzel 2001). The nutrient storage capacity of the rhizome enables the plant to survive suboptimal conditions, including freezing temperatures, drought, or anoxic conditions for some time (Heslop-Harrison 1955, Borysiak et al. 2011).

Preferred *Nuphar lutea* habitat includes organic mud and silt substrates in 2-8 foot deep eutrophic water protected from wind and wave action (Heslop-Harrison 1955, Shroeder 2014). Large *Nuphar lutea* colonies, sometimes many acres in size, occur where habitat is optimal. Isolated individuals may occur where conditions for establishment are hyperlocal in nature (Heslop-Harrison 1955, Hart and Cox 1995).

Life History

Submerged leaves are produced in early spring, followed by floating leaves in April and May. Maximum root and rhizome growth occurs from May to August (Heslop-Harrison 1955). After reaching maturity in 2 to 3 years, *Nuphar lutea* produce up to 15 flowers per plant annually from mid-June to August (Heslop-Harrison 1955). In North America, *Nuphar lutea* can produce over one thousand small, olive green, oval-shaped seeds per flower (Hart and Cox 1995). Seeds ripen in the fall and may be dispersed by water currents either individually or within the fruit before sinking (Hart and Cox 1995); a mucilaginous coating allows the seeds to float for up to 72 hours (Hart and Cox 1995). Seed germination occurs in early winter. It is important to note that seed germination is erratic at best (Heslop-Harrison 1955) and seedlings are rarely observed in natural habitats despite rapid and wide dispersal of floating seeds (Hart and Cox 1995). Heslop-Harrison (1955) found that less than 20% of *Nuphar lutea* seeds germinated in a controlled environment, but determined that germination was most successful in diffuse light relative to low light conditions. Where seedlings have been observed, germination appeared to be most successful in 12 to 24 inches of water (Heslop-Harrison 1955).

There is some indication that sexual reproduction may be the major mechanism for long-distance dispersal (Fer and Hroudova 2008) and intersystem seed dispersal via endozoochory may be possible, although Smits et al. (1989) found that certain water fowl and fish species digest *Nuphar lutea* seeds completely, rendering them unviable. Regardless, low rates of seed germination suggest vegetative reproduction is likely the most probable dispersal and colonization method (Hart and Cox 1995, Barrat-Segretain 1996, Borysiak et al. 2011); local spread can be achieved through branching and growth of the rhizome, while wider dispersal (up to 50 miles in river corridors) is possible via fragmentation of the rhizome (Heslop-Harrison 1955, Fer and Hroudova 2008).

Wetland nutrient cycling and water chemistry

General nutrient cycling characteristics of shallow lakes and wetlands

The primary mechanisms by which wetlands and shallow lakes remove nitrogen include plant and algae uptake, denitrification, and volatilization of ammonia (Wetzel 2001). Typically, plant and algae uptake plays a minimal role in nitrogen removal given the cycle of senescence and growth that recycles nutrients annually. When anoxia dominates in wetland ecosystems, denitrification facilitated by heterotrophic bacteria becomes an important mechanism for the removal of nitrogen from the system (Wetzel 2001). In the absence of oxygen, nitrate is the most efficient electron acceptor available for the oxidation of organic matter via denitrification; denitrification reduces nitrate to nitrogen gas and nitrous oxide, which then escape from the system (Wetzel 2001). In wetlands with aerobic conditions, nitrification dominates and nitrate concentrations can be relatively high.

Phosphorus is removed from the water column of wetlands and shallow lakes via sorption to metal hydroxides-oxides, increases in biomass, and accretion in the sediments (Kadlec 1997). Sorption is typically the dominant process removing phosphorus in recently restored wetlands, while biomass tends to dominate during the first decade following restoration, and accretion is the principal mechanism for phosphorus removal over the long term (Kadlec 1997).

Sorption is mediated by a number of factors including pH, temperature, dissolved oxygen, redox potential, and sediment iron concentrations (Mortimer 1941, Andersen 1975, Penn et al. 2000, Christophoridis and Fytianos 2006). As the sediment becomes anaerobic and redox potential drops, sediment phosphorus binding capacity is decreased through the reduction of iron bound to phosphate; this reduction releases phosphate, increasing soluble phosphorus concentrations in the water column (Mortimer 1941). Conversely, when the sediment-water interface is oxic and redox potential is high, sediment phosphorus binding capacity is increased through oxidation of iron (Mortimer 1941). Phosphorus may be released from oxic sediments if sediment iron concentrations are low; a sediment iron:phosphorus ratio of 10-15 is considered sufficient to avoid saturation of phosphorus sorption sites (Christophoridis and Fytianos 2006). Additionally, an increase in pH under oxic conditions reduces phosphorus sorption to ferric iron, which in turn may facilitate sediment phosphorus release (Andersen 1975, Penn et al. 2000). However, pH in this range may increase the sorption of phosphorus to calcium carbonate, thereby regulating sediment phosphorus release to some extent (Penn et al. 2000). Overall, the role of redox conditions is more dramatic and important in mediating seasonal phosphorus release relative to pH (Penn et al. 2000).

Regardless of sediment conditions, nutrient uptake as a result of increases in aquatic macrophyte and algae biomass may result in short-term sequestration of nutrients in wetland and shallow lake systems. Perennial macrophytes also have the ability to store phosphorus obtained during the growing season in their root systems as above ground tissues senesce (Wetzel 2001). If macrophyte senescence does release substantial amounts of phosphorus and other nurtrients into the water column, epiphyton and periphyton are fully capable of utilizing these released nutrients (Dodds 2003). Benthic or epiphytic algal communities can therefore function as effective phosphorus scavengers outside of the growing season (Wetzel 2001), particularly in areas with mild winters (Liboriussen and Jeppesen 2009). Indeed, as macrophytes, phytoplankton, and cyanobacteria senesce, a reduction in shading allows periphyton to photosynthesize (and take up nutrients) at levels similar to, or higher than, those observed during the growing season in some lakes despite cooling temperatures and reductions in incident radiation during this time period (Liboriussen and Jeppesen 2009). If periphyton growth is seasonal, phosphorus retention may be temporary with sloughing and senescence releasing the majority of phosphorus taken up during the growing season.

Finally, accretion is the principal mechanism for phosphorus removal from wetlands and shallow lakes over the long term (Kadlec 1997). Long-term phosphorus sequestration results directly from phosphorus uptake during growth and incomplete decomposition and subsequent burial of organic matter following senescence (Juston et al. 2013). Wetlands dominated by macrophytes with more easily decomposed tissues, such as floating-leaved species, typically have reduced accretion rates relative to those with high densities of emergent vegetation (Graham et al. 2005).

Regardless of the mechanism by which nutrients are sequestered or cycled in wetlands and shallow lakes, nutrient retention capacity varies widely between systems. While Little Bean Marsh on the Mississippi River is capable of retaining more than 95% of incoming phosphorus (Blevins 2004), some wetlands and shallow lakes are net phosphorus sources given conditions in the water column and sediments conducive to phosphorus release. After wetland restoration or establishment of artificial wetlands, there may initially be high rates of phosphorus retention associated with rapidly growing macrophytes and algae (Dodds 2003). However, if wetlands were established on phosphorus-rich soils, they may be net phosphorus sources. Indeed, flooding soils and restoring wetlands on property formerly used as pasture for dairy cows released a substantial amount of phosphorus into the system (Pant and Reddy 2003). Additionally, breaching levees and flooding former nutrient-rich farmland in Williamson River delta, Oregon released phosphorus (Wong et al. 2011), although this effect was hypothesized to be a temporary result of initial flooding. Even when wetlands are restored or constructed in areas with low or moderate soil nutrient concentrations, the lowest achievable phosphorus concentrations may be relatively high (i.e., around 16 ug/L; Juston et al. 2013), making it difficult to achieve some restoration goals. Additionally, fluctuating water levels may result in less than half the nutrient sequestration capacity of wetlands with static water levels (Dodds 2003). In conclusion, wetland function is highly dependent on site-specific conditions and the results of any one case study may not apply to other systems.

Effects of *Nuphar lutea* on nutrient cycling and water quality parameters

When compared with unvegetated areas of lakes, *Nuphar lutea* had a limited effect on water transparency (Zbikowski et al. 2010), dissolved oxygen concentrations (Moss et al. 1998, Zbikowski et al. 2010, Holmroos et al. 2015), and conductivity (Zbikowski et al. 2010). These findings are not surprising given a lack of complex submerged vegetation in this species, allowing for the free exchange of water in and out of vegetated areas (Zbikowski et al. 2010). Interestingly, there is some disagreement regarding the effects on water temperature with one study (Holmroos et al. 2015) indicating water temperature increases in *Nuphar lutea* stands, while others (Moss et al. 1998, Zbikowski et al. 2010) suggest there is no significant difference relative to unvegetated areas. Despite simple submerged structures, *Nuphar lutea* has large floating leaves and an extensive rhizome system and therefore has the capacity to shade the water column and influence conditions in the sediment. Generally, macrophytes may inhibit the release of phosphorus from sediments and thereby reduce phosphorus internal loading in shallow systems by facilitating particle sedimentation and preventing or reducing sediment resuspension (Wetzel 2001, Jackson 2003). Indeed, several studies found significantly reduced sediment resuspension (Moss et al. 1998, Horppila and Nurminen 2005, Zbikowski et al. 2010) and light penetration in the water column (Zbikowski et al. 2010) during the growing season. The presence of *Nuphar lutea* also significantly increased sediment organic matter and sediment oxygen demand relative to open water habitats (Zbikowski et al. 2010).

Macrophyte senescence can release soluble nutrients to the water column and particulate organic matter to the sediments. It appears that *Nuphar lutea* rhizomes are capable of resorbing approximately 70% of nitrogen and phosphorus contained within leaves during senescence (Kok et al. 1990). Thus, *Nuphar lutea* leaf senescence releases only 30% of nutrients previously associated with leaf tissue into the water column and/or sediments (Kok et al. 1990). The capacity for *Nuphar lutea* to resorb these nutrients does not appear to vary between systems, suggesting that external environmental factors have little influence on the reallocation of nutrients as the plant ages (Kok et al. 1990). Tomaszewicz and Ciecierska (2009) found no increase in water column phosphorus concentrations during *Nuphar lutea* senescence. Additionally, uptake of phosphorus through *Nuphar lutea* roots often decreases phosphorus sediment concentrations such that less is released into the water column when sediment is resuspended (Nurminen and Horppila 2009).

Although Nuphar lutea may be capable of sequestering up to 70% of acquired nutrients in the rhizome, 30% of nutrients in leaf and stem tissue may still be released and utilized by other organisms upon *Nuphar lutea* senescence. This is a key consideration given that *Nuphar lutea* have soft structural tissues relative to emergent macrophytes such as Tule (*Schoenoplectus acutus*) and may therefore decompose more readily upon senescence. Indeed, phosphorus accretion rates are lower in restored wetlands with abundant *Nuphar lutea* relative to those dominated by Tule, indicating that the long-term phosphorus storage capacity for phosphorus may be reduced relative to that in Tule wetlands (Graham et al. 2005).

Effects on aquatic Biota

Macrophytes such as *Nuphar lutea* provide habitat for epibenthic, epiphytic, and mobile invertebrates (Moss et al. 1998, Zbikowski et al. 2010). Specifically, Chironomidae, Ephermeroptera, and Ceratopogonidae densities were significantly higher in *Nuphar lutea* stands relative to unvegetated control plots (Zbikowski et al. 2010). Additionally, *Daphnia* populations were significantly higher and individuals were significantly larger in high density *Nuphar lutea* patches relative to medium or low density patches, suggesting that high plant density limited *Daphnia* predation by Perch (*Perca fluviatilis*) (Moss et al. 1998). Larger populations of large *Daphnia* in high density *Nuphar lutea* beds were also correlated with a reduction in chlorophyll *a* concentrations as a result of *Daphnia* grazing on phytoplankton (Moss et al. 1998). It is reasonable to assume that *Nuphar lutea* beds also provide effective shelter for planktivorous or benthic-feeding fish seeking to avoid piscivorous predators (Moss et al. 1998).

Despite *Nuphar lutea* providing favorable conditions for aquatic animals, this species' submerged vegetative parts have relatively low structural heterogeneity; a lack of complex habitat structure, combined with heavy shading from floating leaves, creates conditions unfavorable to periphyton and epiphyton (Zbikowski et al. 2010). This is an especially important consideration given that attached algal communities may play a central role in cycling nutrients within the littoral community such that fewer are available for cyanobacteria and other undesirable organisms.

Herbivory

Galerucella nymphaeae (water lily beetle) has a mutualistic relationship with *Nuphar lutea*; the beetle pollinates the blossoms, while Wocus provides beetles with food and shelter during all life history stages (Kouki 1991, Bunnell 2014). In the Upper Klamath Basin of Oregon, *Galerucella nymphaeae* egg and larvae density is highest on *Nuphar lutea polysepala* leaves in late May, pupa populations peak in June, and adults are most abundant in July (Bunnell 2014).

Galerucella nymphaeae feed on floating leaves, consuming the upper surface and palisade tissue (Kouki 1991, Bunnell 2014). Beetle herbivory is likely to be highest in large *Nuphar lutea* patches where high leaf density facilitates easy dispersal from leaf to leaf (Setala and Makela 1991). However, even in eutrophic systems with high *Nuphar lutea* densities, *Galerucella nymphaeae* may consume less than 2% of floating leaf biomass (Setala and Makela 1991, Stenberg and Stenberg 2012). Although only a small portion of *Nuphar lutea* is consumed by herbivores, herbivory exposes the interior of leaves to microbial attack and therefore facilitates early leaf senescence (Kouki 1991, Setala and Makela 1991). Indeed, the number of *Galerucella nymphaeae* feeding scars was significantly and positively correlated with the rate and degree of *Nuphar lutea* senescence (Stenberg and Stenberg 2012). Additionally, the presence of *Galerucella nymphaeae* decreased *Nuphar lutea* growth and average leaf density relative to insect-free plots (Stenberg and Stenberg 2012). It is possible that herbivory-induced senescence stimulates new leaf growth, but *Galerucella nymphaeae* tends to preferentially feed on young *Nuphar lutea* leaves so it is unclear if this response is always sufficient to compensate for leaf damage associated with herbivory (Bunnell 2014).

Restoration and Recolonization

A successful *Nuphar lutea* transplanting project in Poland utilized rhizome fragments to colonize uninhabited ponds. Borysiak et al. (2011) collected rhizomes in May before floating leaves were fully developed to ensure rhizome energy reserves were sufficient to aid in acclimation to the transplant site. The authors used only rhizomes with leaf buds and roots present; senesced leaves and soft portions of the rhizome were removed and roots were trimmed to a length of 2-3 inches (Borysiak et al. 2011). Rhizome sections approximately 8 inches long were then placed in mesh bags filled with silt-turf soil and transplanted such that leaf buds were 2 inches above the sediment in water 15-25 inches deep (Borysiak et al. 2011). One year after transplanting, approximately 75-85% of transplanted *Nuphar lutea* individuals were still alive (Borysiak et al. 2011).

In a study examining *Nuphar lutea* recolonization in France, new *Nuphar lutea* individuals were most commonly observed on the periphery of established patches, suggesting extension of rhizomes was responsible for new growth (Barrat-Segretain 1996). Indeed, no seedlings were observed recolonizing the disturbed area for the duration of the study, likely due to high sedimentation rates that contribute to seed burial (Barrat-Segretain 1996). Overall, recolonization of *Nuphar lutea* was a very slow process given that rhizomes can only extend a few inches per year under ideal conditions (Barrat-Segretain 1996). The extensive rhizome system in *Nuphar lutea* generally allows this species to withstand disturbance, but slow growth and low recolonization rates contribute to a low resilience overall (Barrat-Segretain 1996).

Wocus in the Upper Klamath Basin of Oregon

History

Wocus seed was historically one of the most important food sources for TKT. Members of TKT relocated to seasonal villages adjacent to prime seed harvesting areas where seeds were gathered from canoes (Figure 2) (Deur 2009). Harvest and processing took place between June and September with seed harvest occurring primarily in early summer before water levels dropped and wetlands began to dry (Deur 2009). Some harvest did occur in late summer with the help of specially designed footwear, similar to snowshoes, to facilitate travel over recently exposed wetland sediment (Deur 2009). Women typically harvested and processed seeds by parching, hulling, winnowing, and then storing seeds for later use (Deur 2009). Wocus harvest and processing was so central to the role of women in tribal societies of the UKB that the Wocus seed was a symbol of femininity in Klamath culture (Deur 2009).

Figure 2. A Klamath woman harvesting Wocus in Upper Klamath Lake. (Edward S. Curtis 1923)

Wocus seeds varied in quality with those from fully ripe fruit (spokwas) being highest and seeds from under-ripe fruit (chiniakum) being lowest in quality (Colville 1897). Seeds could be processed into a variety of forms including shnaps (parched seed with a flavor similar to popcorn), shlotish (ground shnaps mixed with cold water), and shiwulinz (mush made from seeds of under-ripe Wocus fruit) (Colville 1897).

The Tribes relied on Wocus to such an extent that they actively managed Wocus and wetland habitat to increase future yield. Oral accounts indicate that Klamath tribal members burned the edges of wetlands in the fall to facilitate regrowth of Wocus in areas that would otherwise be dominated by other wetland plants or upland vegetation (Deur 2009). There is also evidence that Klamath tribal members dammed lake outlets specifically to raise water level and increase Wocus habitat (Deur 2009). Tribal communities also had the foresight to manage harvest in such a way as to reduce localized overexploitation of the resource (Deur 2009).

Wocus populations likely began declining in UKAL with the onset of European settlement and conversion of fringe wetlands to agricultural land in the late 1880s. In the late 1800s and early 1900s, farmers constructed dikes and began draining the fringe wetlands to prevent flooding and to increase agricultural yield (Snyder and Morace 1997). In all, over half of the key Wocus habitat associated with UKAL has been drained since 1889 (Snyder and Morace 1997), although some has since been restored.

Distribution

There is very limited information regarding historical Wocus distribution and population estimates in the UKB. Colville (1897) reported that Wocus covered over 10,000 acres of the Upper Klamath Marsh, but such historical accounts do not exist for UKAL. It is reasonable to assume that current Wocus populations in the lakes are only a fraction of what they once were given the extent of wetland reclamation during the 20th century. Currently, Wocus covers approximately 600 acres in UKAL and is largely confined to existing fringe wetlands, particularly those within the Upper Klamath Wildlife Refuge (Figure 3).

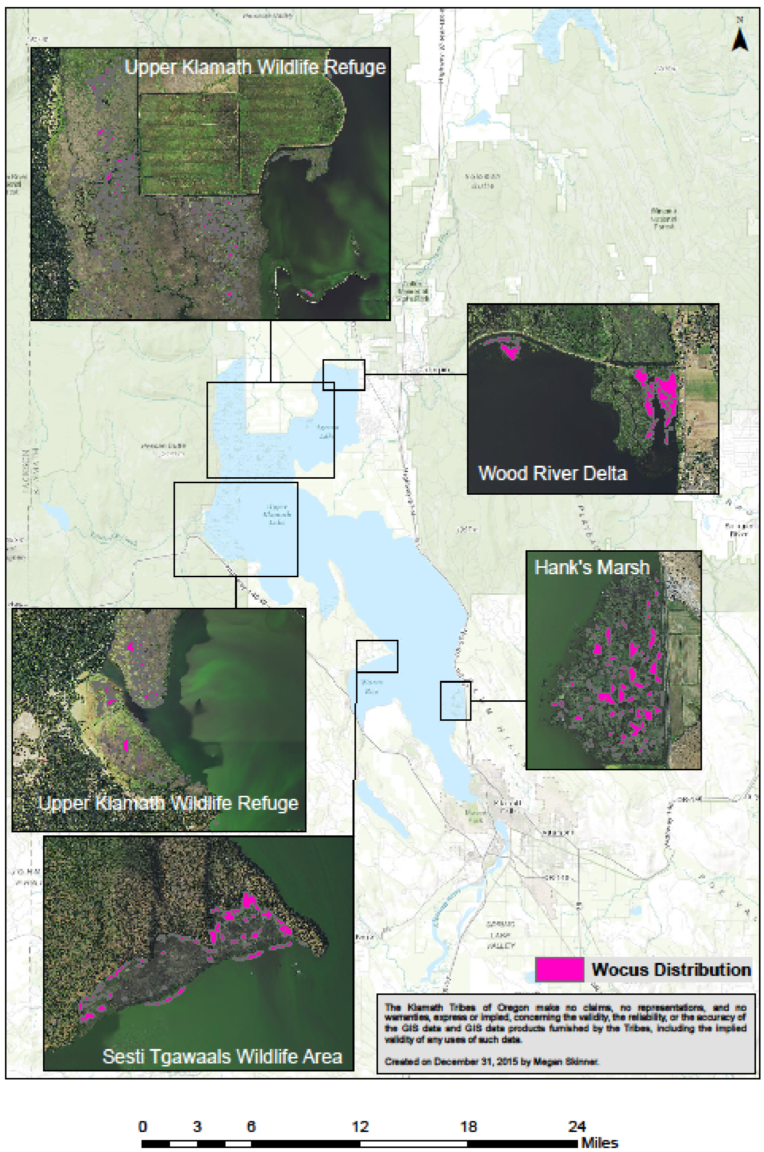

Figure 3. 2014 Wocus distribution in Upper Klamath and Agency Lakes.

Recent restoration efforts

The Nature Conservancy (TNC) has been working to reintroduce Wocus to their 7,509 acre Williamson River Delta (WRD) property since 2004. During this time, several transplanting methods have been utilized with varying results. Tested methods include burying rhizomes in the sediment and tying them to grade stakes; sinking rhizomes to the sediment in weighted burlap bags; burying rhizomes in sediment and tying them to rebar; burying rhizomes in soil above the water line and wiring to rebar stakes; planting small rhizome sections in containers filled with potting soil; propagation from seed at Western Native Planes (formerly Rock Bottom Nursery); burying nursery plants in the sediment and tying them to cinder blocks; attaching rhizomes to coir fabric and weighting with rocks; and pressing rhizomes into the sediment and staking them with bent rebar staples (Shroeder 2014). Early efforts were largely unsuccessful for a number of reasons: excessive wave action that uprooted plants, low water levels leading to desiccation, improper planting leading to rhizome rot, improper seasonal timing of transplanting (spring versus fall), high financial cost of nursery-raised plants, and failure of seeds to germinate. TNC has had greatest success planting large rhizome sections (Figure 4), securing with bent rebar stakes, targeting deeper-water areas, and planting in late spring or early summer. In the fall of 2014, over 66% of transplanted Wocus planted that spring/summer were still alive (Shroeder 2014) and a number of plants survived the winter. After ten years of transplanting efforts, TNC has concluded that whole Wocus plants with rhizomes and several leaves (Figure 4) should be planted in the spring in areas protected from wave action and with at least 3-4 feet of water and secured with bent rebar stakes (Shroeder 2014). TNC is continuing transplanting efforts in the WRD and other entities including U.S. Fish and Wildlife, Trout Unlimited, and TKT are interested in collaborating and expanding the reintroduction program to other parts of the lakes.

Figure 4. Example of a large Wocus rhizome ideal for successful transplanting. (E. Berman 2014)

Future research needs

Despite an emphasis on the role of periphyton in wetland and shallow lake nutrient cycling, it does not appear the relationship between periphyton and *Nuphar lutea* has received any attention. As discussed previously, *Nuphar lutea* rhizomes are capable of sequestering up to 70% of nutrients contained within leaves, but at least 30% of nutrients in senescing tissue will still be released to the water column or sediment. If robust periphyton populations are commonly associated with *Nuphar lutea*, periphyton could play a central role in utilizing any nutrients released during *Nuphar lutea* senescence and could contribute to a tight coupling of nutrients within the macrophtye/periphyton community. It may be that large *Nuphar lutea* leaves lead to shaded conditions unconducive to periphyton growth, but the relationship between these organisms should be studied nonetheless.

For the UKB, further research into the feasibility of seed propagation as a means for reintroduction would be beneficial. Specifically, a study assessing optimal germination conditions may assist restoration practitioners in planning seed plantings in UKAL. The time- and labor-intensive methods currently utilized limit the scope of Wocus reintroduction. Restoration via seed would increase efficiency and scope and reduce labor associated with reintroduction efforts.

Finally, Wocus reintroduction has been offered as mitigation for nutrient loading in UKAL and pilot or laboratory studies assessing the capacity of Wocus to sequester nutrients would assist in restoration planning and implementation. Similarly, it is hypothesized that reintroduced Wocus may provide much needed habitat and cover for the endangered Sucker species and other native fish in UKAL, but further research is needed to test this hypothesis.

Conclusion

Nuphar lutea has the potential to sequester a portion of acquired nutrients in the rhizome and sediments, may benefit zooplankton and benthic macroinvertebrate populations, and may provide habitat and cover for planktivorous and benthic-feeding fish. While the magnitude of these potential benefits in the UKB needs further research, this plant was likely a key component of the historic wetland ecosystem associated with UKAL and Wocus reintroduction may benefit this system in the future. Wocus was also a culturally important plant species and reintroduction will therefore benefit tribal communities in the basin. Much progress has been made since initial reintroduction efforts began ten years ago, and many entities are committed to continuing restoration efforts in the future.

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APPENDIX B

Factors affecting survival and spread of transplanted Wocus (*Nuphar lutea polysepala*) in the Williamson River Delta, Oregon

By

Megan M. Skinner and Christine Adelsberger

Overview

There is growing interest in the reintroduction of native wetland plants in the Upper Klamath Basin of Oregon, specifically in wetland habitats associated with Upper Klamath and Agency Lakes. The hypereutrophic lakes are subject to extensive cyanobacteria blooms and associated large diel fluctuations in oxygen and pH that create hostile conditions for native aquatic biota (Boyd et al. 2002). Additionally, the lakes are critical habitat for ESA-listed Shortnose (*Chasmistes brevirostris*) and Lost River (*Deltistes luxatus*) Suckers (USFWS 2012) and native Redband Rainbow Trout (*Oncorhynchus mykiss newberrii*). It is hypothesized that large-scale reintroductions of wetland plants, particularly Wocus (*Nuphar lutea polysepala*), may provide habitat for native fish and their prey, and alter nutrient cycling such that fewer nutrients may be available for harmful cyanobacteria species (Skinner 2016). Wocus also has cultural value to The Klamath Tribes of Oregon (Colville 1897) and its reintroduction may therefore benefit both the aquatic ecosystem and Tribal culture in the basin. See Skinner (2016) for additional information about the cultural significance and ecology of Wocus in the Upper Klamath Basin.

While The Nature Conservancy (TNC) has been transplanting Wocus in the Williamson River Delta, Oregon (WRD) for over a decade (Shroeder 2014), the effect of habitat variables on survival of transplanted Wocus in Upper Klamath and Agency Lakes has not been quantified in a standardized way. Generally, TNC and collaborators have only had the funding and capacity to develop Wocus transplanting procedures by trial-and-error in the past. As such, transplanting sites, methods, and monitoring procedures were not developed or selected with a standardized assessment of Wocus survival in mind. Despite these challenges, sufficient data is now available to warrant further investigation of transplanting success in the WRD.

TNC has concluded (through trial-and-error) that whole Wocus plants with rhizomes and several leaves should be planted and secured with bent rebar stakes in the spring in areas protected from wave action and with at least 3-4 feet of water (Shroeder 2014). This is a significant refinement of previous transplanting methods, but we believe further investigation is warranted given the desire of The Klamath Tribes, U.S. Fish and Wildlife Service, and other entities to expand Wocus reintroduction efforts beyond TNC's WRD property. Assessing the effects of habitat variables on Wocus survival in the WRD may yield results that can be applied to other areas of the Upper Klamath Basin considered for Wocus reintroduction. Additionally, there are currently no peer-reviewed studies assessing Wocus transplanting methods.

The purpose of this investigation was to (1) quantify the effect of habitat variables on Wocus survival one year after transplanting, (2) quantify the effect of habitat variables on Wocus survival two years after transplanting, and (3) determine if Wocus transplanted in 2014 has spread during the two following years.

Methods

Study Site

The WRD occupies approximately 5,500 acres along the last 6 km of the Williamson River in the Upper Klamath Basin of southern Oregon. Historically, the WRD contained extensive wetlands that provided food and habitat for fish and wildlife, subsistence resources for local tribal communities, and that likely played a key role in nutrient cycling in Upper Klamath Lake. However, during the 1940s, the WRD was leveed, drained, and used for agriculture.

In 1996, TNC began purchasing 7,509 acres of the WRD and surrounding land and created the Williamson River Delta Preserve. TNC breached levees on the western portion of the WRD on October 30, 2007 to facilitate flooding and wetland restoration. Shortly after flooding, The Nature Conservancy initiated Wocus reintroduction efforts on the property (Shroeder 2014). See Wong et al. (2011) for additional details about the study site including nutrient retention rates and soil types.

Transplanting and monitoring

While TNC has been transplanting Wocus in the WRD since 2006, the focus of this study will be transplanting efforts completed in the late spring of 2014 and 2015 with some assistance from The Klamath Tribes and U.S. Fish and Wildlife Service. Previous efforts were only marginally successful and will therefore not be discussed here.

Prior to the 2014 and 2015 transplanting, TNC completed a visual survey of the WRD by boat to identify suitable transplant sites; selected sites represented a variety of substrate types and levels of exposure to wind and waves (Table 1). Once sites were chosen, rhizomes were harvested from a large, self-sustaining Wocus population in the Upper Klamath Marsh, Oregon. The following day, TNC and collaborators transplanted 2 - 15 rhizomes in each selected site (Table 1) following transplanting procedures described in Shroeder (2014).

Table 1. Wocus transplant site characteristics. Note that "exposure level" refers to level of exposure to wind and waves.

Site #	Transplant year	Monitoring year(s)	Substrate type	Exposure level	May water depth (ft)	# of transplants
1	2014	2015, 2016	Soft organic	Low	4.0	6
2	2014	2015, 2016	Firm organic	Moderate	4.0	4
3	2014	2015, 2016	Soft organic	Moderate	4.0	8
4	2014	2015, 2016	Soft organic	Low	4.0	9
5	2014	2015, 2016	Soft organic	Low	4.5	7
6	2014	2015, 2016	Soft organic	Moderate	5.0	8
7	2014	2015, 2016	Soft organic	Moderate	4.0	10
8	2014	2015, 2016	Soft organic	Moderate	4.5	13
9	2014	2015, 2016	Firm organic	Low	3.5	5
10	2014	2015, 2016	Soft organic	Low	4.0	5
11	2014	2015, 2016	Soft organic	Low	3.5	12
12	2015	2016	Soft organic	Moderate	4.0	4
13	2015	2016	Firm organic	High	3.5	2
14	2015	2016	Firm organic	High	5.0	4
15	2015	2016	Firm organic	High	4.5	4
16	2015	2016	Firm organic	High	4.0	8
17	2015	2016	Firm organic	Moderate	4.0	13
18	2015	2016	Firm organic	Moderate	5.0	12
19	2015	2016	Firm organic	Moderate	4.0	8
20	2015	2016	Firm organic	High	4.0	6
21	2015	2016	Soft organic	Moderate	4.0	6
22	2015	2016	Soft organic	Moderate	4.0	2
23	2015	2016	Firm organic	Moderate	4.0	15
24	2015	2016	Soft organic	Moderate	4.0	11
25	2015	2016	Sand	High	4.0	12

TNC monitored transplanted Wocus in May of the year following transplanting. For Wocus transplanted in 2014, TNC has completed two years of spring monitoring (2015 and

2016); for Wocus transplanted in 2015, one spring monitoring event has been completed (Table 1). The spring 2015 monitoring only included the number of plants at each site, but monitoring was expanded in spring 2016 to include level of exposure to wind and waves, substrate type, and May water depth in addition to number of plants at each site. Percent survival was then calculated using number of plants and number of original Wocus transplants for a given site.

Number of surviving plants was determined via visual analysis of the planting site; only Wocus plants with leaves visible at or near the water surface were considered alive. High turbidity associated with suspended sediment and phytoplankton prevents visual assessment of plant parts more than a 0.5 m below the water surface (personal observation). Similarly, level of exposure to waves and prevailing winds from the south and west was assessed visually. Low exposure was defined as areas with still water and limited wind and wave action (canals, protected bays, oxbows, etc); moderately exposed sites were areas with some wind and wave action and some protection from prevailing winds (bays with limited protection, leeward side of islands or levees); and high exposure sites were those in open water with direct exposure to prevailing wind and wave action. Finally, water depth was measured with a stadia rod and substrate type was determined via the "first touch" method (USEPA 2013).

Statistical analyses

Wocus survival one year after transplanting

Analyzing percent Wocus survival data from each plant's first spring monitoring event (2015 and 2016 for Wocus planted in 2014 and 2015, respectively) allowed us to assess percent survival over the full range of substrate types (firm organic, sand, and soft organic) and exposure levels (low, moderate, high); this was necessary given that all levels of each factor were not represented fully in both planting years (Table 1).

We used two one-way ANCOVAs (one each for May water depth and number of transplants) to determine if planting year had a statistically significant effect on percent Wocus survival after controlling for number of transplants and May water depth. Additionally, we used a three-way ANOVA to assess the effect of planting year, level of exposure, and substrate type on Wocus survival.

We recognize that monitoring data collected in two different years for plants transplanted in different years may not be analogous. Including planting year in both the ANOVA and

ANCOVAs allows us to minimize uncertainty by assessing external factors that may differ (i.e., air and water temperature, precipitation, differences in lake-level management, etc.) and lead to variable survival between years.

All data met assumptions of normal error distribution and equal variance (determined via Shapiro tests and visual diagnostics in R).

Wocus survival two years after transplanting

We used a multiple linear regression to assess the effect of water depth and number of transplants on percent Wocus survival for 2014 plants. Additionally, we used a two-way ANOVA to assess the effects of level of exposure and substrate type on Wocus survival for those planted in 2014. Both tests allowed us to determine if habitat variables relevant to 2014 plants (i.e., no exposure level "high") affected survival.

All data met assumptions of normal error distribution and equal variance (determined via Shapiro tests and visual diagnostics in R).

Wocus spread since 2014

Finally, we used a paired t-test to compare 2015 and 2016 percent survival for Wocus planted in 2014. While TNC and collaborators also collected information about the number of plants visible at each site during spring monitoring, percent survival appears to be the appropriate variable for this test given that not all sites received the same number of transplants (Table 1); as such, sites with a large number of transplants, but few survivors, may skew mean 2015 and 2016 plant numbers such that spread would not be apparent. Using percent Wocus survival allows us to analyze spread based on a variable normalized by number of transplants at a given site.

Data met assumptions of normal error distribution and equal variance (determined via Shapiro tests and visual diagnostics in R).

Results and discussion

Wocus survival one year after transplanting

After controlling for water depth, planting year did not significantly affect percent Wocus survival (one-way ANCOVA: $F_{1,21} = 0.031$, $p = 0.861$). Number of transplants was not a statistically significant predictor of Wocus survival for those planted in 2014 and monitored in 2015 (slope not significantly different than zero, $p = 0.274$), while the relationship was statistically significant for Wocus planted in 2015 and monitored in 2016 (slope significantly different than that for 2014 plants, $p = 0.039$, $R^2 = 0.335$) (ANCOVA interaction term: $F_{1,21} = 4.910$, $p = 0.038$) (Figure 1).

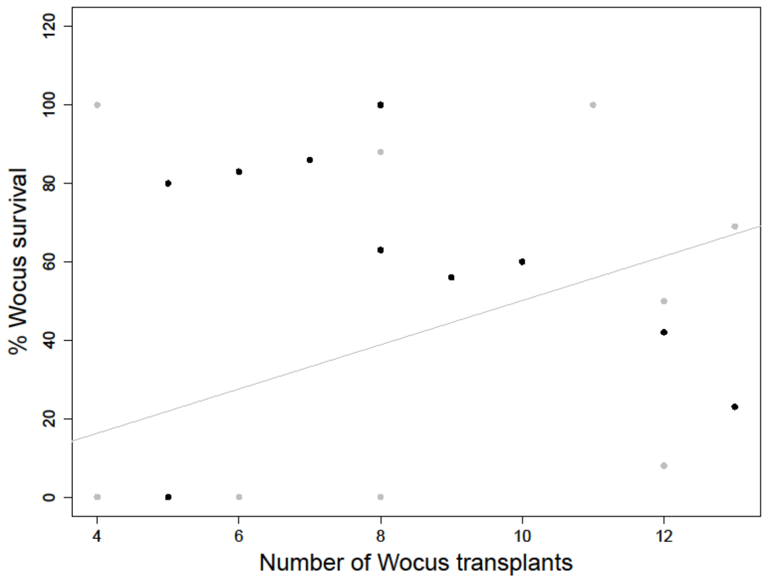

Figure 1. The effect of number of transplants on Wocus survival by planting year. Wocus planted in 2014 are denoted with black circles, while those planted in 2015 are denoted with gray circles and the gray regression line. Note that the slope of the regression line for Wocus planted in 2014 was not significantly different than zero and has been excluded from this figure.

None of the interaction terms in the three-way ANOVA were significant and we therefore interpreted the main effects of exposure, substrate type, and planting year. The main effects had no significant effect on percent Wocus survival (substrate: $F_{2,14} = 0.185$, $p = 0.832$; exposure: $F_{2,14} = 0.389$, $p = 0.122$; planting year: $F_{1,14} = 0.800$, $p = 0.384$).

The results of the ANCOVAs indicate that water depth does not seem to affect percent survival one year after transplanting, but number of transplants may roughly predict percent Wocus survival, particularly for those planted in 2015 and monitored in 2016. Specifically, all 2015 planting sites at which TNC planted more than eight individual Wocus plants had percent survival greater than zero. However, the effect was not significant for Wocus planted in 2014 and monitored in 2015, meaning that the trend may not be strong enough inter-annually to warrant refining of transplant methods. More work is needed to better estimate the magnitude of this effect on percent Wocus survival.

While the ANOVA indicates no categorical habitat variables significantly affected Wocus survival, it is worth noting that the data suggests sites with high exposure levels may result in percent Wocus survival lower than what is acceptable for this type of reintroduction project (Figure 2). It may therefore be beneficial to focus planting efforts on sites with low or moderate exposure.

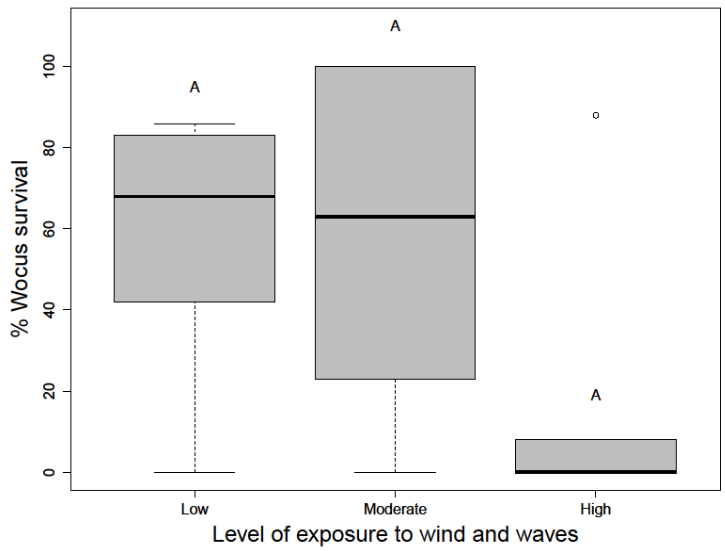

Figure 2. Percent Wocus survival by level of exposure to wind and waves. Boxes represent the first through third quartiles and the median (bold line), whiskers represent the minimum and maximum, and open circles indicate data outliers. Differing letters indicate statistical significance.

Generally, TNC has refined methods such that the Wocus assessed in this investigation were planted in ideal substrates and optimal water depth to encourage establishment and prevent desiccation. As such, it is not surprising that the analysis of these variables yielded negative results. There is a possibility that level of exposure affects percent Wocus survival, but a second year of monitoring for Wocus planted in areas with high exposure in 2015 may clarify this finding. It should be noted that TNC has also established that extremely high exposure to wind and waves will likely result in very low (or no) Wocus survival, so levels of exposure assessed here may be specifically relative to this Wocus transplanting project. Regardless, the results suggest the difference in percent survival between high exposure and the lesser levels of exposure (i.e., low and moderate) is such that focusing transplant efforts on sites with lower levels of exposure may maximize transplanting success in the future.

Wocus survival two years after transplanting

The interaction term in the two-way ANOVA was not significant ($F_{1,7} = 0.073$, $p = 0.795$) and we interpreted the main effects of exposure and substrate type. The main effects were not statistically significant (substrate: $F_{1,7} = 0.192$, $p = 0.674$; exposure: $F_{1,7} = 0.159$, $p = 0.702$).

The multiple linear regression indicated that number of transplants did not have a significant effect on percent Wocus survival two years after transplanting ($t = -1.083$, $p = 0.310$), but water depth did ($t = 2.662$, $p = 0.029$, $R^2 = 0.409$) (Figure 3).

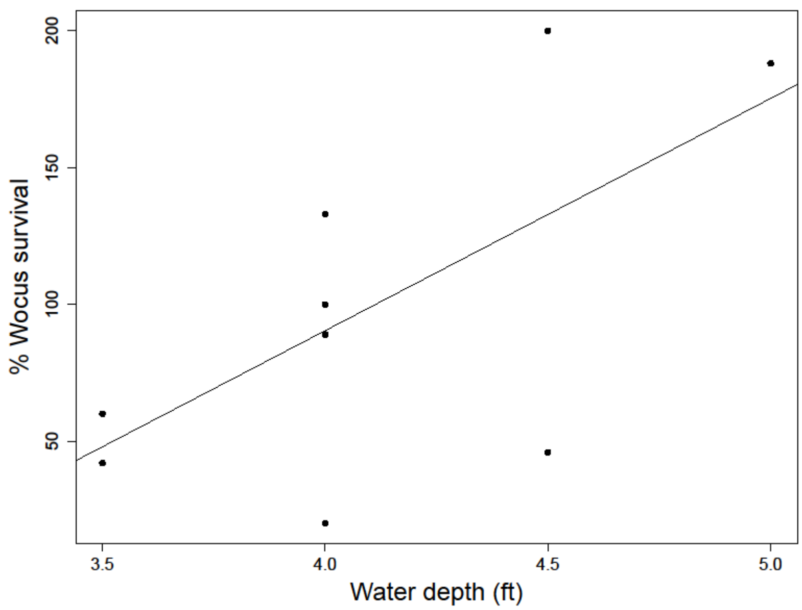

Figure 3. The effect of water depth on Wocus survival two years after transplanting.

The results of the ANOVA suggest that no categorical habitat variables measured significantly affect percent Wocus survival two years after transplanting. As indicated above, TNC has refined transplanting methods to target optimal Wocus growing conditions; it is therefore not surprising that the analysis of some habitat variables yielded negative results. It should also be noted that Wocus transplanted in 2014 were not planted in any sites with a high level of exposure to wind and waves and this may additionally explain the negative result for exposure level. Indeed, the analysis described in the previous section suggests there is not a notable difference in percent survival between low and moderate levels of exposure (Figure 2), so it's reasonable to conclude exposure didn't affect 2014 Wocus transplants.

It appears that Wocus planted in water depths greater than or equal to 4.5 ft had higher percent survival two years after transplanting, relative to those planted in shallower water (Figure 3). However, further research on the effect of water depth is warranted given that this variable did not significantly affect percent Wocus survival one year after transplanting (Figure 1).

Wocus spread since 2014

Percent Wocus survival increased significantly between 2015 and 2016 for Wocus planted in 2014 (paired t-test: $t = -2.494$, $p = 0.032$); mean percent Wocus survival increased by 35 between 2015 and 2016 (Figure 4). This result suggests that, with all inter-site differences in habitat variables aside, percent Wocus survival has increased from a 2015 mean of 63% to 98% in 2016. Specifically, this increase in percent Wocus survival is likely indicative of the presence of new plants appearing at the transplant sites during spring monitoring events. In other words, only 63% of plants survived the first year after transplanting, but during the second year, Wocus spread and multiplied such that mean percent survival increased to 98% of the original transplants. Also note that the 2016 monitoring data indicates some sites had percent survival as high as 200% (or a doubling of Wocus plants since original transplant event), while maximum percent survival in 2015 was 100%.

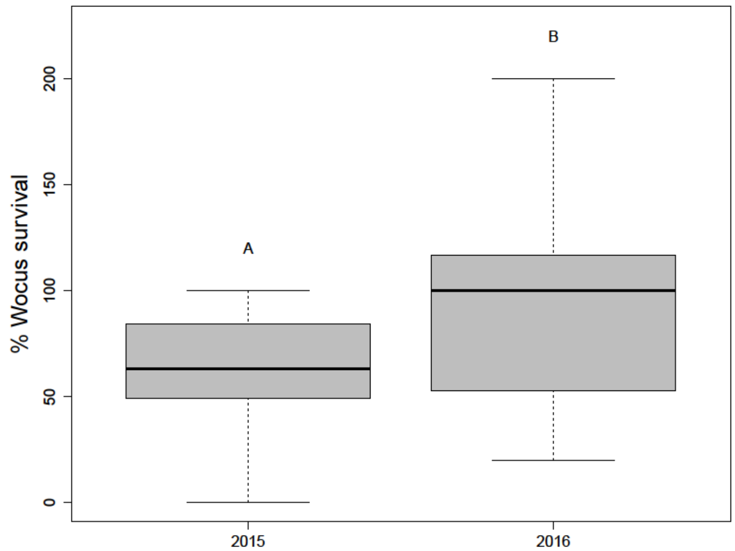

Figure 4. Percent Wocus survival by monitoring year. Boxes represent the first through third quartiles and the median (bold line), whiskers represent the minimum and maximum, and open circles indicate data outliers. Differing letters indicate statistical significance.

Conversely, this increase in percent survival could also be indicative of observer error during the spring 2015 monitoring. This concern is supported by the observation that no sites had 0% survival in 2016, while a number did in 2015 (Figure 4); this suggests even sites with no surviving Wocus in 2015 gained Wocus plants between May of 2015 and 2016. It is unlikely these Wocus plants spread from naturally-occurring colonies or other transplant sites given the distance between transplant sites and a lack of naturally-occurring Wocus colonies to serve as source populations in the WRD. Further research regarding Wocus spread at WRD transplant sites is warranted.

Conclusion

Overall, it seems that substrate type has little effect on percent Wocus survival. However, water depth, number of transplants, and exposure may influence Wocus survival, but the effect is variable between years. Further research regarding the effects of these variables on Wocus survival is warranted. Additionally, it appears that there has been an increase in percent survival between 2015 and 2016 for Wocus planted in 2014, indicating successful establishment and subsequent spread of transplanted Wocus. This should be studied further to determine if higher percent survival was indeed the result of spread rather than observer error during the spring 2015 monitoring event.

Recommendations

Based on the findings of this investigation, we recommend the following to those transplanting Wocus in Upper Klamath and Agency Lakes:

1. Focus transplanting efforts on sites with low to moderate levels of exposure to wind and waves.
2. Specifically in the WRD, continue monitoring efforts of 2015 and future transplants to further investigate the effect of water depth and number of original transplants on survival.
3. Specifically in the WRD, continue monitoring efforts of established transplants to assess spread; this information may be useful in future planting efforts to estimate the time necessary to achieve desirable results.
4. If further Wocus survival studies to refine site selection criteria are desired, sites selected for transplanting each year should represent the entire range of habitat variables examined here.

Acknowledgments

This study would not have been possible without TNC's decade-long dedication to Wocus reintroduction in the Upper Klamath Basin. TNC's efforts have resulted in significant refinements in Wocus transplanting methods, which are of central importance to this study and future reintroduction and research efforts in the basin. In particular, we'd like to acknowledge current TNC employees Heather Hendrixson, Blake Eldridge, and Lori Nussbaum, and past employees Alix Lee, Natanya Hayden, Charlie Erdman, Eli Berman, Carla Ross, Mel Van Scoyc, and Kellen Watson for their Wocus-related efforts. Additionally, assistance from former employees of The Klamath Tribes (Tony LaGreca) and U.S. Fish and Wildlife Service (Ruth Olson and John Reins) has been essential to this restoration process.

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APPENDIX C

The effects of three planting methods on transplanted Wocus (*Nuphar lutea polysepala*) survival in the Williamson River Delta, Oregon

By

Megan M. Skinner and Christine Adelsberger

Overview

There is growing interest in the reintroduction of native wetland plants in the Upper Klamath Basin of Oregon, specifically in wetland habitats associated with Upper Klamath and Agency Lakes. The hypereutrophic lakes are subject to extensive cyanobacteria blooms and associated large diel fluctuations in oxygen and pH that create hostile conditions for native aquatic biota (Boyd et al. 2002). Additionally, the lakes are critical habitat for ESA-listed Shortnose (*Chasmistes brevirostris*) and Lost River (*Deltistes luxatus*) Suckers (USFWS 2012) and native Redband Rainbow Trout (*Oncorhynchus mykiss newberrii*). It is hypothesized that large-scale reintroductions of wetland plants, particularly Wocus (*Nuphar lutea polysepala*), may provide habitat for native fish and their prey, and alter nutrient cycling such that fewer nutrients may be available for harmful cyanobacteria species (Skinner 2016). Wocus also has cultural value to The Klamath Tribes of Oregon (Colville 1897) and its reintroduction may therefore benefit both the aquatic ecosystem and Tribal culture in the basin. See Skinner (2016) for additional information about the cultural significance and ecology of Wocus in the Upper Klamath Basin.

The Nature Conservancy (TNC) has been transplanting Wocus in the Williamson River Delta, Oregon for over a decade (Schroeder 2014). During this time, TNC has significantly refined planting methods and has concluded that whole Wocus plants with rhizomes and several leaves should be planted in the spring in areas protected from wave action and with at least 3-4 feet of water and secured with bent rebar stakes (Schroeder 2014). Harvesting and planting large rhizomes is both time and labor-intensive and therefore may not be suitable for larger-scale reintroduction efforts. Given the desire of The Klamath Tribes (TKT), US Fish and Wildlife Service (USFWS), and other entities to expand Wocus reintroduction efforts beyond TNC’s Williamson River Delta property, it is necessary to determine if other, more efficient methods can be utilized to successfully transplant Wocus. In particular, planting potted Wocus appears to be less labor intensive for restoration practitioners and is thus worth exploring.

The purpose of this investigation was to assess how three planting methods affected Wocus survival in areas with three different levels of exposure to wind and waves. Planting methods included (1) securing large rhizomes to the substrate, (2) pressing Wocus pots into the substrate, and (3) dropping Wocus pots into the water. The ability to simply press or drop pots into the sediment and allow them to biodegrade as the plant establishes not only eliminates the need to harvest rhizomes, but reduces handling time at the transplanting site and does not require a subsequent visit to remove non-biodegradable material from the sediment (as with rebar stakes). If survival of potted Wocus is similar to Wocus rhizome transplants, we believe one of the potted planting methods would represent a significant time and labor savings for restoration practitioners and could facilitate larger-scale Wocus reintroduction efforts in the basin.

Methods

Study Site

The Williamson River Delta occupies approximately 5,500 acres along the last 6 km of the Williamson River in the Upper Klamath Basin of southern Oregon. Historically, the Williamson River Delta contained extensive wetlands that provided food and habitat for fish and wildlife, subsistence resources for local tribal communities, and that likely played a key role in nutrient cycling in Upper Klamath Lake. However, during the 1940s, the Williamson River Delta was leveed, drained, and used for agriculture.

In 1996, The Nature Conservancy began purchasing 7,509 acres of the Williamson River Delta and surrounding land and created the Williamson River Delta Preserve. The Nature Conservancy breached levees on the western portion of the Williamson River Delta on October 30, 2007 to facilitate flooding and wetland restoration. Shortly after flooding, The Nature Conservancy initiated Wocus reintroduction efforts on the property (Schroeder 2014). See Wong et al. (2011) for additional details about the study site including nutrient retention rates and soil types.

Transplanting

Prior to the 2016 transplanting, TNC, USFWS, and TKT completed a visual survey of the Williamson River Delta by boat to identify three suitable transplant sites, each representing one of three levels of exposure to wind and waves. Water depth was consistent across sites and any difference in substrate type between sites was not expected to confound results (Skinner et al. 2016).

Potted Wocus were propagated from wild seed and reared for 1.5 years in black plastic pots in 3 feet-deep pools at Western Native Plants in Bonanza, OR. Before transplanting at the Williamson River Delta, Western Native Plants personnel transplanted Wocus into biodegradable 1-gallon pots in soil mixed with rock and gravel such that pots containing Wocus weighed around 10 pounds. Wocus rhizomes were harvested from a large, self-sustaining Wocus population in the Upper Klamath Marsh, Oregon in June 2016. The following day, TNC and collaborators transplanted 10 rhizomes and 10 pots each from two potted Wocus planting methods at each selected site (Table 1). The “drop” method for potted Wocus planting involved dropping the pot from the water surface such that it would sink through the water column and settle on the sediment surface. The “press” method involved firmly pressing the pot into the sediment.

Table 1. Wocus transplant site characteristics. Note that “exposure level” refers to level of exposure to wind and waves.

Site #	Exposure level	Substrate type	May 2016 water depth (ft)	Planting method	Sample size
1	Low	Soft organic	4	Rhizome	10
1	Low	Soft organic	4	Drop	10
1	Low	Soft organic	4	Press	10
2	Moderate	Soft organic	4	Rhizome	10
2	Moderate	Soft organic	4	Drop	10
2	Moderate	Soft organic	4	Press	10
3	High	Firm organic	4	Rhizome	10
3	High	Firm organic	4	Drop	10
3	High	Firm organic	4	Press	10

Monitoring

At the time of transplanting, TNC and collaborators collected habitat data at each site including water depth, substrate type, and level of exposure to wind and waves following methods described in Skinner et al. (2016). Low exposure to wind and waves was defined as areas with still water and limited wind and wave action (canals, protected bays, oxbows, etc); moderately exposed sites were areas with some wind and wave action and some protection from prevailing winds (bays with limited protection, leeward side of islands or levees); and high exposure sites were those in open water with direct exposure to prevailing wind and wave action.

Number of surviving plants will be determined via visual analysis of the planting sites in May 2017; only Wocus plants with leaves visible at or near the water surface are considered alive. High turbidity associated with suspended sediment and phytoplankton prevents visual assessment of plant parts more than a 0.5 m below the water surface (personal observation).

Statistical analyses

Once we complete the spring 2017 monitoring, we will use a two-way ANOVA (or nonparametric equivalent) to assess the effect of planting method (drop, press, rhizome) and level of exposure (low, moderate, high) on Wocus survival.

Results

Data analysis and interpretation will occur after May 2017 monitoring.

Discussion

If potted plants do not survive and are not visible during the spring 2017 monitoring, we will not know if they failed due to smaller plant size (reduced energy stores), shorter stem length (reduced photosynthetic capability), failure of roots to establish through pot wall, something in the pot material affecting plant health, or if they simply weren't heavy enough and washed/floated away. Additional discussion will be developed after spring 2017 data collection, interpretation, and analysis.

Recommendations

Recommendations will be added after data analysis and interpretation is completed. Data will be collected during the May 2017 monitoring event.

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