H-02 Constructed Wetland Studies

Amphibians and Plants

FY-2008 ANNUAL REPORT

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EXECUTIVE SUMMARY

Construction of the H-02 constructed treatment wetlands adjacent to H-Area on the Savannah River Site (SRS) began during FY-2007. The Savannah River Ecology Laboratory (SREL) initiated amphibian and vegetation surveys at the site in summer 2008. Ecological research conducted by SREL focuses primarily on four questions related to these treatment wetlands: 1) Within 1½ years of construction, what amphibians, reptiles, and plants have become established in the wetlands? 2) Is there any evidence that elevated metals levels in the wetlands (e.g., copper and zinc) affect amphibian success? 3) How do the amphibian diversity and numbers compare to other, more natural, wetlands? 4) As the constructed wetlands age, how will changes in vegetation composition and structure affect the amphibian community?

This report summarizes our initial amphibian and vegetation sampling at the H-02 treatment wetlands from May-September 2008. Permanent plots for monitoring vegetation and amphibians were established in each wetland cell, and drift fences were constructed adjacent to the area to determine amphibian use of the ponds as breeding sites. We recorded 617 captures of 17 amphibian and reptile species at the H-02 treatment wetlands in FY-08, including successful production of juveniles by eight species. This level of reproductive success was higher than at the natural wetland reference site, which dried in mid-May. However, to date no salamander species have been recorded at the H-02 site. Our baseline vegetation sampling documented 18 vascular plant species plus algae in our plots. Our plant coverage, structure and diversity estimates will be used to evaluate community (plant and amphibian) changes over time.

Chapter I. Introduction and Overview

David E. Scott, Rebecca R. Sharitz, J. Whitfield Gibbons & Tracey D. Tuberville

The Savannah River Ecology Laboratory (SREL) initiated ecological studies related to the operation of the H-02 constructed wetlands in May 2008. Constructed wetlands are one method to treat and improve water quality at regulated outfalls on the Savannah River Site (SRS; Bach et al. 2008). Heavy metals such as copper, lead, and zinc are removed by adsorption to organic matter and clay particles, and sulfate reducing bacteria enable the precipitation of metal ions in the anaerobic soils (Nelson et al. 2006). Constructed treatment wetlands proved effective at the A-01 outfall on the SRS, with removal efficiencies > 80% for copper, mercury, and lead within four years. Systematic monitoring has revealed that water quality is improved prior to discharge into streams, but the extent to which these constructed treatment wetlands also serve other "natural wetland" functions, such as providing wildlife habitat, has not been documented.

One taxonomic group for which many types of wetlands are important habitats is amphibians– frogs, toads, and salamanders. The global decline of amphibian species has been discussed in the scientific and popular press for more than a decade. Recent documentation that 32.5% of amphibian species worldwide are threatened and >7% are critically endangered (Stuart et al. 2004) has heightened the interest in understanding why some species are declining and others are not, as well as ways to protect existing populations of species of concern. Some of the main factors implicated in the declines, such as disease (i.e., the chytrid fungus, *Batrachochytrium dendrobatidis*) and pollutants, have been studied in conjunction with natural populations as part of SREL's amphibian ecology program (e.g., Daszak et al. 2005). SREL is recognized internationally for its basic and applied amphibian studies, which include the recovery of local amphibian populations after the construction and start-up of the DWPF facility (e.g., Pechmann et al. 1994, Pechmann et al. 2001).

Isolated wetlands are extremely important habitats for the majority of amphibian species in the Midlands of SC. The natural wetlands on the SRS provide numerous breeding sites for amphibians, including for species of state conservation concern, but restored and created wetlands may also serve that wetland function. A concern related to treatment wetlands that receive process wastewater discharges, however, is that these created wetlands may serve as "ecological traps" for amphibians if adults are attracted to the sites to breed, but the aquatic conditions of the sites are not suitable for eggs and/or larvae, and therefore the reproductive success is zero. Beginning in May 2008, SREL initiated studies to evaluate the habitat suitability of the H-02 wetland complex for amphibians as the wetland increases its contaminant removal efficiency and contaminant load, and as a structurally diverse, mature vegetation community is established.

Water chemistry is extremely important to the successful development of amphibian eggs and young. Of particular interest are factors such as pH, dissolved oxygen (DO) concentration, and concentrations of metal ions such as copper (Cu) and zinc (Zn). The H-02 wetlands, now in their early phase of establishment, exhibit large fluctuations in some of these parameters (Bach et al. 2008). By assessing the response of amphibians to the water quality of the H-02 wetlands over time and comparing amphibian success in created versus natural wetlands, we will better understand the suitability of the H-02 created wetlands for wildlife habitat, especially amphibians. The H-02 wetlands were designed to comply with regulatory guidelines for process and storm water discharge from H-Area facilities, but they may also provide wildlife benefits.

Chapter II of this report contains the results of SREL's amphibian and reptile sampling at the H-02 treatment wetlands from June-September 2008, with comparative data from the reference site, Rainbow Bay. To date the primary effort has been to survey the herpetofauna (amphibians and reptiles) that are using the constructed wetlands. Future efforts will involve experimental tests of the H-02 waters on amphibian development and reproductive success, both in the pre-treatment retention pond and the constructed wetlands.

One objective of the amphibian studies at the H-02 constructed wetlands is to understand the relationship between amphibian community reproductive success and the changes in wetland vegetation structure over time. In Chapter III we present results of the initial vegetation sampling from the summer of 2008.

In summary, wetlands designed to comply with regulatory guidelines for effluent discharge may also provide wildlife benefits. Our initial surveys of the H-02 wetlands documented extensive amphibian use of the wetland cells and successful recruitment of young by eight amphibian species. Assessment of contaminant impacts for this wetland complex may be particularly important, as the state endangered gopher frog (*Rana capito*) and state species of concern tiger salamander (*Ambystoma tigrinum*) breed in natural wetlands near the H-02 wetland complex. The experiments we will conduct in FY-09 on chemical sensitivities of closely related (but not state listed) surrogate species will help quantify the suitability of the H-02 wetlands for amphibians.

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CHAPTER II – AMPHIBIAN AND REPTILE USE OF THE H-02 CONSTRUCTED WETLANDS

David E. Scott, Tracey D. Tuberville, & J. Whitfield Gibbons

INTRODUCTION

Many factors shape the use of aquatic habitats by amphibians, including pond characteristics related to hydrology as well as individual species' characteristics such as physiological tolerances to water chemistry and life history requirements. Ponds that are extremely ephemeral (< 30 day hydroperiod) or permanent (> one year) are used by fewer (and different) species of amphibians than ponds with intermediate hydroperiods (Snodgrass et al. 2000). Pond permanence often negatively affects larval amphibians by permitting many predators, especially fishes, to persist. Because the H-02 constructed wetlands are a flow-through system with a relatively constant water level, the amphibian species richness of the site may be lower than that of similar sized isolated wetlands that dry occasionally, which eliminates predators such as fish.

Once a natural aquatic habitat is colonized by amphibians, numerous factors combine to determine success. In natural ponds, after amphibians mate and deposit eggs, species characteristics such as larval requirements for food, temperature tolerance, predator avoidance, and length of the larval period all interact to determine larval success. In addition, biotic processes such as predation and competition interact to shape communities of pond-breeding amphibians (Wilbur 1987). Salamanders (larval and adult) are carnivorous and frequently occur at high densities, and they can exert strong predation pressure within the amphibian community (e.g., Morin 1981), especially on small herbivorous tadpoles. Predatory salamanders can persist only in ponds with long hydroperiods, and predatory fish only in permanent ponds. These predators can reduce or completely eliminate other species of amphibians (Lawler 1989).

Overlaid on the natural dynamics of amphibian communities, in a constructed wetland additional factors related to water quality and vegetation structure may influence species success. For example, acute toxicity exposures (96-hour LC50s) determined that the LC50 for copper (Cu) concentration for the boreal toad (*Bufo boreas*) is 0.12 mg/L, or 120ppb (Dwyer et al. 2005). In the Argentine toad, *Bufo arenarum*, a Cu concentration of 0.085 mg/L (85 ppb) was lethal to half the embryos within 24 h of exposure (Herkovits and Helguero 1998). In chronic exposures, Cu concentrations of 5.5 µg/L (5.5 ppb) reduced survival of spring peeper tadpoles (*Pseudacris crucifer*) by 41% (Baud and Beck 2005). In some instances there has been no direct effect of low copper concentration on survival or growth, but it has significantly increased the larval period under laboratory conditions (e.g., 3.18 µg/L in the gray treefrog, *Hyla chrysoscelis*; Parris and Baud 2004), which may increase actual mortality under field conditions. Copper in the H-02 influent has ranged as high as 31-37 ppb in summer months, and 7 ppb in the effluent exiting the treatment wetlands (G. Mills and N. Etheridge, *unpublished data*); these levels may be of concern for normal amphibian development.

The potential toxicity of various metal species to amphibians and the concentrations at which effects are observed is a complex phenomenon that is influenced by pH, dissolved organic carbon (DOC) levels, the presence of other metal ions and/or acid-volatile sulfides (AVS), as well as the developmental stage and species of amphibian. For example, Cu may inhibit larval growth at concentrations of 60 µg/L in one species but not another, eggs may show no effect even at 150 µg/L Cu, and effects may be reduced by the presence of either Zn or DOC or by a lowered pH. This is a key point for the H-02 wetlands, which have both Zn and relatively high levels of DOC— the bioavailability and toxicity of metals are not necessarily proportional to total concentration but may be influenced by other components of the system. In the H-02 wetlands, which were amended with gypsum, it is also likely that AVS is an important pathway for binding metals and reducing bioavailability and toxicity to amphibians.

Project Goal --We will assess the evolution of the suitability of the H-02 wetlands for amphibians in two ways: 1) We will use a variety of capture/census techniques to monitor amphibian use of the wetlands, particularly to determine which species are able to successfully produce juveniles (i.e., indicating that the wetland is not a likely ecological trap for that species), and 2) We will use manipulative experiments to examine the egg and larval success of select amphibian species under the range of Cu concentrations found in the H-02 system (i.e., retention pond and constructed treatment cells). We will combine these results with the findings in Chapter III, relating the vegetation in the wetlands to suitability for amphibian growth and development.

METHODS

The H-02 study site -- Drift-fence/pitfall trapping (Dodd and Scott 1994), aquatic trapping, and call surveys are standard techniques to monitor amphibians – we are using all three to assess amphibian use of the H-02 wetland cells. Site use approval (SU-08-13-R) was granted in mid-July 2008, and by mid-August we installed three partial drift fences (20 m in length per fence) along the north edge of the H-02 constructed wetlands to capture adult amphibians as they enter the wetlands (from the adjacent forest) to breed and juveniles as they emigrate from the wetland after completing larval development (see description of drift fences in the Rainbow Bay sampling section). Captured animals are released on the opposite side of the fence to continue their movement. Data on species richness and juvenile recruitment can be compared to surveys from reference wetlands on the SRS to assess the performance of the H-02 wetlands as suitable aquatic breeding habitat.

We supplemented the drift fence technique with aquatic trapping (i.e., using minnow traps) to assess larval species richness, numbers, and health. In FY-08 we conducted three aquatic trapping sessions (one per month in June, July, and August). Each session consisted of 15-24 traps set for four days/three nights in the two constructed treatment wetlands. Traps were checked daily and species, number of individuals, and life stage were recorded. In August 2008 we established 24 permanent trap locations (12 per wetland cell) to facilitate vegetation sampling (see Chapter III) and allow comparison of successional changes in vegetation with amphibian populations.

Planned studies in FY-09 – In FY-09 we plan to continue the drift fence and aquatic trap sampling, and supplement these techniques with night call surveys to estimate numbers of breeding individuals [in FY08 most amphibians species had either completed breeding or were nearly finished prior to the initiation of this project]. Some larvae will also be tested for the presence of the chytrid fungus to compare to levels of this pathogen found in bullfrog (*Rana catesbeiana*) larvae at the A-01 created wetlands. Additional samples will be taken of selected species to assay copper levels in tissues. In conjunction with the study by G. Mills and others, we will use *in situ* toxicity tests to examine the effects of H-02 water chemistry on several amphibian species (e.g., representative frog, toad, and salamander species).

The Rainbow Bay Study Site -- The amphibian community has been monitored for 30 years at Rainbow Bay (RB) in Barnwell County on the SRS, approximately 3 km from the H-02 wetlands. Rainbow Bay is a relatively undisturbed freshwater wetland known as a Carolina bay (Sharitz 2003). Carolina bays are natural elliptical depressions that vary in size (long axis extremes from 50 m to 8 km; Sharitz and Gibbons 1982) and in the degree to which they retain water.

Rainbow Bay differs from the H-02 constructed wetlands in many respects – it does not have stream water input, and thus is filled by rainfall; it is a temporary pond with a surface area of approximately 1 ha and a maximum water depth of 1.04 m. Although it was once an herbaceous wetland dominated by rush *(Juncus repens),* spike-rush *(Eleocharis sp.),* bulrush *(Scirpus cyperinus),* panic grass *(Panicum verrucosum),* and knotweed *(Polygonum sp.)*, several prolonged drought periods have resulted in a more closed canopy wetland dominated by sweetgum *(Liquidambar styraciflua),* swamp tupelo *(Nyssa biflora),* and red maple *(Acer rubrum).* This study site is not unusual in any obvious manner relative to other amphibian breeding ponds of its size in this region (Sharitz 2003), except for its relatively undisturbed condition and protected status during the last 57 years. The number of species of amphibians (27) we have observed in our study at RB is representative of the diversity found in southern regions of the U. S. For example, Dodd (1992) collected 16 species of amphibians during a six-year study at a small pond in the north Florida sandhills. The similarity that RB has with the H-02 wetlands that make RB of value as a reference site is that both sites are currently fish-free and in close proximity (< 3 km). Because all permanent water habitats on the SRS contain fish, and fish have such a strong impact on amphibian species success, long-term data from a fish-free reference site are of value.

Drift Fence Sampling -- We have sampled the amphibians migrating to and from RB using a terrestrial drift fence with pitfall traps (Gibbons and Semlitsch 1981). The pond was encircled by a drift fence of aluminum flashing (440 m long, 50 cm high, buried 10-15 cm deep in the ground) in September, 1978. Pitfall traps (40-L buckets) were buried inside and outside the fence flush to the ground and next to the fence at 10-m intervals. These traps have been checked daily from 21 September 1978 through the end of FY-08. For many species, this sampling technique has provided a nearly complete annual census of the number of breeding adults and of juvenile recruitment, and thus a thorough understanding of “natural” amphibian population dynamics.

RESULTS

H-02 wetland captures – We recorded 617 captures of 17 amphibian and reptile species at the H-02 treatment wetlands from June through September 2008 (Table II-1). Amphibian species comprised most captures, as only 12 captures of reptiles have been recorded to date. All amphibian captures were of frog and toad species; i.e., no salamander captures have been recorded at the wetlands. Of the ten frog and toad species captured, the most common were bullfrogs (*Rana catesbeiana*), eastern narrowmouth toads (*Gastrophryne carolinensis*), and bronze/green frogs (*R. clamitans*). During the aquatic sampling periods no fish were captured in the wetlands, but predaceous water bugs (*Abedus* sp.), a potential predator of amphibian larvae, were relatively common (54 captures).

Eight species of frogs and toads produced juveniles in the wetlands. In addition to the three most common amphibian species, we recorded juveniles of three species of treefrogs (*Hyla cinerea*, green treefrog; *H. gratiosa*, barking treefrog, and *H. squirella*, squirrel treefrog), the southern toad (*Bufo terrestris*), and the southern leopard frog (*R. sphenocephala*) (Table II-1).

Species at the Reference Site, Rainbow Bay – Rainbow Bay was sampled from 1 October 2007 to 31 September 2008, a continuation of the previous 29-yr study. We captured 4601 individuals of 32 species of amphibians and reptiles at the RB reference site during FY-08. These captures included five salamander species, seven frog and toad species, two turtle species, six lizard species, and 12 snake species. The most common species were the marbled

TABLE II-1. The 17 species of amphibian and reptiles captured at the H-02 constructed wetlands from May-September 2008. Monthly aquatic sampling was conducted from June-August using minnow traps at 24 systematically chosen sample locations; terrestrial sampling was conducted using three 15-m drift fences with pitfall traps on the northwest side of the wetlands.

Species	Capture Method	Adults	Larvae/Immature (Juveniles)*
Reptiles:			
Anolis carolinensis	fence	2	1
Eumeces fasciatus	fence	1	0
Sternotherus odoratus	fence	1	0
Trachemys scripta	fence	0	1
Tantilla coronata	fence	3	1
Thamnophis sirtalis	fence	0	1
Virginia valeriae	fence	1	0
Amphibians:			
Acris gryllus	aquatic trap	0	2 (0)
Bufo terrestris	fence	5	35 (3)
Gastrophryne carolinensis	fence	42	13 (81)
Hyla cinerea	fence & aquatic trap	5	4/1 (42)
Hyla gratiosa	fence	0	0 (2)
Hyla squirella	fence	0	0 (2)
Rana clamitans	fence & aquatic trap	2	92/17 (62)
Rana catesbeiana	fence & aquatic trap	1	59/18 (49)
Rana sphenocephala	fence & aquatic trap	12	1/24 (27)
Scaphiopus holbrookii	fence	4	0 (0)
TOTALS (all species)		79	156/114 (268)

** The "Larvae" category indicates captures of amphibian tadpoles; "Immature" represents amphibian or reptile individuals that were too young to sex accurately; "Juveniles" are amphibians that were identified as having recently metamorphosed from the wetlands based on their size and/or the presence of a small tail nub.*

salamander (*Ambystoma opacum*), southern toad, eastern spadefoot toad (*Scaphiopus holbrookii*), and narrowmouth toad. The RB wetland began filling on February 1, 2008, and dried on May 19, 2008 (i.e., a hydroperiod of 108 days). Only two amphibian species successfully produced juveniles under these short hydroperiod conditions – marbled salamanders (12,630 juveniles) and spring peepers (*Pseudacris crucifer*; 18 juveniles).

Relationship of Species Diversity and Reproductive Success to Hydroperiod – At the RB reference site, the long-term data illustrate that the number of species that produce metamorphosed juveniles is positively correlated with total hydroperiod of the wetland (r = 0.81, $P < 0.0001$, n = 30, Fig. II-1). In addition, the total number of metamorphs (all species combined) increases directly with total hydroperiod at RB (r = 0.54, $P < 0.01$, n = 30, Fig. II-2). Longer hydroperiods allow a greater diversity of seasonally reproducing species to breed in the pond. Longer hydroperiods also permitted a greater number of larvae the opportunity to reach the critical size to initiate metamorphosis (Wilbur 1987), and hence allowed more juveniles of more species to be recruited into terrestrial adult populations.

DISCUSSION AND CONCLUSIONS

Our 30-year study of amphibians at a natural temporary pond, Rainbow Bay, documented that hydroperiod is a primary source of variation in the community structure of amphibians in natural, relatively undisturbed wetlands, as different species perform better under different hydroperiod regimes. Competition among larvae and predation by aquatic insects and other amphibians in the

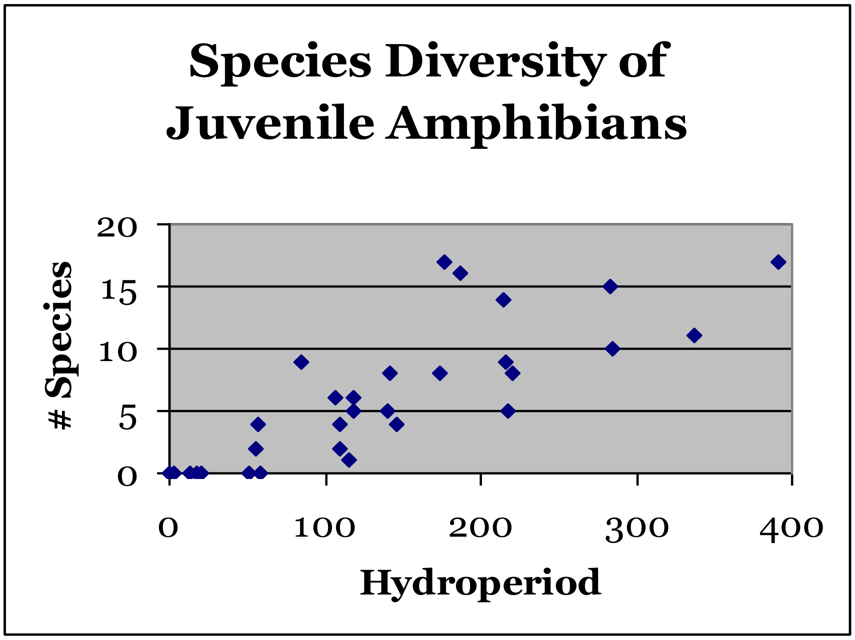

Fig. II-1. Relationship between the diversity of metamorphosing juvenile amphibians and total hydroperiod at Rainbow Bay. Each data point represents one year between FY-1979 and FY-2008.

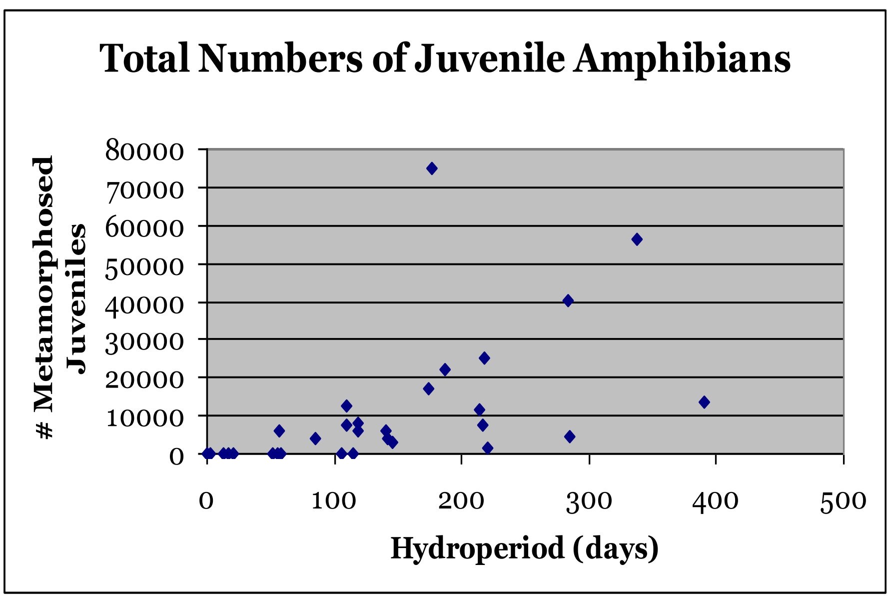

Fig. II-2. Relationship between the total numbers of metamorphosing juvenile amphibians and total hydroperiod at Rainbow Bay. Each data point represents one year between FY-1979 and FY-2008.

aquatic environment also influence a species' success, but these biotic processes are mediated by pond hydroperiod. At a seasonal wetland such as RB, regulation of community structure occurs through the interaction of rainfall, hydroperiod, competition, and predation. Our expectation is that, at the permanently pooled H-02 wetlands, competition and predation will play increasingly important roles as the community ages.

At RB, juvenile recruitment of all species is often limited by a short hydroperiod in the driest years. The production of juveniles is episodic, with large numbers of metamorphs being produced in only a small number of the 30 years. Because RB filling is dependent on annual variation in the amount and timing of rainfall, the hydroperiod is highly variable; nonetheless, it was still a significant predictor of the number and diversity of metamorphosing amphibians in the bay. Our data show that temporal variation in pond conditions may favor the reproductive success of different species in different years at RB; future sampling at the H-02 wetlands will reveal whether the permanent-pond amphibian community is more stable (but less diverse) over time.

Comparison of the RB data to the H-02 wetlands is tempered by the unique nature of the H-02 wetland (i.e., a permanent water but fish-free wetland), the entire drift fence at RB (compared to partial fences at H-02), and the fact that the H-02 sites are newly created. Nonetheless, our 5-mo sampling at the H-02 wetlands revealed that, based solely on wetland hydrology combined with the absence of fish populations, the treatment wetlands have high potential as amphibian breeding habitat. Rapid pond drying often causes complete reproductive failure in amphibian species, and early pond drying is a fairly common event at RB that represents a pervasive risk (e.g., Taylor at al. 2006). Similarly, the presence of fish populations in permanent hydroperiod wetlands also poses lethal risk to most amphibian species—the H-02 wetlands provide an uncommon habitat that has both a permanent hydroperiod and is fish-free.

At Rainbow Bay, in general the longer hydroperiod years produced a greater diversity of amphibian juveniles than the shorter years because long hydroperiods allowed species that breed late or have long larval periods to produce metamorphosed juveniles (Pechmann et al. 1989). This hydroperiod/recruitment connection is especially important for salamander species, which have not been found in the H-02 wetlands. If salamanders successfully colonize the H-02 system, then the diversity at the treatment wetlands will approximate that of the “natural” wetlands in the longer hydroperiod years.

Although a longer hydroperiod (especially in the absence of fish) generally has a positive effect on species diversity, it likely has a negative effect on some particular species due to increased competition and/or predation with or by other species. For example, at RB long hydroperiods have been associated with decreased recruitment of the ornate chorus frog, *Pseudacris ornata*, due to the accompanying predation by salamanders. As the H-02 system matures, it will be of interest to examine whether competitive and predatory relationships change.

Aside from documenting the use of the H-02 treatment wetlands by the amphibian community, our study plans to examine the potential effects of elevated copper concentrations on eggs and larvae of select species (see "Planned studies for FY-09"). A concern related to treatment wetlands that receive process wastewater discharges, however, is that these created wetlands may serve as "ecological traps" for amphibians if adults are attracted to the sites to breed, but the aquatic conditions of the sites are not suitable for eggs and/or larvae, and therefore the reproductive success is zero. The extreme *natural* fluctuations of amphibian populations can make impacts due to human activity difficult to discern (Pechmann et al. 1991); therefore, ecotoxicological experiments will be our primary method of assessing the overall suitability of these wetlands to amphibians.

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CHAPTER III – VEGETATION COMMUNITY OF THE H-02 WETLANDS – IMPORTANCE TO AMPHIBIANS

Rebecca R. Sharitz, Paul Stankus, & Linda Lee

INTRODUCTION

In general, natural wetlands tend to have greater vegetation species richness and cover than created wetlands, at least initially following construction. In many cases, created wetlands may be planted with one or more species to establish early vegetation cover, as was the case with the H-02 wetlands. These wetland cells were planted in FY-07 with the giant bulrush (*Schoenoplectus californicus*), a species that had been successfully established in the A-01 wetlands. As the H-02 wetlands mature and undergo vegetation succession, we anticipate that the complexity of the giant bulrush community will become greater as the *S. californicus* plantings spread and as additional native species become established. As the wetlands age, the levels of dissolved organic carbon (DOC) also may become more similar to natural wetlands. The higher, perhaps more stable, levels of DOC and organic matter in an older constructed wetland may translate to greater contaminant removal efficiency, and as a result a more suitable wildlife habitat.

Even in an engineered aquatic system dominated by one plant species such as the giant bulrush, the structural complexity of the habitat can be an important component of amphibian success. Many amphibian species require variety in vegetative structure for egg laying. Once hatched, many amphibian larvae need vegetation as cover to hide from predators; and, as noted above, the DOC associated with a well-established plant community will reduce metal bioavailability.

In FY-08 we established baseline measures of vegetation in the H-02 wetlands, both from the standpoint of the success of the bulrush plantings and overall plant cover and diversity. As the wetlands mature, we will use standard metrics such as plant density and species richness to determine changes in the plant community over time. The giant bulrush is expected to remain the dominant species in the constructed facility; however numerous other species are expected to become established on the shallow and more open boundary around the edge of each wetland cell. Vegetation plots will be used to document the increase in bulrush density, and the arrival and establishment of additional species. Vegetation will be sampled at several periods during the growing season and compared from year to year; these data can also be compared with similar created wetland systems (e.g., the A-01 system) and natural wetlands on the SRS.

METHODS

The H-02 wetland cell grid – A vegetation/amphibian sampling grid was established in the wetlands on August 25, 2008. Twelve 5-m^2 circular plots were systematically placed across each wetland cell at approximate 30-m (length) by 10-m (width) intervals. Metal-free 75-cm PVC pipe was used to establish permanent mid-points of each plot. These plots were the focal points of the vegetation sampling and amphibian minnow trapping and will be resampled in the future.

Vegetation sampling – The goal of our initial sampling on August 26-29, 2008, was to quantify plant species presence, surface coverage of each species, and stem density of the giant bulrush (*S. californicus*). We measured 1.25 m from the mid-point of each plot and circumscribed the 5-m^2 area to be sampled. Within each plot we identified each species, and visually estimated its coverage in seven cover class categories: 1 (<1% coverage), 2 (1-<10%), 3 (10-<25%), 4 (25-<50%), 5 (50-<75%), 6 (75-<95%), and 7 (95-<100%). We combined the duckweed species, *Lemna minor* and *Spirodela polyrrhiza*, into one group as they usually occurred together and it was very difficult to estimate their separate coverages. We could not determine the species of *Utricularia* as it was not in flower, and we combined all algae into a single group. We also counted the number of stems of *S. californicus* within each plot as a measure of stem density and estimate of structural complexity.

RESULTS

Species occurrence – We recorded 18 species (or species groups) of aquatic plants in the H-02 treatment cells. Six species (including the planted *S. californicus*) were ubiquitous, occurring in 20 or more plots (Table III-1). Eight species were relatively uncommon, occurring in four or fewer plots, and the rest were intermediate. Eight species were found in one of the wetland cells but not in both.

TABLE III-1. Plant species sampled in the H-02 constructed wetlands in August 2008. Twelve 5-m^2 plots per cell were sampled. Number of plots is the number per cell in which each species occurred; average % cover is based upon cover estimates within sample plots extrapolated to the entire cell. Number of stems of the planted California bulrush is used as a measure of vegetation structural complexity.

Species	# Plots		Average % Cover		# Stems/m^2	
	Cell A	Cell B	Cell A	Cell B	Cell A	Cell B
Algae sp.	12	12	46.54	32.25	–	–
Alternanthera philoxeroides (alligatorweed)	1	1	0.46	0.04	–	–
Azolla caroliniana (Carolina mosquitofern)	0	7	0.00	2.38	–	–
Cynodon dactylon (Bermuda grass)	10	10	21.46	17.13	–	–
Cyperus erythrorhizos (redroot flatsedge)	0	1	0.00	0.46	–	–
Hydrocotyle ranunculoides (floating marsh pennywort)	12	12	26.88	9.33	–	–
Juncus diffusissimus (slimpod rush)	3	0	3.38	0.00	–	–
Lemna minor & Spirodela polyrrhiza (duckweeds)	12	12	18.83	18.83	–	–
Leptochloa fusca ssp. uninervia (Mexican sprangletop)	4	3	1.00	0.96	–	–
Limnobium spongia (American spongeplant)	3	7	15.67	27.96	–	–
Lindernia dubia (yellowseed false pimpernel)	1	0	0.04	0.00	–	–
Panicum anceps (beaked panicgrass)	1	0	3.13	0.00	–	–
Pistia stratiotes (water lettuce)	0	5	0.00	3.88	–	–
Sagittaria filiformis (threadleaf arrrowhead)	9	6	24.04	10.08	–	–
Sagittaria latifolia (broadleaf arrowhead)	2	0	0.08	0.00	–	–
Schoenoplectus californicus (California bulrush)	12	12	18.85	16.92	32.4±24.4	30.1±35.5
Scirpus cyperinus (woolgrass)	2	0	0.08	0.00	–	–
Utricularia sp. (bladderwort)	1	3	*	*	–	–
Open Water	12	12	12.88	16.96		

*present but submerged

Vegetation cover – Average cover estimates per species typically ranged from less than 1% to greater than 20%. Species with the greatest cover in both cells included *Cynodon dactylon*, *Hydrocotyle ranunculoides, L. minor* and *S. polyrrhiza* combined, *Limnobium spongia, Sagittaria filiformis,* and the planted *S. californicus* (Table III-1). Species of algae covered one-third to nearly one-half of the water surface in both cells. Additional species that were noted but were not sampled in the plots were *Eclipta prostrata, Juncus effusus* ssp. *solutus*, *Paspalum dilatatum, Typha latifolia* and *Verbena brasiliensis*. Both wetland cells also had open water (13-17% of area).

Stem density of ***Schoenoplectus californicus*** **–** The giant bulrush occurred in all plots, with average densities of 30-32 stems/m^2. There was great variation in stem densities among the sample plots, ranging from 6 -72 stems/m^2 in Cell A and 4-137 stems/m^2 in Cell B. Thus, standard deviations were also high. Percent cover estimates of this species were 17-19% (Table III-1).

DISCUSSION AND CONCLUSIONS

The dominant plant species were generally similar between the two cells of the H-02 wetland complex. Most differences in species composition reflected differences in the less abundant species. Most of the colonizing plants are typical wetland species of the region that may have been dispersed into the wetland cells by windblown seed or by other vectors such as waterfowl. We expect that additional wetland plants will continue to become established in these wetlands as they mature, although it is likely that the dense growth of the giant bulrush will restrict their spread in deeper water areas. The species composition and the habitat structure provided by these species should support populations of pond-breeding amphibians if other habitat conditions, such as water chemistry, are suitable.

Densities of the planted bulrush (*S. californicus*) varied greatly among our sample plots in both wetland cells. In a more intensive study of the planting success of this species, substantially higher stem densities were found (E. Nelson, SRNL, personal communication). For example, on a similar sampling date in August, Nelson reported densities of 52.6±29.0 in Cell A and 66.7±25.8 in Cell B. These differences likely reflect the different locations of the sample plots. Whereas our plots were placed in both edge and center locations in the cells, Nelson's study was designed to determine establishment success and focused on more interior areas of the wetland cells.

It is likely that propagules of some of the plant species that have colonized the wetlands were present in the sediment surrounding the roots of the planted *S. californicus*. Such inadvertent introduction of non-native or potentially undesirable species is a common problem associated with obtaining plant material for wetland construction or restoration (Maki and Galatowitsch 2004). Two of the plants in the H-02 wetlands, *Alternanthera philoxeroides* and *Pistia stratiotes*, are on the South Carolina Department of Natural Resources Aquatic Nuisance Species list (http://www.dnr.sc.gov/invasiveweeds/illegal1.htm). Only small amounts of *A. philoxeroides* were found in each of the wetland cells, and this species has been reported in other wetland and aquatic sites on the SRS over the years (e.g., Batson et al.1985). However, *P. stratiotes* is a new and highly invasive species that is abundant in one of the wetland cells but not the other. In addition, one of the more common plants in both wetland cells, *Limnobium spongia*, is considered a weedy invasive in South Carolina and has not been reported previously in Aiken County. The plants of *S. californicus*, which does not naturally occur in South Carolina, may have been acquired from southern sites (e.g., Florida) where these other species also occur.

Efforts are being made to remove *P. stratiotes* from the H-02 wetland cell in which it occurs (E. Nelson, personal communication). This species reproduces rapidly by vegetative offshoots formed on short, brittle stolons, but it seldom flowers and produces seed, so it may be possible to control this species by continued manual removal. In addition, it is not cold-tolerant, so winter temperatures may also cause a decline in its abundance.

The presence of these non-native species demonstrates one of the persistent problems associated with using non-local plant materials in wetland construction or restoration. In an examination of aquatic plant materials ordered from vendors in 17 states, Maki and Galatowitsch (2004) reported that 93% of the orders contained a plant or animal species not specifically requested. In wetland construction or restoration efforts, there is often an initial period of invasion by undesirable species. Typically, if proper hydrologic conditions are imposed, such invasions are temporary (Mitsch and Gosselink 2000), although selective removal may be necessary in the beginning.

Although there are reports of invasive plant species negatively affecting amphibians either directly or indirectly (e.g., a preference by bullfrogs for habitats with the invasive common reed, *Phragmites australis*, which consequently has negative effects on other frog species – Clarkson and Devos 1986), we are unaware of any negative effects of the H-02 invasive plant species on amphibians in a flowing water system such as the H-02 wetlands. Some aquatic invasive plants may increase transpiration and decrease hydroperiod (Zedler and

Kercher 2004) in some wetlands, however, and shortened hydroperiods have the potential to impact the amphibian community.

LITERATURE CITED

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