Journal of Freshwater Ecology

ISSN: 0270-5060 (Print) 2156-6941 (Online) Journal homepage: https://www.tandfonline.com/loi/tjfe20

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To cite this article: Emma F. Zeitler, Kristen K. Cecala & Deborah A. McGrath (2018) Evaluation of anuran diversity and success in tertiary wastewater treatment wetlands, Journal of Freshwater Ecology, 33:1, 475-488, DOI: 10.1080/02705060.2018.1524334

To link to this article: https://doi.org/10.1080/02705060.2018.1524334

JOURNAL OF FRESHWATER ECOLOGY
2018, VOL. 33, NO. 1, 475–488
https://doi.org/10.1080/02705060.2018.1524334

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Evaluation of anuran diversity and success in tertiary wastewater treatment wetlands

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ABSTRACT

Constructed wetlands (CWs) are a multifunctional environmental technology capable of supporting plant and wildlife communities and removing excess nutrients and other pollutants. Tertiary treatment wetlands have also been proposed as one solution to remove persistent pharmaceuticals and personal care products (PPCPs) that remain after conventional wastewater treatment. Though aquatic wildlife is generally sensitive to environmental contaminants, it is unknown whether CWs can serve dual purposes supporting wildlife habitat and polishing wastewater. Our objective was to assess the capacity of a newly established CW for tertiary wastewater treatment to support amphibians. Specifically, we assessed adult anuran occupancy and tadpole and adult body size and condition relative to nearby unimpacted ponds. We found that a diverse community of adult anurans rapidly colonized the wetlands where successful reproduction was documented. Adult frogs and tadpoles were observed to have variable sizes among ponds with some life stages in better body condition at the tertiary treatment wetlands. Preliminary investigations suggest that tertiary treatment wetlands provide habitat for successful colonization and reproduction of anurans, but carryover effects need to be evaluated to determine if tertiary treatment wetlands serve as sinks or suitable habitat that supports stable populations.

ARTICLE HISTORY
Received 5 June 2018
Accepted 12 September 2018

KEYWORDS
Community ecology; constructed wetland; effluent; growth; nitrogen; tadpole; wastewater

Introduction

Habitat loss has become one of Earth's most prevalent environmental issues (Brooks et al. 2002; Cardinale et al. 2012; Primack and Sher 2016), with wetland loss impacting a multitude of economic and biological benefits (Greb et al. 2006; Vörösmarty et al. 2010). Wetlands support highly diverse species assemblages that are undergoing significant declines (Groombridge and Jenkins 1998; Dudgeon et al. 2005; Strayer and Dudgeon 2010; Vörösmarty et al. 2010). Amphibians have been negatively affected by this loss in habitat, with 32% of amphibian species being threatened by extinction compared to birds (12%) or mammals (23%; Dudgeon et al. 2005). Additionally, extinction rates of freshwater animals in North America have increased at a rate of 4% per decade (Ricciardi and Rasmussen 1999) underscoring the need for conservation practitioners to focus on freshwater ecosystems.

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Anurans are often used as indicators of environmental conditions because of their physiology and unique biphasic life cycles (Henry 2000; Degarady and Halbrook 2006). Anurans' complex life cycles consist of a larval, growth-focused stage and an adult, dispersion-focused stage (Werner 1986). Their permeable eggs, gills and skin make them sensitive to aquatic contaminants in their surroundings (Laposata and Dunson 2000). Larval stages are particularly important because size at metamorphosis or abnormalities have life-long consequences for fitness (Alford and Harris 1988; Chelgren et al. 2006). Additionally, transitions between aquatic and terrestrial environments increase exposure to environmental challenges (Todd et al. 2011). As predators and prey, changes in anuran densities could have consequences throughout a food web (Wilbur 1997; Laposata and Dunson 2000; Beard et al. 2003; Whiles et al. 2006). Ultimately, anuran populations are good indicators of wetland conditions and may serve as umbrella species for wetland biodiversity (Vitt et al. 1990; Pollet and Bendell-Young 2009; Guzy et al. 2012).

One of the most effective mitigating strategies for aquatic habitat loss is constructed wetlands (CWs; Kivaisi 2000). CWs are engineered systems designed and constructed to promote growth and development of native wetland communities (Vymazal 2010). The ecosystem services, such as nutrient retention or wildlife habitat, provided by CWs can be determined by principles governing their construction (Vymazal 2007; Verhoeven et al. 2011; Li et al. 2013). As wildlife habitat, studies have shown that while species richness varies across regions, CWs often support populations of higher densities (Balcombe et al. 2005; Hsu et al. 2011). Their presence has even resulted in the delisting of threatened anuran and avian communities (Strand and Weisner 2013). However, CWs also have documented negative effects on wildlife populations by reducing the diversity and promoting invasive species or reducing larval survival (DiMauro and Hunter 2002; Porej and Hetherington 2005; Denton and Richter 2013). Studies conducted on amphibian populations showed that CWs favored predatory species but became a population sink for others (Denton and Richter 2013; Richter and Drayer 2016). This ambiguity of the ability for CWs to sustain biodiversity is largely unexplored and requires more research (Hsu et al. 2011).

Although wildlife support is a benefit of CWs, it is often a byproduct of wetlands constructed for a different purpose like wastewater effluent treatment. Modern municipal wastewater treatment systems are effective at removing excess nutrients, yet recent attention has highlighted deficiencies in removing pharmaceutical and personal care products (PPCPs; Koplin 2002). The consequences of PPCPs in receiving streams include negatively impacted ecosystem processes and wildlife behavior (Koplin 2002; Richmond et al. 2017). A low-cost solution for advanced treatment of effluent and removal of PPCPs are CWs (Ghrabi et al. 2011). Studies demonstrate that CWs can remove 40–55% of total nitrogen and 40–60% of total phosphorus, while significantly depleting biochemical oxygen demand, chemical oxygen demand, and fecal coliform bacteria (Vymazal 2010). Planted vegetation is effective at removing excess nutrients and provides an environment where microbes that facilitate or degrade PPCPs can grow (Brix 1997; Price and Probert 1997). Storage of excess nutrients and byproducts of PPCPs in CWs could therefore negatively impact the suitability of CWs for wildlife (Ruiz et al. 2010).

While controversy remains about the sensitivity of amphibians to environmental contamination (Kerby et al. 2010), a host of studies demonstrate negative effects of aquatic contaminants on amphibians including PPCP's, phenols, pesticides and heavy metals (Blaustein et al. 2003; Storrs and Semlitsch 2008; Todd et al. 2011; Säfholm et al. 2014). Additional studies of amphibians in contact with effluent document a range of negative effects on clutch size, larval survival and developmental abnormalities that could ultimately affect population trajectories (Laposata and Dunson 2000; Ruiz et al. 2010; Smith and Burgett

2012). In some habitats, as many as 98% of tadpoles demonstrated developmental abnormalities including malformed/extra limbs, open limb slits, missing eyes, edema, scoliosis and calcinosis (Keel et al. 2010; Ruiz et al. 2010). In this particular instance, the frequency of malformations declined with distance from effluent introduction points (Ruiz et al. 2010). Amphibian developmental sensitivity to effluent and their importance in the ecosystem suggest that this taxon is a powerful indicator of habitat suitability of CWs built for tertiary treatment of effluent. Our objectives were to assess the ability for effluent treatment CWs to support a diverse anuran community. Specifically, we evaluated differences in amphibian diversity and size between an effluent treatment CW, and rain-filled reservoirs. In concordance with previous studies, we expected to find differences in growth and frequency of abnormalities between inhabitants of CWs and rain-fed ponds.

Methods

Study area

The Cumberland Plateau is located at the southern part of the Alleghany Plateau and extends from northern Kentucky to northern Alabama (Evans et al. 2017). The elevation is approximately 585 masl with 142 cm of annual average precipitation. Pennsylvanian sandstone is the bedrock providing a fine sandy loam that is shallow and well-drained. The forest is mainly comprised of mixed pine and deciduous species, with *Quercus* and *Carya* species dominating the canopy. The Cumberland Plateau is host to the one of the highest predicated reptile and amphibian diversity regions in Tennessee and one of the most diverse vascular plant communities in the eastern United States (Barrett et al. 2014; Evans et al. 2016). As the only naturally occurring standing water on top of the Cumberland Plateau, ephemeral wetlands are essential habitats for amphibians, yet urban sprawl and pine plantations have dramatically reduced the density and quality of these habitats (Evans et al. 2017). Supplementing natural ephemeral wetlands, private land-owners have established a high density of rain-filled reservoirs that also provide habitat for breeding amphibians (Kirchberg et al. 2016).

Control sites

The majority of our control sites were rain-filled reservoirs: Lake Bratton, Lake Cheston, Leaky Pond, and St. Mary's Pond. These sites were chosen for ease of access, proximity to the experimental site, and to represent the diversity of pond types and upland conditions available in the region. While we used St. Mary's Pond for call surveys, restricted access prevented us from performing active surveys. The three remaining reservoirs are man-made, which were completely filled by 1960. Each has forested uplands with variable degrees of bank clearing, with Lake Bratton and Leaky Pond having the most shoreline forest cover (>80% forested). Lake Cheston has more shoreline development but both it and Leaky Pond have considerable emergent vegetation. These reservoirs retain water year-round. In contrast, we also surveyed one fully forested ephemeral wetland – Breakfield Wetland – that dries seasonally and has no emergent vegetation. All sites were within 5 km of one another.

Sewanee constructed wetland (SCW) complex

The University of the South installed a 0.18-ha CW complex comprised of three basins to provide tertiary treatment (or "polish") effluent conventionally treated in lagoons at the municipal wastewater treatment facility in 2016 (Figure 1). The three SCW basins,

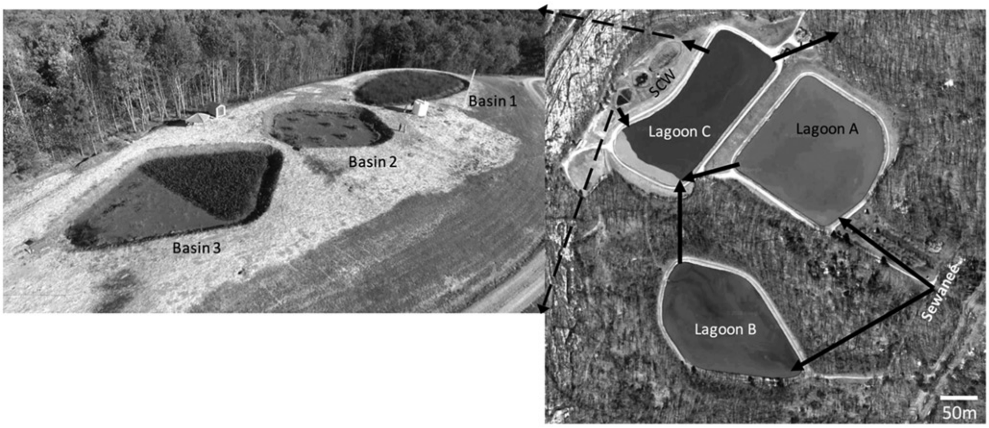

Figure 1. Aerial photo of the Sewanee Utility District where wastewater is treated and the SCW built to evaluate the efficiency of this biotechnology to treat conventionally-treated effluent. Sewage enters the facility from the Sewanee, Tennessee municipality and is diverted into Lagoons A and B for microbial breakdown. Primary treatment occurs before transfer to Lagoon C for secondary treatment, for a total lagoon treatment time of approximately 40 days. Treated effluent is pumped from Lagoon C outflow into the inflow of SCW Basin 1 where tertiary treatment occurs as water moves from Basin 1 to Basin 3. Treated effluent leaving Basin 3 is returned to Lagoon C before discharge from the facility. Thick black arrows indicate effluent movement through the facility. Photos by Brandon Moore.

designed with a free-water surface flow system, are attached to the secondary treatment lagoon (Lagoon C). Tertiary treatment begins when treated effluent from the outflow of the secondary lagoon is pumped into the first SCW basin (Basin 1). Basin 1 has a surface area of 837 m^2 and a depth of 38 cm. It is a long, narrow basin beginning with a plant-free zone, but also containing a section of soft stem bulrush (*Schoenoplectus tabernaemontani*). The effluent then enters Basin 2, a diamond-shaped basin that contains nine soil mounds planted with native wetland vegetation. Basin 2 has a surface area of 352 m^2 and a depth of 13 cm. Finally, the effluent enters Basin 3, whose first half was planted with pickerel weed (*Pontederia cordata*), and second half serves as a plant-free zone. The basin's surface area is 378 m^2, with a depth of 30 cm. As an experimental system, treated effluent is characterized and returned to Lagoon C (Figure 1). Preliminary data indicate that the SCWs are effective at removing nutrients from conventionally-treated effluent (Hopson et al. 2018). PPCP removal is more complicated with lower concentrations only documented in Basins 1 and 3 (Hopson et al. 2018).

Study design

Anuran diversity

We documented adult anuran occupancy using call surveys at each location. We conducted bimonthly surveys of the calling community from February–May 2017 with monthly surveys in June and July 2017 for a total of 10 calling surveys. Following the North American Amphibian Monitoring Program protocol (NAAMP; Weir and Mossman 2005), we were still for 2 minutes after arrival before documenting all species calling for 5 minutes. Surveys began 60 minutes after sunset and took less than 2 hour to complete surveys at each site. We evaluated community similarity among our surveyed locations to determine if the amphibian community was different at the SCW or Lagoon C relative to rain-filled waterbodies. We conducted non-metric multi-dimensional scaling to characterize Sørensen dissimilarity among sites using presence-absence information for all 10 surveys (Sørensen 1948; Gardner 2014). Differences among waterbodies with and without treated effluent were evaluated using an analysis of similarity (Clarke 1993; Warton et al. 2012). Analyses were conducted in R using package vegan (R Core Team 2016; Oksanen et al. 2017).

Body size and condition

Though documentation of adult frogs indicates that they have colonized a site, it does not indicate successful reproduction. To complement calling surveys, we surveyed tadpoles at each of the same study locations except St. Mary's Pond for which we did not have physical access. Bimonthly surveys were conducted from April to July 2017. Initial surveys in April included minnow traps, but few captures resulted in this method being abandoned in favor of active dip-net surveys. Dip-net surveys included 4 hour of surveying at each site. After two surveys yielded no tapdoles, we removed two rain-filled sites (Breakfield Wetland and Leaky Pond) and the wastewater treatment lagoon (Lagoon C) from our surveys. High densities of bulrush prevented effective surveying of Basin 1 at the SCW, but sampling did take place in both Basins 2 and 3 (Figure 1).

Upon capture, tadpoles were identified to species using methods described in Altig and McDiarmid (2015), measured, weighed, aged, and released at its capture location. We quantified snout-vent length (SVL), total length and aged tadpoles using Gosner stages (Gosner 1960). From these data, we calculated body condition using the scaled mass index (Peig and Green 2009). The scaled mass index, a comparison of individual SVL to body mass relative to the entire sample population, is recommended for small vertebrates to assess body condition when direct quantifications of fat are unavailable (Peig and Green 2009). For the most commonly detected species (*Lithobates catesbeianus* and *L. clamitans*), we compared SVL and body condition between the remaining two rain-filled reservoirs (Lake Cheston and Lake Bratton) and two effluent treatment basins (SCW Basins 2 and 3) using a linear mixed model with capture day as a random effect and location as a fixed effect. Analyses were carried out in R using package lme4 (Bates et al. 2015; R Core Team 2016).

Finally, we evaluated the adult morphology of an easy-to-capture adult species, *Hyla chrysoscelis*, at the SCW and one of the rain-filled reservoirs (Leaky Pond) where they are abundant. Collections were conducted at night in a two-week period in June and July 2017. Individuals were captured by hand and returned to the lab for measurements. We measured individual SVL, mass, and leg length before returning individuals to their capture locations. Leg length was used to assess if there were differences in morphology between recently colonized sites versus well established sites (Hudson et al. 2016). Body size (SVL) and leg length corrected for body size were compared between sites using a Wilcoxon rank sum test because our data violated normality assumptions of *t*-tests (Hollander et al. 2013). Analyses were carried out in R (R Core Team 2016).

Results

We detected 14 species of anurans calling from waterbodies in our area. These species included *Acris crepitans, Anaxyrus americanus, A. fowleri, Gastrophryne carolinensis, Hyla chrysoscelis, H. cinerea, H. gratiosa, Lithobates catesbeianus, L. clamitans, L. palustris, L. sphenocephalus, Pseudacris crucifer, P. feriarum,* and *Scaphiopus holbrookii* (Table 1). Analysis of similarity results indicated that the SCW and Lagoon C were similar to the other rain-filled reservoirs ($R = -0.155$, $p = 0.638$). Visual evaluation of the NMDS plot

Table 1. Descriptions of terminology and occupancy of adult (X) and larval (*) anurans at waterbodies in our study area on the southern Cumberland Plateau. Surveys determined that effluent-filled sites (Lagoon C and the SCW) did not have different adult anuran communities than rain-filled reservoirs.

Source	Effluent-filled		Rain-filled				
Hydrology	Permanent						Ephemeral
Type	2° Treatment Lagoon	3° Treatment Wetland	Reservoir				Wetland
Name	Lagoon C	SCW	Lake Bratton	Lake Cheston	Leaky Pond	St. Mary's Pond	Breakfield Wetland
Acris crepitans		X		X*	X	X	X*
Anaxyrus americanus	X	X	X	X	X	X	
A. fowleri	X			X			
Gastrophryne carolinensis	X	X		X			
Hyla cinerea		X*			X		
H. chrysoscelis	X	X*	X	X	X	X	X
H. gratiosa					X		
Lithobates catesbeianus	X	X*	X*	X*	X	X	
L. clamitans	X	*	X*	X*	X	X	
L. palustris	X	X	X	X		X	
L. sphenocephalus		X		X	X	X	
Pseudacris crucifer	X	X	X*	X*	X*	X	X*
P. feriarum	X	X		X	X		X
Scaphiopus holbrookii	X	X	X				

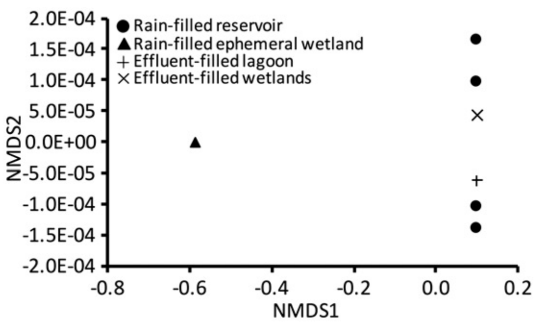

Figure 2. Non-metric multidimensional scaling plot indicating that the calling anuran community at the effluent-fed secondary treatment lagoon (Lagoon C) and the effluent-fed SCW were not different from rain-filled reservoirs (Lake Bratton, Lake Cheston, Leaky Pond, St. Mary's Pond). The only site with a different calling anuran community pattern was the rain-filled ephemeral wetland (Breakfield Wetland) that dries seasonally unlike the other sites that are perennial.

supports this conclusion and indicated that the natural ephemeral wetland (Breakfield Wetland) was the only site with a different community composition (Figure 2).

We detected only six species of tadpoles including *A. crepitans, H. chrysoscelis, H. cinerea, L. catesbeianus, L. clamitans,* and *P. crucifer.* Only tadpoles of *L. catesbeianus* (N = 236) and *L. clamitans* (N = 251) were detected in both the SCW and rain-filled reservoirs. Consequently, these were the only species used to compare size and condition among sites. Location was significantly associated with SVL and body condition in *L. catesbeianus* ($\chi^2 = 12.79$, $p = 0.005$; $\chi^2 = 15.57$, $p = 0.001$) and *L. clamitans* ($\chi^2 = 12.65$, $p = 0.006$; $\chi^2 = 12.59$, $p = 0.006$). *Lithobates catesbeinanus* body size was highest in Lake Cheston and lowest in Basin 3 with Lake Bratton and Basin 2 demonstrating intermediate values. Body size of *L. clamitans* was most divergent between the two control sites with the two SCW basins demonstrating intermediate values (Figure 3b). *Lithobates*

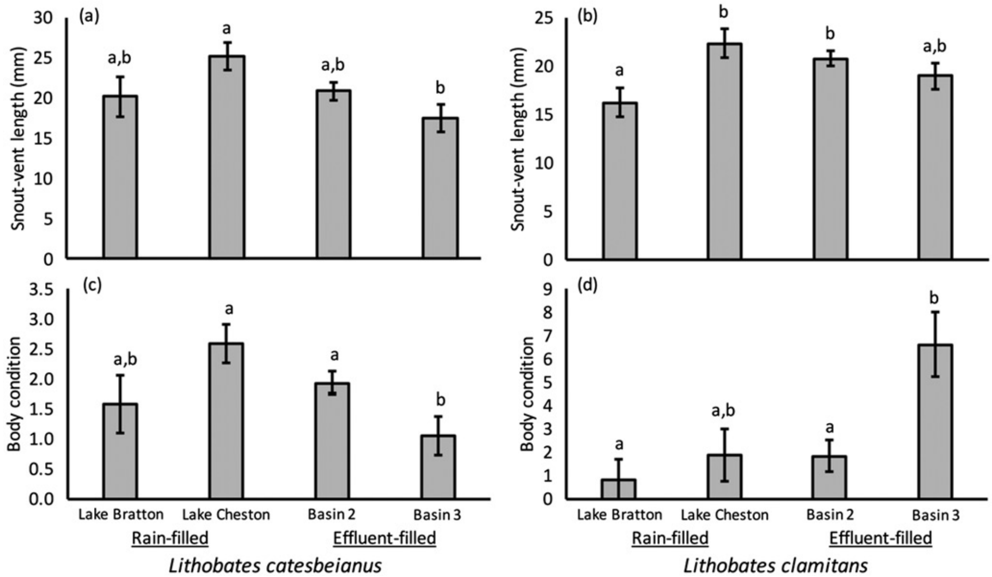

Figure 3. Mean body size (a, b) and condition (c, d) of *Lithobates catesbeianus* (a, c) and *L. clamitans* (b, d) tadpoles captured in four waterbodies on the southern Cumberland Plateau. Lake Bratton and Lake Cheston receive rain water whereas Basins 2 and 3 of the SCW receive wastewater effluent. Body condition was calculated using the scaled mass index (Peig and Green 2009). Letters indicate significance from a post-hoc analysis, and error bars represent ±1 SE.

catesbeianus body condition was highest in Lake Cheston and lowest in Basin 3 (Figure 3c). Body condition of *L. clamitans* was considerably higher in Basin 3 than Basin 2 or either of the two rain-filled reservoirs (Figure 3d).

We quantified body size (SVL) and body-size corrected leg length for 54 adult *H. chrysoscelis* from the SCW and for 61 adults of the same species from Leaky Pond. Adult *H. chrysoscelis* were larger at the SCW than at Leaky Pond ($W = 1183$, $p = 0.009$; Figure 4a), but size-corrected leg length did not differ between sites ($W = 1649$, $p = 0.993$; Figure 4b).

Discussion

We describe rapid and successful colonization of the SCW by adult anurans with similar diversity as rain-filled reservoirs. The only difference observed in community composition was between the permanent reservoirs (rain or effluent-filled) and the ephemeral wetland, with the latter demonstrating lower diversity. Although fewer species of tadpoles were detected, their discovery at the SCW indicates successful reproduction occurring at the newly established site. In contrast, the absence of tadpoles at the wastewater treatment lagoon may indicate that tertiary treatment and/or emergent vegetation is necessary for successful amphibian development. Moreover, in the SCW, we did not find evidence of malformations or developmental abnormalities, as have been observed in other taxa exposed to effluent (e.g. Ruiz et al. 2010; Galus et al. 2013; Park et al. 2014). However, variation in body condition observed at the SCW suggested differences in habitat quality between Basins 2 and 3. Individuals found at the SCW tended to be larger though variability existed among species and sites making it challenging to make broad conclusions about the suitability of the SCW for amphibians.

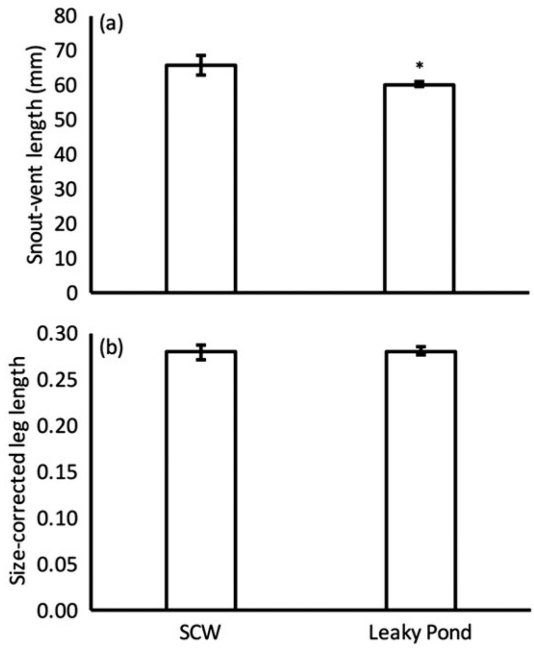

Figure 4. Mean *Hyla chrysoscelis* body size (a; SVL) and body size-corrected leg length (b; leg length corrected for SVL) for individuals collected at rain-filled Leaky Pond and the SCWs treating wastewater effluent. Error bars represent ±1 SE and * indicates a significant difference.

Anuran diversity

While we detected high diversity of calling anurans at the SCW, we did not detect similar diversity in tadpoles, which may be due to methodological or ecological reasons. Adult anurans colonizing the newly established SCW necessarily would have developed in a different water source because the SCW was completed after their larval development. Therefore, as adults, they are not solely reliant on water in the SCW which could minimize the effects of treated effluent on adult life stages (Todd et al. 2011). Egg and tadpole life stages were exclusively reliant on treated effluent, which has been documented to reduce egg and larval survival rates, as well as increase rates of scoliosis and calcinosis in populations (Laposata and Dunson 2000; Keel et al. 2010; Ruiz et al. 2010). Despite the potential for negative effects of treated effluent on egg or tadpole development, *Lithobates*-dominated tadpole communities were described at all sites perhaps suggests that consistent methodological biases among sites may explain detection of low tadpole diversity (Wassens et al. 2016; but see Guzy et al. 2014). For example, tadpole surveys beginning in April may have begun after winter-breeding *Pseudacris* spp. metamorphosed, and fast-developing tadpoles of species like *Anaxyrus* spp., *S. holbrookii*, and *G. carolinensis* could have hatched and transformed in between our surveys or hatched after our surveys were completed (Lanoo 2005). We recommend future controlled experiments understanding the effects of treated effluent on egg and tadpole development and higher temporal resolution of future tadpole surveys.

Body size and condition

Tadpole body size and condition appeared to be highly variable among sampling locations making it challenging to draw conclusions about the effects of development in treated effluent. Variation in tadpole body size and condition among sites may suggests that (i) habitat variation among rain-filled reservoirs may be larger than any potential effect of treated effluent, or (ii) body size is unaffected by treated effluent. Conversely, adult *H. chrysoscelis* were 8.3% larger at the SCW. Though it is possible that only older and larger individuals dispersed to the SCW (Phillips et al. 2006), smaller, juvenile frogs are typically the dispersing life stage for anurans (Semlitsch 2008). While we are unaware of other studies comparing body size at wastewater treatment facilities, researchers have documented higher density and diversity of insectivores (i.e. bats and birds) around wastewater treatment facilities (Kalcounis-Rüppell et al. 2007; Naidoo et al. 2013). Higher prey volume may explain larger body sizes at the SCW, but we acknowledge that without surveys at other locations, this difference may simply be representative of variation in adults among locations. We recommend that future studies investigate body size and condition at a broader range of sites to encompass variation present on the southern Cumberland Plateau.

Each of the basins at the SCW has different vegetative and morphological characteristics, which may contribute to patterns of success in tadpoles. This is particularly relevant between Basin 2 and 3, as Basin 2 is shallow with limited emergent aquatic vegetation relative to Basin 3 that is deeper and dominated by pickerelweed. Between Basins 2 and 3, we observed opposite patterns of change in the body condition of our focal tadpole species potentially reflecting competitive interactions between the two species where context is known to mediate the direction of competitive outcomes (e.g. Werner 1991, 1994; Werner and Anholt 1996). We observed declines in *L. catesbeianus* body condition from Basin 2 to Basin 3 suggesting that Basin 2 is of higher quality. Conversely, body condition of *L. clamitans* was highest in Basin 3 relative to rain-filled reservoirs and Basin 2 suggesting that Basin 3 is of high-quality habitat. We hypothesize that differences between the two SCW basins are due to structural differences associated with shifts in depth and vegetative structure.

Species-specific responses among *Lithobates* species to various stressors could contribute to the different patterns of body condition as they respond to abiotic and biotic conditions of their environment. Removal of nutrients in Basins 1 and 2 results in significantly lower nitrogen (ammonia, ammonium, and organic N) availability in Basin 3 (Hopson et al. 2018) that could promote *L. clamitans* performance. Concurrently, *L. catesbeianus* have been demonstrated to be resistant to poor water quality such as the higher concentrations of PPCPs found in Basin 2 (Descamps and de Vocht 2017, Lo′pez et al. 2017; Hopson et al. 2018). However, another study in the absence of interacting contaminants found that *L. clamitans* performed better at elevated nitrate levels (Smith et al. 2006), but nitrate concentrations in the SCW are low throughout making this mechanism unlikely (Hopson et al. 2018).

Variable responses of anuran communities to shifting nutrient contexts are consistent with others describing reversal of competitive relationships among larval anurans in nutrient enriched environments (Smith and Burgett 2012). First, *L. catesbeianus* is a generalist that often displaces other species of tadpoles in shallow wetlands (Snow and Witmer 2010; Da Silva et al. 2011; Cloyed and Eason 2016). *Lithobates catesbeianus* also tends to be resistant to predation, which may occur at higher rates in Basin 2 relative to Basin 3 because of the terrestrial mounds that could provide hunting platforms for predators such as birds or snakes. In Basin 3, increased aquatic structure and shade from emergent vegetation could have reduced the competitive effects of *L. catesbeianus* on *L. clamitans* (Herrick 2013). Similar to Basin 3, the rain-filled reservoirs in our survey have extensive areas of emergent wetland vegetation, which may be a condition that improves

L. clamitans performance particularly in the presence of predators (Tarr and Babbitt 2002). While fish are present in all of the surveyed sites, the only fish documented at the SCW are *Gambusia affinis,* which may mediate interactions between the two *Lithobates spp*. observed in this study (Werner 1991; Smith et al. 2008; Smith and Dibble 2012; Smith et al. 2013). Because *L. catesbeianus* do not appear to react to the presence of *Gambusia* spp. (Smith et al. 2008), predation by *Gambusia* spp. on *L. clamitans* may be maximized in a simple aquatic environment without emergent vegetation as found in Basin 2 relative to Basin 3 (Hartel et al. 2007; Smith et al. 2013).

These data indicate that CWs built for wastewater effluent treatment were rapidly colonized by adult anurans, but subsequent effects on anuran egg development or future reproduction and survival are unknown. No harmful short-term effects were detected in CW inhabitants; however latent physiological effects need to be assessed. While increased foraging opportunities from excess nutrients could have positive impacts on anuran fitness (Werner 1986; Smith et al. 2006; Smith and Dibble 2012), effects of PPCPs could have latent physiological effects with negative impacts to fitness (Hayes 1997; Egea-Serrano et al. 2012; Melvin et al. 2014). To resolve the long-term effects of effluent treating CWs on wildlife, we recommend studies to describe the interactive effects of excess nutrients and PPCPs on individual development and fitness to determine the probability of long-term population persistence in effluent dominated aquatic systems.

Acknowledgements

We thank Erin Gill and Ansley Murphy for assistance with data collection, Brandon Moore for the use of his photograph, and Scott Torreano for his management of the Sewanee Constructed Wetlands. Ben Beavers and the Sewanee Utility District supported this project and provided access to the wastewater treatment facility and Sewanee Constructed Wetlands.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The Coca-Cola Foundation, Coca-Cola Bottling Company United, the Riverview Foundation, Tennessee Department of Environment and Conservation, and the Yeatman Fund and McCrickard Award at the University of the South provided support for this project.

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