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Research Article

Abundant feces from an exotic armored catfish, *Pterygoplichthys disjunctivus* (Weber, 1991), create nutrient hotspots and promote algal growth in a Florida spring

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Abstract

Florida springs are relatively unique, stable systems that have experienced increasing and synergistic threats from reductions in water flow, nutrient additions, and invasions of exotic species. The purpose of this study was to evaluate the effects of the egesta of one common exotic loricariid catfish species on nutrient availability and periphyton growth in a central Florida spring. *Pterygoplichthys disjunctivus* produces copious feces that, unlike excreted nutrients, continue to contribute to nutrient loading when the fish are not present. Fecal abundance, measured using photographs of randomly distributed quadrats, was found to be high in the spring run (total estimates for the spring run averaged 6.1×10^5 cm^3 feces). We measured extractable fecal nutrients ($N\text{-}NO_3^-$, TKN, $P\text{-}PO_4^{3-}$) from feces collected both from the guts of live fish and from the spring benthos, and leaching rates of nutrients from feces in laboratory flumes. We also measured direct algal growth of feces incubated in nutrient broth and the potential of fecal nutrients to stimulate algal growth downstream in arrays deployed *in situ*. We found that although each fecal sample leached minute quantities of nutrients, the total quantity of nutrients released in the spring run from local accumulations of feces was substantial. Furthermore, even the relatively small quantities of nutrients released from fecal material from one fish were sufficient to increase algal growth in the *in situ* experimental arrays. Leachate from *P. disjunctivus* fecal deposition could create transient biogeochemical hotspots lasting considerably longer than those described for excreta. Although the contributions of the extractable fecal nutrients were small relative to the overall nutrient load to the spring (TKN = 0.18–3.64%, $P\text{-}PO_4^{3-}$ = 2.78–22.2%), these fecal hotspots typically occurred in low-flow areas with structure where algae tend to accumulate and nutrient influx of spring water is slow. Therefore, it is likely that *P. disjunctivus* fecal leachate significantly alters nutrient availability for algae in this spring system.

Key words: armored catfish, feces, nutrient leaching, invasive species

Introduction

Exotic species invasions as a whole may be considered one of the most significant modern drivers of ecosystem change (Mooney and Hobbs 2000). Although most invasions likely have minimal impact, others affect the structure or function of individual ecosystems or whole landscapes (e.g. Nile perch and zebra mussels) (Simberloff 2011; Strayer 2012). These impacts may be transitory, as in the case of boom-and-bust invasions or seasonal movements of invasive exotics (Williamson 1996; Moore et al. 2012; Capps and Flecker 2013a, b), or localized, as in the case of production of biogeochemical hotspots by aggregations of exotics (Capps and Flecker 2013a, b). Alternatively, these changes may be both persistent and widespread if extraordinarily tolerant invaders become widely distributed (Strayer et al. 2006). Strayer (2012) argued that although the scientific community has recognized that exotic invasions impact ecosystem function, the role of invasions has not been sufficiently incorporated into ecosystem management. Without comprehensive recognition of the role of these invasions, management approaches may not achieve their targets.

The influence of exotic species on aquatic ecosystems will depend to a large degree on their roles in their new ecosystems. Grazers, in particular, can influence algal biomass and composition through

both direct (consumption) and indirect (nutrient excretion/egestion) pathways (Knoll et al. 2009). Excretion (via the gills or urine) produces transiently available, labile nutrients that can be either quickly (minutes to hours) utilized by algae or transported downstream (Vanni 2002; Liess and Haglund 2007). The products of egestion (feces), on the other hand, are retained in lotic systems longer than the products of excretion, as they contain particulate matter (including viable algal fragments in herbivores) (McDonald 1985; Vermeij et al. 2013), and, if pelletized, will remain intact for days or weeks (Hood et al. 2005). Pelletized feces leach nutrients relatively slowly, particularly since decomposition of the feces depends on colonizing bacteria to convert nutrients into more labile forms (Wotton and Malmqvist 2001; Vanni 2002). The dominant components of nutrients released from grazers may differ between excreta and egesta; Liess and Haglund (2007) found that snail excreta provided the bulk of the N contribution to the periphyton from snails, whereas egesta were the source of most of the P taken up by the periphyton. Although the effects of nutrient excretion by fish have been studied (e.g., McIntyre et al. 2008; Capps and Flecker 2013a), little attention has been paid to the role of egesta, particularly pelletized feces, in nutrient cycling or ecosystem change (André et al. 2003).

The importance of grazing fishes to nutrient cycling has been highlighted by dramatic changes in nutrient availability induced when fish either migrate into an ecosystem (Meyer and Schultz 1985) or are lost from a system (McIntyre et al. 2007). It has also been suggested that an uneven distribution of animals (and fecal deposition) throughout an ecosystem may not only lead to high nutrient loading in the vicinity of the animals (McIntyre et al. 2008), but also generate localized areas of unusually high rates of nutrient cycling (biogeochemical hotspots as coined by McClain et al. 2003). Exotic grazers in particular may alter nutrient cycling pathways, and the contribution of these pathways to the overall nutrient budget of a system, if their internal stoichiometry differs from that of the environment they have invaded (Hall et al. 2003; Glibert et al. 2011; Capps and Flecker 2013a, b). For example, grazers with particularly low body N : P ratios may have a greater effect on N-limited systems as these grazers consume relatively more P and release relatively more N than grazers with a high body N : P ratio (André et al. 2003; Hood et al. 2005).

In recent decades, Florida freshwater springs have been threatened by persistent invasions of exotic species (USGS 2015), declining springflows (Neubauer et al. 2008), increasing anthropogenic nitrate inputs to surface waters and aquifers (Katz 2004; Heffernan et al. 2010), and nuisance algae blooms (e.g. *Vaucheria* (de Candolle) and *Lyngbya wollei* (Farlow ex Gomont)) (Notestein et al. 2003; Stevenson et al. 2007; Brown et al. 2008; Quinlan et al. 2008). The increase in algal coverage seen in springs has often been associated with a reduction in submerged aquatic vegetation, such as *Vallisneria americana* (Michx) (Brown et al. 2008; Cowell and Dawes 2008; Sickman et al. 2009). Although there are large numbers of freshwater exotic fish species in Florida (USGS 2015), the contribution of these exotic invasions to declines in spring ecosystem quality and function has been largely undocumented.

Here we focus on one of the most abundant exotic fish species in Volusia Blue Spring: *Pterygoplichthys disjunctivus*, a relatively large loricariid armored catfish species from the Amazon basin (Fuller et al. 1999). This species became widespread in springs and rivers throughout Florida, beginning in the late 1990's (Gibbs et al. 2008). When this species is abundant in Volusia Blue Spring, the number of individuals can be as high as 600–1000 in the 650 m run (M Gibbs, unpubl. data). Stable isotope analysis has demonstrated that the diet of this species is broad, and diet and gut structure are plastic (German et al. 2010); in Volusia Blue Spring, *P. disjunctivus* ingests large quantities of algae. Others have reported that high densities of *P. disjunctivus* can denude substrates (Power 1983; Hoover et al. 2004) and alter nutrient patterns via excretion (Scott et al. 2012; Capps and Flecker 2013 a, b; Datri et al. 2014). We have observed that *Pterygoplichthys disjunctivus* produces relatively large, distinct egesta (fecal material) that can be readily identified in the spring run throughout the year (K Work and M Gibbs, pers. observ.). We have also noted that catfish feces in Volusia Blue Spring contain roughly 40% sand, are held together by mucus, and can be deposited in strands as long as 20 cm (M Gibbs, unpubl. data). The two other common, large herbivores in the spring run (mullet and tilapia) only egest loose waste, and we have not found any other pelleted fish fecal material besides that produced by *Pterygoplichthys* during 15 years of snorkel surveys in the spring run. Two key observations about the feces of *Pterygoplichthys* inspired the current study: (1) we observed abundant catfish feces in the spring run, and (2) during the past six years, we noticed that even though the average numbers of catfish in the spring run have dropped dramatically (from many 100s to 100 or fewer) in daytime observations, the amount of fecal material appears to have stayed the same, suggesting that catfish are entering the spring run at night, and leaving during the day. If

nutrients are leaching from feces, the effect could be large, and if fecal material persists beyond the presence of the individual fish that deposited the material, the effect could be relatively long-lasting.

In short, unlike any other fish species in the spring run, *Pterygoplichthys disjunctivus*' grazing and digestive processing repackages periphyton with sand into a fecal structure that remains relatively intact in the spring, instead of flowing downstream and out into the river. It is important, therefore, to consider armored catfish egestion separately from excretion, since egested material will remain where deposited (if pelletized), but requires some processing before it can be used by periphyton; excreta, on the other hand, are both quickly flushed from its point of release (on the order of hours) and immediately available to periphyton (André et al. 2003; Hood et al. 2005). Furthermore, the feces of many herbivores contain undigested material, which for aquatic organisms eating algae (grazers) includes viable algae (Wotton and Malmqvist 2001). If the repackaged fecal periphyton produced by *P. disjunctivus* leaches nutrients, the addition and retention of large quantities of potentially nutrient-rich feces should increase internal nutrient loading in the spring (Vanni 2002; Hahn et al. 2004; Glibert et al. 2011). This increase in nutrient loading may stimulate algal growth which could be further exacerbated by live cells in the feces. Therefore, we asked: (1) what is the abundance of armored catfish feces in the spring? (2) how much extractable fecal nutrients are in catfish feces and how quickly does leaching occur? (3) do fecal samples contain viable algal cells? and (4) does the presence of feces stimulate new algal growth?

Based on our anecdotal observation of abundant feces in the run, we hypothesized that not only would fecal nutrients stimulate new algal growth from viable algal cells in the feces, but fecal density would be high enough to release sufficiently large quantities of these nutrients to create biogeochemical hotspots in the spring run.

Material and methods

Study site

Volusia Blue Spring (28º56′51.0″N, 81º20′22.5″W) is a first magnitude spring that discharges 1.8–6.1 $m^3 s^{-1}$ of 23 °C water daily from the Floridan aquifer into the St. Johns River in Volusia County, FL. The run is 650 m long, approximately 20–30 m wide, and has a benthic area of roughly 17,400 m^2 (Neubauer et al. 2008), with virtually no submerged macrophyte vegetation, and a partial canopy of live oak (*Quercus virginiana* (Miller, 1768)) and sabal palm (*Sabal palmetto* (Walter, 1830)) (Scott et al. 2004). Algal coverage of the sediments of Volusia Blue Spring is high; Stevenson et al. (2007) estimated that 80% of the substrate was covered with benthic algae, 60% of which belong to the filamentous nuisance taxon, *Vaucheria*. In addition, the spring has been categorized as Impaired due to extremely low dissolved oxygen concentrations (Brown et al. 2008), although increasing nitrate also has been a persistent problem in the spring (Holland and Bridger 2014). Dissolved oxygen averages 0.1 mg O_2/L at the headspring, and slowly increases to 0.7 in the mid-channel of the lowest reaches of the spring run (Work et al. 2010). Dissolved oxygen, however, can be much higher along the banks of the spring run where algal growth is heaviest and water flow is slow enough to pick up oxygen from the algae (average = 0.4–6 mg O_2 L^{-1} along the banks). The spring also has experienced increases in nitrate loading over the course of 40 years, with a low of 100 μg L^{-1} in the mid-1970s to a high of ~700 μg L^{-1} in 2008. Phosphorus, on the other hand, has varied between ~50 and 75 μg L^{-1}, although more randomly than nitrate (Holland and Bridger 2014). In winter, tarpon (*Megalops atlanticus* (Valenciennes, 1847)) and endangered Florida manatee (*Trichechus manatus latirostris* (Harlan, 1824)) use the run as a warm water refuge from the river, whereas in summer the run is heavily used by recreational swimmers. Several exotic fish species, including blue tilapia (*Oreochromis aureus*), two South American armored catfish species (*Hoplosternum littorale* (Hancock, 1828) and *P. disjunctivus*), and a South American pirapatinga (*Piaractus brachypomus* (Cuvier, 1817)), have invaded Volusia Blue Spring from the St. Johns River and both *O. aureus* and *P. disjunctivus* can be observed in the spring throughout much of the year (Work et al. 2010).

Fecal abundance in the spring run

We surveyed fecal abundance nine times between June 2008 and May 2012; five times during summer months (April–September) when catfish numbers were generally lower, and four times during winter (October–March) when catfish were more likely to be using the spring run as a thermal refuge. We divided the spring run into 84 grid sections of 10 m^2 each, most of which were sampled during each survey (Figure 1). To sample a grid section, we tossed three weighted flags to somewhat haphazard locations within the grid section, dropped a 0.51 m^2 quadrat where each flag landed, and took photographs for later analysis. Each survey took place over 2–3 days,

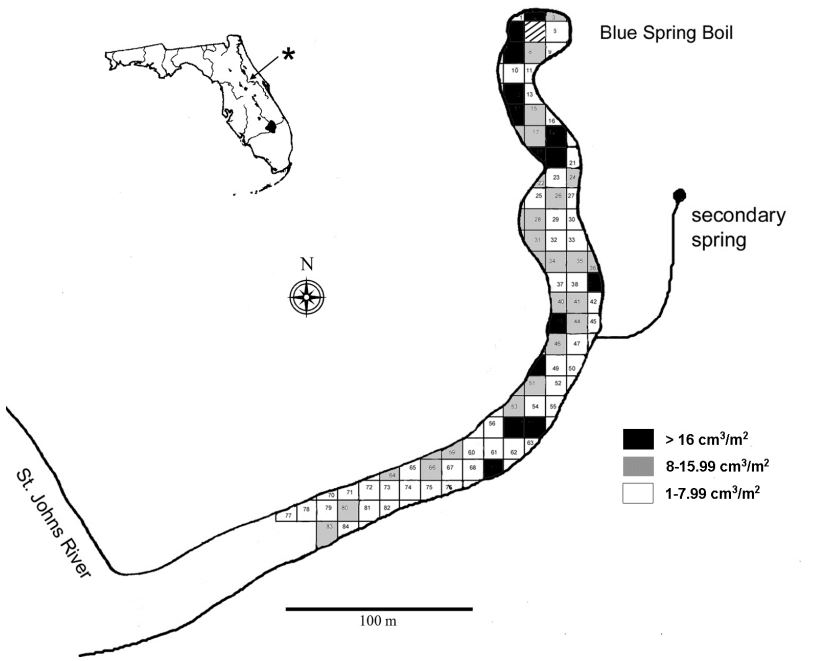

Figure 1. Map of Volusia Blue Spring, indicating sampling grid for *P. disjunctivus* fecal abundance surveys. The hatched grid at the headspring was not sampled due to extreme depth. Unnumbered grids along the edges of the spring run were too small to be sampled adequately. Grid shading represents the average density for that particular section across quadrats sampled in all sample periods.

and we chose the grid sections sampled each day using the random number generator function in Microsoft Excel. We did not sample the lower 100 m of the spring run due to the presence of large alligators (*Alligator mississippiensis* (Daudin, 1802)).

Upon return to the lab, we edited the photographs for color balance and to standardize size (the quadrat had a 10 cm scale bar inscribed on it). We identified, highlighted, and measured all feces for length using a ScaleMasterClassic Digital Plan Measure (Calculated Industries, Carson City, Nevada). We then calculated averages of summed fecal lengths from all 3 quadrats in each grid section. Feces were nearly always the same diameter, and so we were able to estimate fecal volume using a standard cross-sectional area of 0.1256 cm^2, based on a diameter of 0.4 cm. We then calculated fecal volume both in terms of unit area and for the entire spring run.

Collection of fecal material

Between the fall of 2010 and spring of 2013, we harvested 31 individual *P. disjunctivus* using pole spears. We sacrificed the fish with an overdose of MS222 (tricaine methansulfonate) and dissected them for fecal samples (FDEP Permits 06180813, 07080913, 09141013, 100400013, handled according to Stetson University IACUC-approved methods). We took fresh fecal samples from the rectum or, if that wasn't possible, the distal 5% of the 800–900 cm intestine, where the intestinal contents were highly compacted and most closely resembled the fecal samples that we collected from the spring benthos.

Fecal production rates

We captured 6 individual *P. disjunctivus* using cast nets, and brought them back to the lab, where they were housed for 24 hours in large tanks fitted with wire platforms. After 12 hours, fish and platforms were removed, and the fecal strands left on the bottom of the tank were either measured *in situ* and then removed, drip-dried, and weighed, or removed with a dip net, drip-dried, and weighed. The fish were returned to the tanks for a further 12 hours, but as they were not fed, did not produce significant amounts of feces thereafter. The process was repeated a second time one week later with 6 more fish. All data from both trials were pooled and an hourly rate of linear fecal production was calculated based on the first 12 hours of captivity. Catfish were sacrificed after 24 hours, following the methods described above.

Extractable fecal nutrients

We measured the extractable nutrients in *P. disjunctivus* feces from the 31 freshly caught fish as well as 42 fecal samples of unknown age collected from the Volusia Blue Spring substrate. We suspended each 5 cm long (0.63 cm^3) fecal sample (equivalent to 1–2

average sized strands of field collected feces) in 100 ml of distilled water, crushed it by hand within a Ziplock bag, and then immediately vacuum filtered the crushed sample through coffee filters to remove solids. Once the solids were removed, we filtered the samples through 0.45 μm GFF filters and put them on ice. For the first 33 samples (including both freshly caught and substrate samples), we tested the filtered leachate in house for [$P\text{-}PO_4^{3-}$] (Soluble Reactive Phosphorus) using the ascorbic acid method (American Public Health Association (APHA) 1998) and for [$N\text{-}NO_3^-$] using either a cadmium column (APHA 1998) or the nitrate test kit for the Thermo Orion high performance ammonia electrode (Thermo Fisher Scientific, Inc., Chelmsford, Massachusetts), which reduces nitrate to ammonia using titanium chloride and sodium hydroxide. We sent the remaining 40 fecal samples (including both freshly caught and substrate samples) to Environmental Conservation Laboratories, Inc. (Orlando, Florida), which analyzed the samples for [$P\text{-}PO_4^{3-}$], [$N\text{-}NO_3^-$], and [TKN] (Total Kjeldahl Nitrogen). Minimum detection levels for nutrients for both in house and Environmental Conservation Laboratories analyses were the same: $P\text{-}PO_4^{3-}$ = 11 μg L^{-1}, $N\text{-}NO_3^-$ = 52 μg L^{-1}, TKN = 33 μg L^{-1}.

Nutrient leaching from feces

To measure the leaching rate of nutrients from *P. disjunctivus* feces, we deposited 1.25 cm^3 of intact feces (equivalent to three average field measured fecal strands) collected from freshly caught *P. disjunctivus* in a loose pile 5 cm from the upstream end of two one-way plexiglass flumes (9.7 × 4.2 × 11.6 cm); two other flumes without feces served as controls. We connected the flumes to a laboratory tap to generate a one-way flume flow comparable to the springflow in regions where fecal samples are commonly deposited (~0.1 m s^{-1}). From the downstream end of each flume, we collected 200 mL effluent samples every 24 hours for a maximum of seven days. We then analyzed the leachate for extractable fecal nutrients using the methods described above. We repeated this process four times so that there were a total of eight control measurements and eight experimental measurements. We subtracted the nutrient concentrations in control samples ($P\text{-}PO_4^{3-}$ = ~20 μg L^{-1}, $N\text{-}NO_3^-$ = ~80 μg L^{-1}, TKN = ~40 μg L^{-1}) from those of the experimental samples for each of the four replicate runs of the experiment, and multiplied these by the flow rate in the flume, to determine the leaching rates in μg per unit time (Equation 1). Because fecal samples can differ in volume, we standardized these measurements per unit fecal volume (Equation 2). Finally, we calculated the total amount of extractable nutrients in two ways: 1) we multiplied the leaching rate by leaching duration to calculate the total amount leached (Equation 3a) and 2) we calculated the amount of nutrients lost (which we assumed leached out) by measuring the difference between the extractable nutrients in fresh feces vs. samples from the sediment (Equation 3b). This measurement is likely conservative because the feces from the sediment differ in age to unknown degrees.

Equation 1: Leaching rate per sample (μg s^{-1}) = ([control] – [experimental] (μg L^{-1})) × flow rate (L s^{-1})

Equation 2: Leaching rate per unit volume of feces (μg cm^{-3} s^{-1}) = (([control] – [experimental] (μg L^{-1})) × flow rate (L s^{-1})) / volume of feces sampled (cm^3)

Equation 3a: Total amount leached per sample for flume samples (μg) = leaching rate per sample × time period of leaching

Equation 3b: Total amount leached per sample for crushed samples (μg) = average extractable fecal nutrients in samples from fish – average extractable fecal nutrients in samples from substrate

We then applied leaching rates (averaged over the four replicate runs) to the estimated volumes of feces in areas of high and low fecal densities to determine the magnitude of the potential impact of *P. disjunctivus* feces on spring nutrient dynamics (Equation 4).

Equation 4: Leaching rate of all feces within one square meter of substrate (mg m^{-2} day^{-1}) = (leaching rate per unit volume of feces (μg cm^{-3} s^{-1}) × density of feces within one square meter of substrate (cm^3 m^{-2})) × 86,400 (s day^{-1})

Fecal stimulation of algal growth

To determine whether *P. disjunctivus* feces stimulated algal growth, we constructed an acrylic array, based on a design by Notestein et al. (2003), to deploy fecal samples in the field. The array was composed of four open tubular chambers (7 cm diameter × 19 cm long) secured to a larger sheet of plexiglass. Each of the chambers contained two smaller plexiglass platforms that each held five glass slides mounted perpendicular to the platform and parallel to the flow (Figure 2). Two of the four chambers served as the experimental treatment and the other two chambers served as the control treatment. To deploy the array, we loaded the platforms with clean slides and inserted them into the chambers. For the experimental treatments, we placed the equivalent of three average strands of

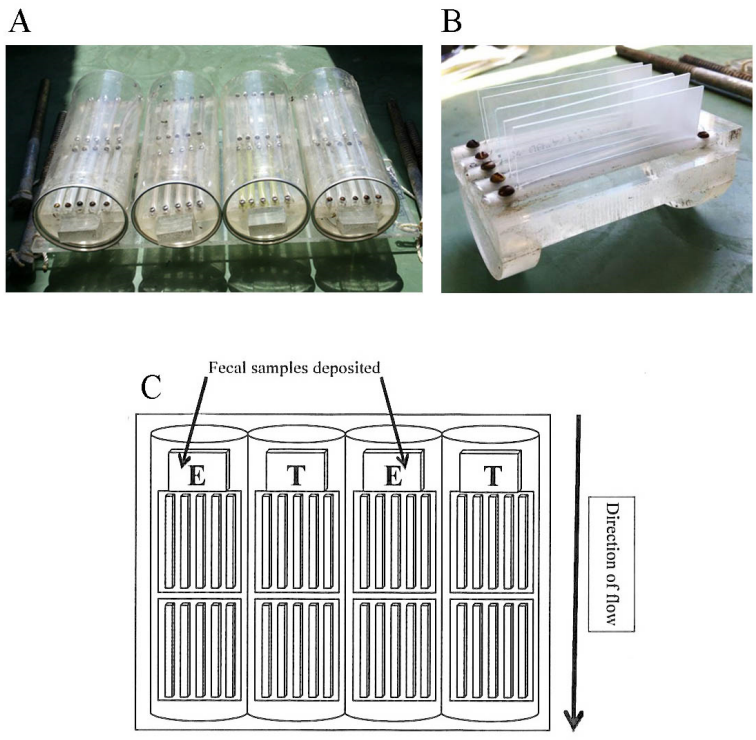

Figure 2. The array for measuring algal growth in the presence of *P. disjunctivus* feces (A) and one of the platforms contained within the array (B). The diagram of the array (C) indicates the arrangement of the treatments. Platforms labeled "E" were the experimental platforms and received no feces, whereas the platforms labeled "T" were the treatment platforms and received a 10 cm sample of feces. Photographs by the authors.

fresh feces (1.25 cm^3), on a small shelf projecting off the upstream end of each chamber (Figure 2). The platform slides served as a substrate for new algal growth. We loaded the control chambers with the platforms and slides, but with no fecal samples. We loaded and deployed the array during three phases of the algal growing season during the summer of 2013: May (early in the growing season), June (peak growing season) and August (late in the growing season).

We deployed the array approximately 4 m from the spring bank in an area with little debris or canopy cover, so that it would receive both adequate sunlight and flow. We secured the array to the substrate with four large zinc bolts and left it submerged for 1 ½ to 2 weeks during each of the three trials to capture the peak growth rates of the algae prior to either algal overgrowth of the entire array or senescence of the colonizing algae. We evaluated the arrays at one week and then every other day for algal colonization and growth. At each observation, we cleaned the outside of the array to maintain maximum light exposure to the slides, and if algae in and on the array had become so abundant that it threatened to overflow from one treatment to the next (at 1 ½ to 2 weeks), we removed the array. At the end of each trial, we collected the array and removed the slides for analysis. We put each slide into its own ziplock bag with 100 ml of deionized water and abraded the slide to remove the algae. We filtered the contents of the Ziplock bags onto 0.45 micron glass fiber filters and then estimated accrued algal biomass with standard chlorophyll *a* analysis (APHA 1998) after extracting the pigments with 90% aqueous acetone during a two-hour incubation. We then measured absorbance with a Genesys20 ThermoSpectronic spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Viability of algal cells in feces

To determine the viability of algal cells in feces, we homogenized each of ten 0.63 cm^3 samples of feces (equivalent of 1–2 average strands of feces) from ten freshly caught *P. disjunctivus* separately in 3 ml of distilled water, subdivided each sample into four separate 0.55 g subsamples, and then placed each subsample in a 50 ml Pyrex screw top culture tube containing 40 mL of Alga-Gro Freshwater Medium (Carolina Biological, Burlington, NC). We incubated these replicate culture tubes under fluorescent light on a 12 : 12 light : dark schedule at approximately 21°C. For each of the ten original samples, we analyzed one of the four replicate tubes for chlorophyll *a* each

week for four weeks using the methods described above. To calculate the increase in algal biomass over the four week period, we subtracted the initial chlorophyll *a* measure of algal biomass from the final chlorophyll *a* measure of algal biomass and divided by the time interval.

Fecal nutrients vs. nutrient loading from springwater

To determine the potential magnitude of impact of *P. disjunctivus* feces on spring nutrient dynamics, we calculated the total nutrient loading of the spring from the nutrient concentration in water exiting the spring vent (obtained from Holland and Bridger 2014) multiplied by the discharge of the spring (obtained from USGS Real Time Water Data, http://waterdata.usgs.gov/nwis/rt). We calculated the percentage contribution of our estimates of fecal leaching to total nutrient load of the spring using the most extreme values for discharge and leaching; we used the highest discharge and nutrient concentration and the lowest leaching rate calculated to find the low end of the range, whereas we used the lowest discharge and nutrient concentration and the highest leaching rate to find the high end of the range (Equation 5).

Equation 5: % contribution of extractable fecal nutrients = loading rate of extractable fecal nutrients ($\mu g\ day^{-1}$) / loading rate of spring vent ($\mu g\ day^{-1}$)

Due to the high volume and velocity of Blue Spring, however, most of the water discharging from the spring vent was unlikely to come into contact with periphyton on the substrate (Vogel 1989; Nowell and Jumars 1984), so we also calculated the boundary layer of low flow water near the substrate of the spring using the following equation from Vogel (1989):

Equation 6: $\delta = 5\times\sqrt{(x \times \mu)/(\rho \times U)}$

where d = boundary layer (cm), x = distance from spring vent (m), μ = dynamic viscosity ($g\ cm^{-1}\ s^{-1}$), ρ = density of water ($g\ cm^{-3}$), U = water flow rate ($m\ s^{-1}$)

For this calculation, we used values for viscosity of 20 ^{o}C water (very close to the water temperature in the spring) and the flow rate collected by USGS Real Time Water Data program (http://waterdata.usgs.gov/nwis/rt), which likely represents much higher values than the flow rates near the substrate of the spring. Furthermore, we calculated the boundary layer using a distance of 500 m (close to the end of the run), so these calculations likely underestimate the impact of fecal nutrient loading near the substrate. We then recalculated the percentage contribution of fecal nutrients to spring water using the nutrient loading rate ($\mu g\ day^{-1}$) in the volume of this boundary layer to make rough estimates of the extremes of impact of fecal nutrients on the nutrient loading of the spring.

Statistical analysis

We tested whether there were significant differences in fecal densities between grid sections in different regions of the spring (the grid sections were divided into five regions from the spring vent to the vicinity of the St. Johns River) with a Kruskall Wallis test due to non-normality in the fecal density data. We evaluated differences in nutrient concentrations between substrate and fish samples, and between experimental and control flumes, with non-parametric Independent Samples Median tests. We evaluated differences between control and experimental treatments in the field array experiment using a two-way ANOVA with time and treatment as factors. We ran all tests in the statistics program SPSS (IBM, Armonk, New York).

Results

Fecal abundance in the spring run

The density of feces within the run was highly variable from site to site and ranged from 0 to 64.4 $cm^3\ m^{-2}$ (Figure 1). Feces were abundant in the run; the scaled-up volume of feces in the entire run was estimated to range from 3.5×10^5 to 1.2×10^6 cm^3 (average = 6.2×10^5 cm^3) over the five years that benthic abundance was assessed. Fecal densities differed among regions with the highest densities near the spring vent ($p < 0.0001$). In general, feces tended to be most concentrated in areas with structure outside of the center channel and upper half of the run. The average strand of feces was just over 3 cm long (0.4 cm^3), and normally ranged from 0.5 cm (0.06 cm^3) to 9 cm (1.13 cm^3) in length, although strands up to 20 cm (2.5 cm^3) were occasionally measured. Fecal samples also appeared to vary highly in age with some samples appearing green and freshly released, whereas others appeared nearly white or covered in algae (Figure 3).

Fecal production rates

On average, unfed adult catfish in the lab produced 5.55 cm (0.70 cm^3) of feces every hour. This fecal production rate represented the fecal output of seven female and five male catfish averaging 43 cm standard body length and weighing 1181 g.

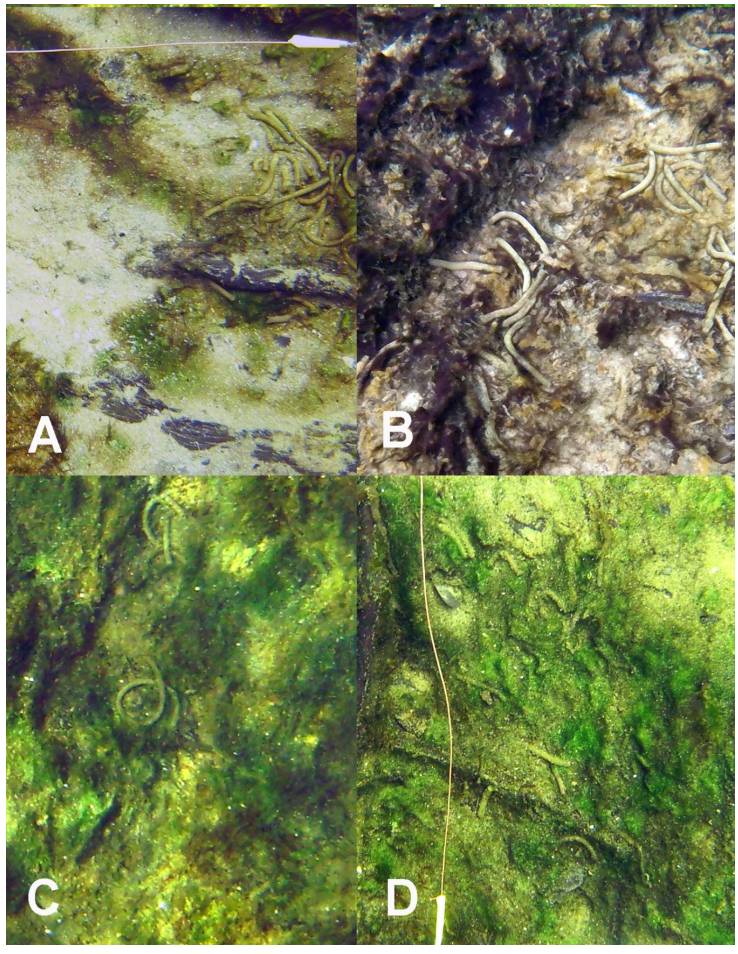

Figure 3. Fecal strands in Volusia Blue Spring. (A) Fresh fecal samples, probably deposited within a day. (B) Relatively fresh fecal samples, a few days old. (C and D) Old fecal samples, somewhat covered in debris and newly growing algae, one to two weeks old. Photographs by the authors.

Extractable nutrients from feces

We were able to extract significantly less $P\text{-}PO_4^{3-}$ ($p < 0.0001$), $N\text{-}NO_3^-$ ($p < 0.0001$), and TKN ($p < 0.006$) from fecal samples taken from the spring substrate than from fresh samples taken from live fish. Estimated loss of nutrients from freshly released feces was high and inversely related to the abundance of extractable fecal nutrients in the feces (Figure 4); extracts from substrate samples contained on average 94% of the $N\text{-}NO_3^-$, 64% of the $P\text{-}PO_4^{3-}$, and 46% of the TKN of fresh fecal samples.

Nutrient leaching from feces

In the flume leaching experiment, fresh fecal samples leached detectable ($p < 0.004$) quantities of $P\text{-}PO_4^{3-}$ and TKN in five of six replicates. Fecal samples remained intact and appeared unchanged throughout the weeklong experiment. Although the quantity of $N\text{-}NO_3^-$ that leached from fresh fecal samples was not detectable in any flume samples (Table 1), when we estimated nutrient loss from feces using the difference in extractable fecal nutrients between fresh samples collected directly from fish and substrate samples of unknown age collected from the spring benthos, the substrate fecal samples clearly had lost $N\text{-}NO_3^-$, $P\text{-}PO_4^{3-}$, and TKN. The nutrient losses estimated in this way were 35–107% of the measurements of nutrient leaching from fresh samples in the flumes. In the flumes, samples in five of the six replicates leached an order of magnitude more TKN than $P\text{-}PO_4^{3-}$ and the TKN leached more than three times as rapidly as $P\text{-}PO_4^{3-}$ (2 vs. >7 days, Figure 5). When these leaching rates were applied to the actual fecal densities in the spring, the leaching rates per unit area were highly variable, but the estimated leaching from high density areas was considerable ($P\text{-}PO_4^{3-}$: 2.9–1,880 mg m^{-2} day^{-1}; TKN: 53.3–34,341 mg m^{-2} day^{-1}, Table 2). The leaching from the estimated total density of feces in the run represented a small fraction of the total

Table 1. Mean nutrient content and estimates of nutrient loss from feces (with standard deviations) as measured 1) directly from flume nutrient leaching samples and 2) indirectly as the difference in concentration between fecal samples from live fish and from the substrate. Nutrient concentrations from Wetland Solutions (2010) are provided for comparison.

Parameter	$P\text{-}PO_4^{3-}$	$N\text{-}NO_3^-$	TKN
Total extracted nutrients of fecal sample from fish (μg cm^{-3})	74.4 ± 11.0	33.5 ± 5.7	1430 ± 188
Total extracted nutrients of fecal sample from substrate (μg cm^{-3})	26.6 ± 4.23	2.08 ± 0.20	786 ± 201
Quantity leached per fecal sample as estimated by loss (μg cm^{-3})	47.8	31.5	639
Quantity leached per fecal sample as estimated in the flumes (μg cm^{-3})	137.0 ± 26.0		597 ± 179
Nutrient concentration of spring water (μg L^{-1})	71	400	175

Table 2. Estimates of nutrient loading (with standard error) from *Pterygoplichthys disjunctivus* feces in areas with varying fecal densities. The average leaching rate was calculated from the flume studies. Fecal densities were estimated from the areal surveys and ranged from 0.10–64.4 cm^3 m^{-2} (average = 35.6 cm^3 m^{-2}). Data for the nutrient concentrations of Volusia Blue Spring water were taken from Wetland Solutions (2010).

Rate	$P\text{-}PO_4^{3-}$	TKN
Leaching rate per fecal sample from flumes (μg day^{-1})	18.31 ± 8.36	334.9 ± 71.2
Leaching rate per unit volume feces (μg cm^{-3} day^{-1})	29.2 ± 13.3	533.2 ± 133.3
Leaching rate for low density areas (μg m^{-2} day^{-1})	2.9 ± 0.54	53.3 ± 6.5
Leaching rate for average density areas (μg m^{-2} day^{-1})	1,040 ± 193	18,900 ± 2,330
Leaching rate for high density areas (μg m^{-2} day^{-1})	1,880 ± 349	34,341 ± 4,210
Leaching rate for run (μg day^{-1})	$6.47\times10^6 \pm 5.33\times10^6$	$1.04\times10^8 \pm 7.97\times10^7$
Nutrient loading by spring vent (μg day^{-1})	$8.78\times10^9 - 4.08\times10^{10}$	$1.42\times10^{10} - 1.14\times10^{11}$
Nutrient loading in estimated boundary layer (μg day^{-1})	$2.87\times10^8 - 1.33\times10^9$	$4.66\times10^8 - 3.72\times10^9$
% of nutrient loading by spring vent	0.016 – 0.074	0.090 – 0.72
% of nutrient loading by estimated boundary layer	0.18 – 3.64	2.78 – 22.2

nutrient loading to the spring ($P\text{-}PO_4^{3-}$: 0.016–0.074%; TKN: 0.090–0.72%). However, this contribution increased considerably when we considered only the nutrient loading to the estimated low flow boundary layer where the algae typically reside and water flow is reduced ($P\text{-}PO_4^{3-}$: 0.18–3.64%; TKN: 2.78–22.2%).

Fecal stimulation of algal growth

Algal biomass, measured as chlorophyll *a*, was significantly greater in the presence of fresh *P. disjunctivus* feces than in its absence over the course of the growing season in the *in situ* array experiment (treatment: $p = 0.01$, Figure 6). In addition, the concentration of chlorophyll *a* in both the control and experimental treatments increased comparably throughout the growing season (season: $p = 0.002$; interaction between season and treatment: $p = 0.17$). The fecal samples remained intact through the duration of the experiment (up to two weeks).

Viability of algal cells in fresh feces

All but one of the ten fecal samples that were collected from live fish and grown in the laboratory contained viable algae that proliferated in the test tubes. Chlorophyll *a* accumulated, on average, at a rate of 0.042 ± 0.024 μg day^{-1} (range = 0.0057 – 0.075 μg day^{-1}).

Discussion

Our hypotheses that *P. disjunctivus* feces would be abundant in Volusia Spring run and that these feces contained sufficient nutrients and live algal cells to stimulate algal growth *in situ* were supported. The feces distribution was highly clumped, particularly around structures, so that some areas contained very high densities, whereas other areas contained none. Feces were long lasting (at least two weeks) in the spring run. The fecal production rates we measured in the lab looked impressive, but likely were underestimates of the true rates, as we did not have the capacity to feed adult fish in the lab. In comparison, small juveniles (5 cm standard body length) fed and maintained in the lab fully and rapidly evacuated their 70 cm guts within two hours (Hood 2000).

Although the quantities of fecal nutrients leached from each individual sample of feces were tiny, the composite leaching rate was substantial in areas with high densities of feces. The flume measurements that we made of the nutrients leaching from intact feces

were higher for P relative to the measurements for lab-analyzed extractable fecal nutrients from crushed and filtered fecal samples, either because crushing the samples did not release all available nutrients, continued remineralization occurred as the samples sat in the flumes for a week, or the flume measurements overestimated leaching because a constant leaching rate was assumed between flume water sampling events. Further investigations into the nuances and longevity of P leaching from the feces of *P. disjunctivus* are clearly warranted. Not surprisingly, the largest nitrogen component of the feces was organic nitrogen, although the feces also contained small amounts of nitrate, a trend found in other fish fecal samples (Meyer and Schulz 1985). Although organic nitrogen in feces is not immediately available to algae, mineralization by sediment bacteria should eventually occur, thus increasing its bioavailability (Brookshire et al. 2005). The feces also leached a smaller, but still substantial amount of phosphorus. Katz (2004) suggested that phosphorus may be limiting in systems that experience persistent anthropogenic nitrogen loading like Florida springs. Although Volusia Blue Spring has experienced an increase in nitrate loading over the past 50 years (Holland and Bridger 2014), as have many springs in Florida, the N: P ratio has been relatively low (~7, Wetland Solutions 2010) and our measurements of nutrient leaching suggested that *P. disjunctivus* feces also contributed nutrients in a relatively low N : P ratio ($N\text{-}NO_3$: $P\text{-}PO_4^{3-}$ = 0.66). Despite the low ratio, P additions could be important if the addition of new nutrients is low due to low flow rates in backwater areas where both feces and algae accumulate. At the same time that *Pterygoplichthys* feces were leaching nutrients, however, they also were likely releasing viable algal cells, since the feces we harvested directly from fish clearly contained living algae. The combination of slowly leaching nutrients and live algae release likely produced the stimulation of algal growth that we measured in the field array experiment.

Many studies have examined the effect of grazers on nutrient recycling (Meyer and Schulz 1985; Sterner 1990; Vanni and Layne 1997; Vanni 2002; Hood et al. 2005; Liess and Haglund 2007; McIntyre et al. 2007; Small et al. 2011; Atkinson et al. 2013; Capps and Flecker 2013a, b); however, among the studies that have involved fish, most have focused on the role of excretion in increasing nitrogen availability (Meyer and Schulz 1985; Hood et al. 2005; McIntyre et al. 2007; Small et al. 2011; Capps and Flecker 2013 a, b), probably because excretion releases nitrogen in a labile form easily used by algae (Liess and Haglund 2007). Egestion, on the other hand, produces partially processed organic matter with some portion of the nutrients removed and assimilated by the herbivore (Wotton and Malmqvist 2001). Herbivores are notoriously inefficient digesters, however, and so the degree of processing and cohesion of the feces after release varies with species (Wotton and Malmqvist 2001). It could, therefore, be postulated that the amount of labile nutrients released from feces might be relatively low for most species and, perhaps as a result, most studies of fish feces have been related to the huge volumes of fecal material produced by aquaculture businesses (Lin et al. 2002; Brinker et al. 2005; Stewart et al. 2006). The pelletized nature of *P. disjunctivus* feces has allowed the feces to persist beyond the presence of the fish. The clumped nature

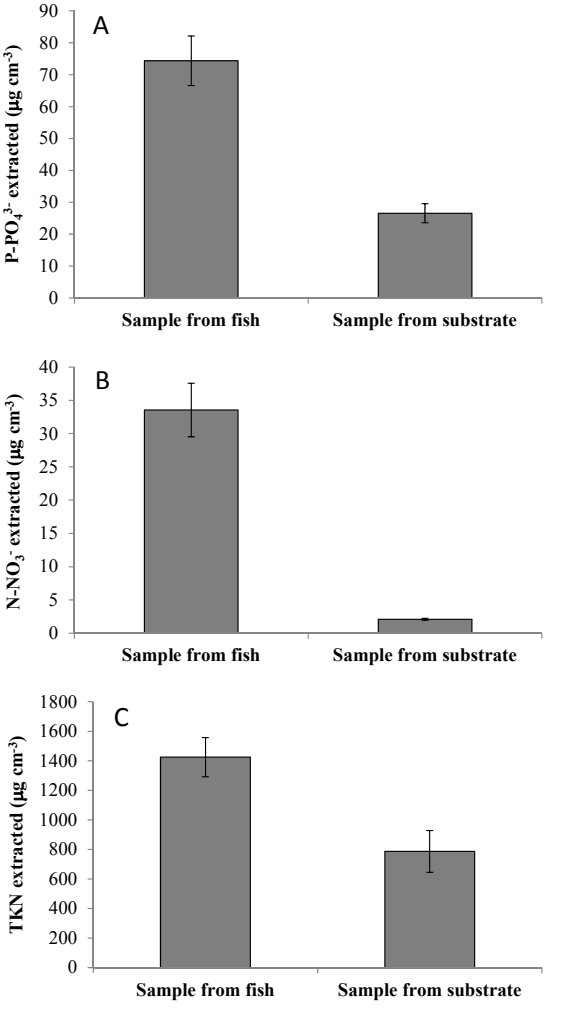

Figure 4. The $P\text{-}PO_4^{3-}$ (A), $N\text{-}NO_3^-$ (B), and TKN (C) content of samples taken directly from freshly caught *P. disjunctivus* and collected from the substrate. The age of the samples collected from the substrate was variable. Samples were suspended in water, filtered, and the filtrate was measured for nutrient content. Error bars represent standard error.

of the distribution of the feces and the small, but significant, quantities of nutrients leached from the feces indicated that *P. disjunctivus* feces may contribute to biogeochemical hotspot formation, as defined by McClain et al. (2003).

Loricariid catfish have long been known to affect periphyton biomass and productivity in their native habitats (Power 1990), and more recent studies have indicated that in addition to mechanically removing cells and sediment, loricariid catfish also may alter nutrient cycling in streams. Capps and Flecker (2013a, b) found that *Pterygoplichthys* sp. in Mexico excreted nutrients at rates comparable to (P), or in excess of (N), algal uptake, and the excretion rate of both nutrients by *Pterygoplichthys* sp. was much higher than the nutrient excretion rates for native fishes, more than matching the nutrient demands of the system. Hood et al. (2005) produced mass balance models that predicted that *Ancistrus triradiatus* (Eigenmann, 1918) and *Chaetostoma milesi* (Fowler, 1941) both excrete and egest material at a higher N : P ratio than the surrounding biofilm, potentially changing the availability of nutrients and the nutrient limitation patterns of the periphyton. These studies clearly suggest that loricariid catfish can play a significant role in controlling periphyton abundance both directly, through grazing, and indirectly, through alteration of sediment accumulation and nutrient availability. The disproportionate effects of excretion nutrient deposition by a single species in aquatic environments, also has been observed for a characid fish (*Astyanax aeneus* (Günther, 1860)) in low nutrient streams in Costa Rica (Small et al. 2011) and for an exotic snail (*Potamopyrgus antipodarum* (Gray, 1853)) in a Wyoming stream (Hall et al. 2003). Our study adds to this body of literature by contributing the additional role of fecal nutrients to nutrient cycles in lotic systems. Although the vast majority of the nutrient release from *Pterygoplichthys* feces was in the form of organic nitrogen, the feces also contributed small quantities of nitrate and phosphate. The quantity of phosphate released from areas with high fecal densities (0.82 μmol m^{-2} hr^{-1}) was lower than the total dissolved phosphorus released through excretion from aggregated *P. disjunctivus* in a Mexican stream (4.5–18 μmol m^{-2} hr^{-1}), but higher than the excretory releases of phosphorus by native fish (0.18 μmol m^{-2} hr^{-1}) (Capps and Flecker 2013a, b). Furthermore, since we know that feces remain in the spring run for an extended period of time (on the order of weeks), the continuous nutrient leaching from feces (particularly P) should have a greater nutrient impact than the more transient excreta. These two processes, excretion and egestion, can be

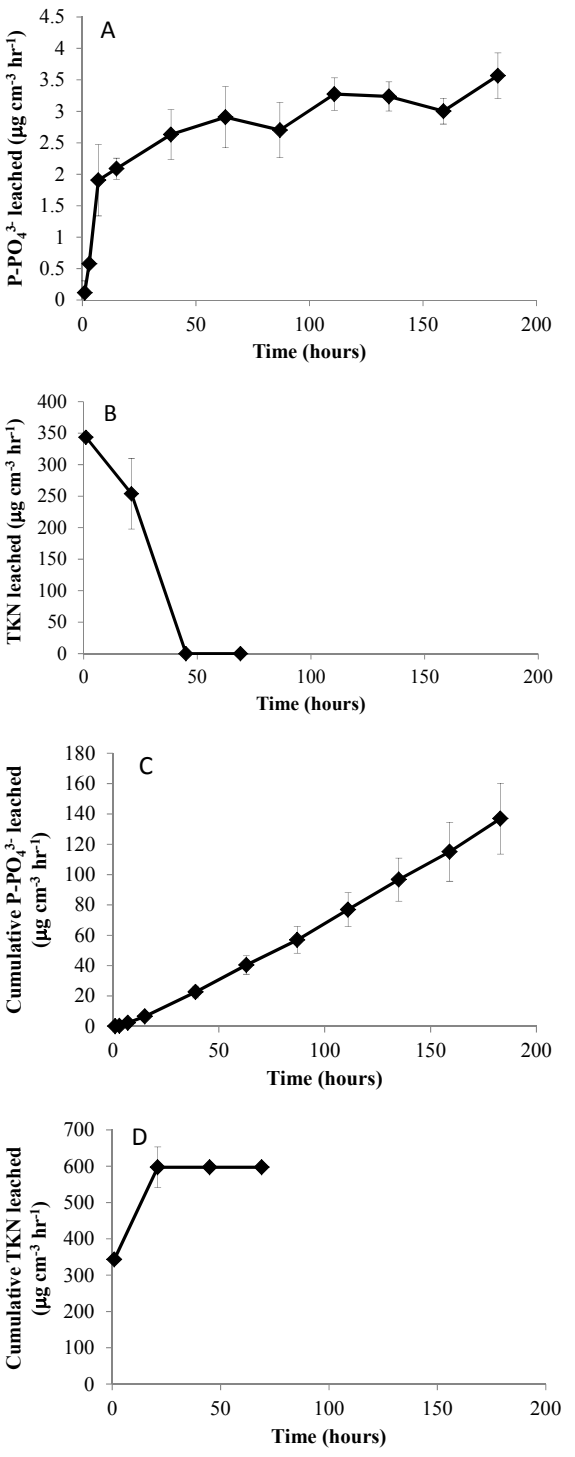

Figure 5. Hourly rate of $P\text{-}PO_4^{3-}$ (A) and TKN (B) leaching per unit volume of feces per day and cumulative leaching of $P\text{-}PO_4^{3-}$ (C) and TKN (D) over the course of 7.5 and 3 days, respectively. Fecal samples from freshly caught *P. disjunctivus* were allowed to leach into flumes in which tap water flowed at a rate comparable to spring flow rates. Samples were taken from downstream effluents. Error bars represent standard error.

considered as additive impacts on the nutrient cycles of a stream or spring; however, the release of phosphorus from feces is sustained beyond the presence of the fish.

A comparison of the exact exposure of benthic algae to nutrients leached from *P. disjunctivus* feces to their exposure to nutrients from spring water is challenging, as flow rates were much lower in the areas where feces are deposited (i.e. on the substrate, within existing algal mats, and in low flow backwater areas) than in most of the rest of the cross-sectional area of the spring (Vogel 1989; Stevenson and Glover 1993; Work et al. 2010). When we scaled up fecal leaching rates to the entire spring run, the amount of extractable fecal $P\text{-}PO_4^{-3}$ leaching from catfish feces is estimated to range from 6.89×10^3 μg day^{-1} to 4.46×10^6 μg day^{-1}, and TKN between 1.26×10^5 μg day^{-1} to 4.48×10^7 μg day^{-1}. Volusia Blue Spring is wide, relatively high velocity, relatively deep, and releases, on average ~315,000 μg s^{-1} of $P\text{-}PO_4^{3-}$ (71 μg L^{-1} × 4446 L s^{-1}), however, in much the same way that fast flowing spring water fails to pick up much oxygen as it passes over algal beds, the majority of nutrients in spring water are not available to benthic algae as the water flowing out of the spring vent likely has little interaction with the benthos (Vogel 1989). The exposure of any given alga on the substrate to the nutrient concentrations of the main spring channel, therefore, only represents a tiny fraction of that total load of $P\text{-}PO_4^{3-}$ to the spring, and our calculation of the contribution of fecal nutrients to the total nutrient load of the spring was exceedingly conservative. The slow flow rate of backwater areas around logs, and areas near the substrate where catfish deposit feces should increase the impact of fecal-derived nutrients through 1) reduced flushing rates for leached nutrients, 2) more opportunity for remineralization of organic nitrogen into useable forms of nitrogen, and 3) reduced recharge of spring vent-derived nutrients.

In addition to $P\text{-}PO_4^{3-}$, $N\text{-}NO_3^-$, and TKN, the feces in this study contained viable algal cells. The phenomenon of algae passing through the guts of herbivores and remaining viable has been described in invertebrates (Porter 1976; Cuker 1983; van Donk and Hessen 1993), coral reef fish (Vermeij et al. 2013), and some freshwater fish (McDonald 1985; Kolmakov and Gladyshev 2003; Lewin et al. 2003). The survival of cyanobacteria during gut passage, in particular, may be quite high; Lewin et al. (2003) found that 60% of *Microcystis aeruginosa* cells were viable after passing through roach guts. In some cases, algal cells that pass through the guts of grazers have had higher growth rates than ungrazed cells (Porter 1976; McDonald 1985; Kolmakov and Gladyshev 2003). The combined effect of leaching nutrients and the release of live algal cells from catfish feces likely produced the significant algal growth we measured downstream of feces in the field array experiment. Although we cannot quantify the relative importance of nutrients vs. live algae released from feces, we can say that *P. disjunctivus* feces do stimulate algal growth in their vicinity. Given that one sample of feces (approximately 0.63 cm^3) produced measureable growth of algae downstream, and that feces persist for weeks at a time, it is likely that high fecal density areas (64.4 cm^3 m^{-2}) would have a significant effect on algal mat production.

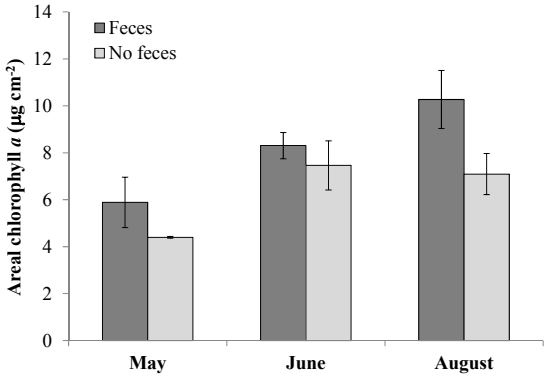

Figure 6. The effect of *P. disjunctivus* feces on algal accumulation in the array experiment. Algal accumulation at the end of each deployment was calculated as the summed algal biomass on the five slides divided by the area of the five slides available for algal attachment. Error bars represent standard error.

Pterygoplichthys disjunctivus feces are not, of course, the only source of nutrient recycling in Volusia Blue Spring. Although the feces of *P. disjunctivus* are the only visible, persistent feces in the spring run, other fish species (both native and exotic) must be contributing nutrients via diffuse egested materials and excretion. Additionally, algal mats, regardless of their initial origin, may provide their own nutrient recycling in lotic systems (Mulholland et al. 1994; Sickman et al. 2009). The success of *P. disjunctivus* feces at stimulating algal growth *in situ*, however, would suggest that the feces, undoubtedly in conjunction with excreta, have an effect on algal accumulation in Volusia Blue Spring and other ecosystems where *Pterygoplichthys* is found.

Conclusion

Whether by excretion (Capps and Flecker 2013a, b) or by egestion (this study), it is likely that the large numbers of loricariid catfish in Volusia Blue Spring and elsewhere redistribute nutrients and affect nutrient

cycling as they graze in one area, move on to another, and excrete and defecate along the way. The patchy distribution of feces suggests that these abundant exotic catfish could produce biogeochemical hotspots, as seen in the patterns of excretion by Capps and Flecker (2013a, b). In this study, we also showed that the combination of a change in nutrient availability and the presence of viable algae liberated from nutrient-rich feces could increase algal biomass in a spring that already has experienced nutrient enrichment. As Strayer (2012) argued, successful management of impacted ecosystems must take the role of exotic species into account. Volusia Blue Spring has been impacted by increasing anthropogenic nitrate loads, as well as intense recreational use, but the magnitude of the impact of the invasion of *P. disjunctivus* may be, by some measures, just as large. Although the nutrients leaching from the feces may not be "new" nutrients if they originate from production in the spring, the leaching lifespan of these fecal nutrients is longer and a greater proportion of the nutrient load likely is retained within the spring near algal substrates when processed by *P. disjunctivus*. Furthermore, some as yet unmeasured portion of the feces is likely to contain algae and nutrients translocated (Vanni 2002) from the St. Johns River as fish regularly move into and out of the spring run. Focusing on anthropogenic nutrient load to the system alone, therefore, is unlikely to be sufficient for management.

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