WATER QUALITY AND PLANKTONIC MICROBIAL ASSEMBLAGES OF ISOLATED WETLANDS IN AN AGRICULTURAL LANDSCAPE

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Abstract

Wetlands provide ecosystem services including flood protection, water quality enhancement, food chain support, carbon sequestration, and support regional biodiversity. Wetlands occur in human-altered landscapes, and the ongoing ability of these wetlands to provide ecosystem services is lacking. Additionally, the apparent lack of connection of some wetlands, termed geographically isolated, to permanent waters has resulted in little regulatory recognition. We examined the influence of intensive agriculture on water quality and planktonic microbial assemblages of intermittently inundated wetlands. We sampled 10 reference and 10 agriculturally altered wetlands in the Gulf Coastal Plain of Georgia. Water quality measures included pH, alkalinity, dissolved organic carbon, nutrients (nitrate, ammonium, and phosphate), and filterable solids (dry mass and ash-free dry mass). We measured abundance and relative size distribution of the planktonic microbial assemblage (< 45 µm) using flow cytometry. Water quality in agricultural wetlands was characterized by elevated nutrients, pH, and suspended solids. Autotrophic microbial cells were largely absent from both wetland types. Heterotrophic microbial abundance was influenced by nutrients and suspended matter concentration. Agriculture caused changes in microbial assemblages forming the base of wetland food webs. Yet, these wetlands potentially support important ecological services in a highly altered landscape.

Introduction

Intermittently inundated wetlands of the southeastern USA provide important ecosystem services and values (Golladay et al. 1997, Semlitsch and Brodie 1998, Battle and Golladay 1999, Kirkman et al. 1999); these wetlands have been shown to support speciose plant (Kirkman et al. 2004, Kaeser and Kirkman 2009) and amphibian communities (Liner et al. 2008), and are important for movement and breeding of reptiles (Subalusky et al. 2009). Many of these wetlands, often referred to as geographically isolated (e.g. Martin 2010), occupy shallow basins entirely surrounded by upland land cover. Their hydrology is variable, but they tend to have ponded water during periods when rainfall exceeds evapotranspiration (Kirkman et al. 1999). In the USA, their apparent lack of connection to perennial waters has resulted in little recognition and protection compared to other wetlands under Section 404 of the Clean Water Act (National Research Council 1995, Federal Register 1996). Isolated wetlands enhance water quality (Knox et al. 2008, Brown et al. 2010) and support biologically rich communities (Kirkman et al. 2004, Liner et al. 2008), however the potential for these processes and communities to persist in agriculturally altered landscapes is largely unknown.

Isolated wetlands are easily drained and have been significantly altered by agriculture practices (e.g., channelization, center pivot irrigation, runoff, and agrochemical application) (Bennett and Nelson 1990, Moreno-Mateos 2008). Globally, substantial wetland areas have been lost due to drainage and development. Over 50% of the area of depressional wetlands, riparian zones, floodplains, peatlands, and lake littoral zones has been lost mostly due to land conversion into intensive agriculture in North America, Europe, and Australia (Millennium Ecosystem Assessment 2005). The impact of wetland drainage on water storage and nutrient retention has received a great deal of attention, but changes and associated structural alteration of

wetlands in developed landscapes has not been well-studied. The impact of structural changes and nutrient supply on wetlands can be large (Armentano 1980, McCarty and Ritchie 2002). Previous studies have indicated that primary production in unaltered wetlands is nutrient-limited (Watt and Golladay 1999, Craft and Casey 2000, Battle and Golladay, 2001), thus wetland functioning is likely altered by elevated nutrient inputs.

Bacteria are likely the most abundant organisms in wetlands (Boon 2006), and are important contributors to biogeochemical functions (nutrient cycling, decomposition, assimilation of dissolved organic carbon, etc.) in intermittent wetlands (Palmer et al. 1997, Boon 2006). Planktonic bacteria are important in food webs because they are consumed by zooplankton and, in turn, other macroinvertebrates (Boon and Shiel 1990, Thouvenot et al. 1999). However, the influence of wetland alteration on planktonic microbes has been understudied. We examined water quality and associated planktonic microbial community within reference and agricultural wetlands of the Gulf Coastal Plain of the southeastern USA to determine the impact of agriculture (notably, elevated nutrients) on the microbial community. Our goal was to examine whether intensive agriculture influenced the abundance of planktonic microheterotrophs and what water chemistry variables best predicted microbial abundance.

Methods

Study Sites:

Our study was conducted in the Dougherty Plain physiographic district of the Coastal Plain of Georgia, USA. The wetlands we sampled are considered geographically isolated, meaning that they are surrounded by upland vegetation/land use and are not directly connected by surface drainage to streams, lakes, or other permanent water bodies (Kirkman et al. 1999).

The isolated wetlands of southwestern Georgia often occupy shallow catchments that extend beyond the jurisdictional wetland boundary (Watt and Golladay 1999). The climate in this region is humid subtropical (Christensen 1981), with an average annual precipitation of 131 cm that is distributed evenly throughout the year. Mean daily temperatures range from 21˚ to 34˚C in summer and 5˚ to 17˚C in winter (National Climate Data Center, Asheville, NC). The area contains extensive agriculture dominated by peanut, cotton, corn, and cattle production (Golladay et al. 2000). We sampled 10 wetlands impacted by agriculture on privately owned working farms (center pivot irrigation, row crops, and cattle) and 10 reference wetlands on Ichauway, a 119-km^2 ecological reserve and site of the J.W. Jones Ecological Research Center, Baker County, Georgia (Fig. 1). Ichauway Reserve is a remnant longleaf pine (*Pinus palustris*) forest that has been relatively undisturbed since the 1930s, and has been managed with low intensity, dormant season prescribed fires (frequency of 1 to 3 years) for several decades.

***Field Collection*:**

Wetlands (Table 1) were sampled three times during the hydroperiod in 2009: in the winter before leaf-out (February), following a large rain event after leaf-out (April), and another leaf-out sampling period (June, during seasonal drying). In February, one of the sites could not be sampled because the wetland was dry (SA39.W2). In April, water over roads prevented us from sampling one site (SA39.W20). In June, we were able to sample all wetlands. Wetland sampling devices were constructed out of 5.1 cm diameter PVC pipe and placed in all the wetlands prior to the beginning of sampling so sample collection could be done with minimal disturbance to the water column. Devices were a vertical pipe embedded into wetland sediments until the pipe was stable and not subject to vibration during sampling (~ 30cm). A horizontal pipe was connected to the vertical pipe using a 90 degree connector. Small holes in the vertical

pipe above the sediment surface allowed free exchange of water with the water column. Clean, flexible tubing was inserted inside of the devices the day before sampling and then connected to an ISCO peristaltic pump the day of sampling. This enabled us to obtain water from the wetland water column without disturbing sediments. Samples for water chemistry and filterable solids (dry mass, DM and ash-free dry mass AFDM) were collected in 500 ml and 1000 ml acid washed and rinsed sample bottles. Three 10 ml samples for each wetland were collected during each sampling period for characterization of planktonic microbial assemblages via flow cytometry. Samples were placed on ice immediately following collection and kept refrigerated (4 ± 1°C) until analysis.

***Laboratory*:**

Water Chemistry –

Samples were transported to the lab on ice and then filtered (Gelman A/E, GFF, 1- μm nominal pore size). Dry mass (DM) and ash-free dry mass (AFDM) were determined gravimetrically (Wallace et al. 2006). Water chemistry was determined according to standard procedures (see Battle and Golladay, 2001b). We measured dissolved organic carbon (DOC) and dissolved inorganic carbon (IC) with a Shimadzu TOC-5050 analyzer (Shimadzu Scientific Instruments, Kyoto, Japan). We determined NH_4-N, NO_3-N, and soluble reactive phosphorus (SRP) with a Lachat Quikchem 8000 flow-injection colorimetric method (Lachat Instruments, Milwaukee, Wisconsin). Using unfiltered water, alkalinity and pH were assessed with a Mettler DL12 titrator (Mettler-Toledo Inc., Columbus, Ohio).

Flow cytometry –

Samples for flow cytometry were passed through a 45 μm mesh sieve to remove large particles. Samples were preserved in formalin (2% final concentration) and kept at 4±1° C in the dark (samples were analyed within 1 month of collection). Samples were stained with the nucleic acid stain, SYBR Green II (SYBR, Invitrogen, final concentration 5X), for at least 30 minutes prior to analysis.

Flow cytometry was performed on a FACS Calibur flow cytometer (Becton Dickinson) using a 488 nm laser and a 635 nm red diode laser (for detection of chlorophyll autofluorescence). All parameters were logarithmically amplified and parameter values were displayed ranged four orders of magnitude on a log scale. Fluorescent beads (Calibrite, Becton Dickinson) were run periodically to verify that fluorescence intensity values remained consistent during sample analysis. SYBR Green II (SYBR) fluorescence was detected in a FL1 photomultiplier tube (530/30 nm bandpass filter) and objects were first gated based upon FL1 fluorescence (>50 channels). Several samples were filtered (0.22 μm) and analyzed to verify that particles smaller than this size (e.g. colloids, viruses) were not detected using this threshold fluorescence value. Objects meeting the minimal FL1 threshold value were then gated based upon forward (FS) and side (SS) angle light scattering (>2 channels).

To determine the sample volume analyzed, we measured the sample weight lost during analysis. This method yields accurate measurements of sample volume by adding a known quantity of beads to the sample (Rose et al. 2004). Between 70 and 100 μl of sample was analyzed. Generally, the flow rate was set to keep the count rate below 1000 objects per second. For one of the agricultural wetlands (Striplings), samples were diluted 1:20 to keep the count rate near this limit.

Data generated via flow cytometry were analyzed using MatLab (V7.7, The Mathworks). Cells were gated according to chlorophyll *a* fluorescence, detected in FL3 (Chl *a* positive cells were >425 fluorescent channel values). The threshold value was determined to be appropriate for discriminating between autotrophic and heterotrophic bacteria based upon comparative analysis of cyanobacterial and heterotrophic bacterial cultures. This threshold value was sufficiently low to detect cyanobacterial Chl *a* fluorescence without detecting SYBR labeled heterotrophs. Large phytoplankton (e.g., phytoflagellates) produce greater Chl *a* florescence signals, therefore, were also capable of detection using this threshold. Fluorescence overlap between SYBR green and Chl *a* fluorescence was determined to be minimal. Cells without detectable Chl *a* fluorescence signals were described as heterotrophs. Both Chl *a* positive and negative cells were further classified based upon FS signal, which is a proximal measurement of cell size. The size gates were set based upon the modal FS value of 1.0, 2.0, 4.0 μm diameter beads. For example, objects with a FS signal between the modal signal ≥1.0 and <2.0 μm beads were grouped into the 1–2 μm size class. This categorization provides an estimate of cell size, however, because difference in morphology between spherical beads and cells, this estimate should not be used to calculate biomass. The final cell concentration for each group was calculated by adjusting for the flow rate, dilution (when samples were diluted), and the addition of small volumes of SYBR and internal reference bead suspensions.

Statistical Analysis-

Water chemistry data were compared and analyzed separately by sample date due to the large variations in rainfall over the course of the study (Fig. 2). Water chemistry data were analyzed using a Mann Whitney Rank Sum Test since the majority of the data violated the

assumptions of normality. Dunn's test was used for pairwise comparisons. Principal Components Analysis (PCA) was used to illustrate the data in multivariate space and to reduce the number of water chemistry variables. Pearson's correlation was used to discern the variables that were related to the PCA scores.

Surprisingly, there were few cells containing chlorophyll in the water column, and only the heterotroph data were used for our analyses. Cell concentration (rather than biomass) was used to avoid biases caused by converting concentration to biomass without a thorough microscopic analysis of the microbial community (e.g., determining cell morphology). Heterotroph concentration data (cells mL^{-1}) were natural log-transformed to meet the normality and the homogeneity of variance assumptions implicit in parametric analysis. Two-way ANOVAs were conducted to examine variation in number of small heterotrophs with wetland type and sampling period being the main effects. Tukey's Honest Significant multiple comparisons ($\alpha = 0.05$; Littell et al. 2002) followed significant ANOVAs. However, this analysis does not indicate the strength of environmental factors influencing heterotrophy abundance. We were also interested developing predictor variables for the number of heterotrophs in a wetland. Water chemistry parameters differentiating reference and agricultural wetlands in the PCA were used in Akaike's Information Criterion (AIC) to determine the best linear model predicting the number of heterotrophs in the wetlands. We used pH, DM, AFDM, dissolved inorganic carbon (IC), NO_3, PO_4, and total dissolved carbon in the model building process. Several multiple linear models were calculated and compared using AIC. Based on maximum-likelihood estimates and the number of model parameters, AIC provides a measure for selecting among competing models of a given data set. The model having the lowest AIC is considered the best model because it provides the optimal compromise between predictive power and model complexity (see Johnson

and Omland 2004). AIC analysis allowed for determination of the water chemistry variables that likely lead to higher numbers of heterotrophic bacteria. An AIC was run for each individual sampling date to account for temporal differences. Then, an AIC was performed with all sampling dates with the cumulative rainfall 30 days preceding sampling added as a variable to the model. All analyses were done in SAS v9.2 (SAS Institute, Cary, NC).

Results

Water chemistry-

Water chemistry varied significantly between reference and agricultural wetlands (Fig. 3). Suspended solids ($p < 0.001$), pH ($p < 0.001$), alkalinity (not shown; $p < 0.001$), and soluble reactive phosphorus ($p = 0.002$) were significantly higher in agricultural wetlands. Dissolved organic carbon was significantly different ($p < 0.01$, main effects model) and more variable in agriculturally impacted wetlands, however multiple comparisons were not able to distinguish differences among wetlands.

The PCA showed a strong grouping pattern distinguishing reference and agriculturally disturbed wetlands (Fig. 4). PC1 axes for all dates were generally related to higher pH, total suspended solids, nitrate, and phosphate. PC2 axes for all dates were related to higher dissolved carbon. Reference wetlands tended to show lower dispersion along both axes on all dates in comparison to agricultural wetlands.

Heterotroph abundance-

Heterotrophic cells (i.e., cells without chlorophyll fluorescence) dominated the water column in all wetlands. The majority of cells were small (< 4 μm) and were likely bacteria

219 rather than heterotrophic eukaryotes. However, because the heterotrophic assemblage structure
220 was not directly measured via microscopy, we used the term heterotrophic cells to describe this
221 portion of the microbial assemblage. Yet, most are likely bacteria, with the larger cells consisting
222 of flagellates. Greater than 94% of the heterotrophic assemblage was <4 μm at all sites, and
223 larger cells became more abundant later in the season when the temperature increased; particles
224 < 2 μm composed 93% of the heterotroph assemblage in February (Fig. 5a), 80% in April (Fig.
225 5b), and 84% in June (Fig. 5c). The total number of heterotrophs varied significantly with
226 wetland type (agricultural vs. reference) and sampling period (Fig. 5; ANOVA, $p < 0.0001$).
227 Tukey's HSD indicated that heterotrophic bacterial abundance was greater in agricultural
228 wetlands and that the April sampling date (Fig. 5b) was significantly different (i.e. lowest values
229 in February (Fig. 5a) and highest in June (Fig. 5c)) in the number of heterotrophs than the other
230 two sampling dates.
231 For the February sampling period, a model with DM, AFDM, NO_3, and PO_4 best
232 predicted the number of heterotrophs in the wetlands ($w_m = 0.214$, $R^2 = 0.98$; Table 2), with the
233 next two models having a combination of those predictors. The April sampling followed a
234 period of above-normal rainfall, which diluted the standing water (Fig. 2). This dilution affect
235 increased the number of variables that distinguished the reference from the agricultural wetlands
236 (Fig. 4) and increased the number of variables used in the AIC model selection. Heterotrophic
237 bacterial concentrations sampled during April were best predicted by a model that included DM,
238 AFDM, IC, NO_3, and PO_4 ($w_m = 0.452$, $R^2 = 0.923$). The heterotroph assemblage present in June
239 was best characterized by the amount of DM ($w_m = 0.139$, $R^2 = 0.85$). When sampling periods
240 were combined into one model and an estimate for the 30 day total rainfall was added, a model
241 that included DM, AFDM, IC, NO_3, and the total amount of rain over the previous 30 days best

242 predicted the heterotroph concentration (w_m = 0.255, R^2 = 0.839). Overall, the concentration of
243 suspended matter and nutrients were drivers of heterotrophy cell concentration in these wetlands.
244
245 **Discussion**
246 Our study identified several key water chemistry variables that differentiated reference
247 wetlands from agricultural wetlands (pH, suspended matter, nutrients, and dissolved carbon).
248 Nutrient concentrations were generally elevated and showed a greater range of variability in
249 agricultural relative to reference wetlands. Elevated nutrient levels are an indicator of fertilizer
250 and animal waste runoff (Dorioz and Ferhi 1994, Carpenter 1998, Anctil et al. 2009). In addition,
251 disturbed wetlands had higher pH and total alkalinity compared to reference sites, which we
252 attribute to application of agricultural lime ($CaCO_3$), a common practice on fields in
253 southwestern Georgia (*personal observation*). Alkalinity and pH levels could also be influenced
254 by irrigation, another common practice in the region. A majority of water used in irrigation
255 comes from the upper Floridan Aquifer, which has higher pH and alkalinity than rainwater
256 (Hicks et al. 1987). Cumulatively, these data suggest that agricultural areas in southwestern
257 Georgia are contributing non-point source pollutants to adjacent isolated wetlands causing major
258 changes in water quality.
259 Agricultural (row crop and pasture) wetlands showed further evidence of water quality
260 alteration by having increased suspended sediment concentration. Sediment particles can have
261 absorbed pollutants attached (Knox et al. 2008) and are a common non-point source pollutant
262 associated with soil erosion from land development (Gaynor and Findlay 1995, Anctil et al.
263 2009, Makarewicz et al. 2009). Agricultural wetlands had greater suspended particle

concentration than reference sites, which is evidence of increased runoff within and adjacent to the wetland boundary.

The concentration of heterotrophic microbial cells was generally greater in agricultural wetlands than reference sites. The planktonic heterotroph assemblage was dominated by organisms in the bacterial size range (i.e., < 4µm in cell size). In isolated wetlands, bacteria play key roles in major biogeochemical functions (nutrient cycling, decomposition, assimilation of dissolved organic carbon, etc.) (Boon 2006). Bacterial concentrations were an order of magnitude lower than found in previous studies of wetlands (Boon 1991). However, our study only investigated water column heterotrophs and did not include sediment bacteria. Due to these higher numbers of heterotrophs in the bacterial size range, water column process rates (e.g., microbial uptake of nutrients, microbial transformations to gaseous forms, etc.) within these wetlands may be high and contribute substantially to ecosystem function.

This study identified several factors (suspended matter, nutrients, and over a longer time scale, cumulative rainfall) that influenced planktonic heterotrophic cell abundance. Many of these variables differentiated reference wetlands and agricultural wetlands (PCA; suspended sediments and nutrients), yet some variables did not seem to influence the abundance of heterotrophs (pH). In a previous study within the region, soil analysis indicated P or N/P co-limitation of isolated wetlands of southwestern Georgia (Craft and Casey 2000). Our results indicated that nutrients were important variables influencing planktonic heterotrophy abundance. We suggest that the timing of nutrient subsidies from agricultural fields, along with suspended sediments and rainfall are important drivers of planktonic heterotrophs in Coastal Plain isolated wetlands.

Numerous studies have demonstrated the importance of freshwater wetlands for maintaining water quality through remediation of nonpoint runoff (Johnston 1991, Mitsch and Gosselink 1993, Craft 1997, Knox et al. 2008). The importance of riparian and floodplain wetlands in sequestering N and P derived from fertilizer has been well documented (Brinson et al. 1981a, b, Naiman and Decamps 1997). Yet, biogeochemical functions of isolated wetlands are not well quantified. Isolated wetlands often occupy depressions in the landscape where they serve as focal areas concentrating non-point source runoff (Leibowitz 2003). Isolated wetlands assimilate nutrients associated with runoff through uptake in plant biomass and subsequent deposition in sediments, or through microbial denitrification in wetland soils (Whigham and Jordan 2003). While wetland soils are typically chemically reduced and contain ample organic C for denitrification (Craft 1997), this may not be the case in agricultural settings in which soil tillage and drainage aerate soils and promote loss of soil organic matter. Such practices are also likely to alter the chemical quality of organic carbon, which may alter rates of key microbial processes including denitrification. While it is well established that planktonic wetland communities (which here includes microbes, plants, and animals) can assimilate nutrients, their potential contribution to improving water quality of non-point source runoff to isolated wetlands remains poorly understood. Agricultural land management often results in reduction or elimination of rooted perennial vegetation, thus uptake by planktonic organisms may be a major pathway of nutrient assimilation and water purification. Our results show a clear response of planktonic microorganisms to non-point source runoff associated with intensive agriculture.

Agricultural land use has caused significant changes in the water chemistry and the associated heterotroph assemblage of isolated wetlands. Agricultural wetlands tended to have higher concentrations of planktonic bacteria, which seemed to be linked to availability of carbon

and nutrients. However, additional studies are needed to understand the impacts of other water quality variables (e.g., pH) on wetland planktonic communities. Further studies into nutrient processing and assimilation by wetland planktonic microbial communities will lead to greater insight on their potential for nutrient removal by isolated wetlands. Wetlands are known for their environmental remediation properties (Knox et al. 2008, Brown et al. 2010), however the ecosystem services (e.g., nutrient retention and cycling, water quality improvement) provided by isolated wetlands within altered landscapes are not well quantified. Excess fertilizer and manure on agricultural lands create surplus N and P, which is mobile in many soils and often leaches to downstream aquatic ecosystems and groundwater (Carpenter 1998). However, wetlands that remain in agricultural areas potentially trap nutrients might otherwise runoff into adjacent streams or into the groundwater (Knox et al. 2008). Our study indicates greater planktonic cell numbers in wetlands influenced by agriculture, which may be crucial to assimilation and cycling of nutrients (Knox et al. 2008). Thus, isolated wetlands potentially provide valuable ecosystem services within highly disturbed agricultural landscapes, although their potential remains largely unrecognized.

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Table 1: The size and the surrounding land use of the wetlands used in this study. Land use is delineated by a 100-m buffer surrounding each of the wetlands. Reference wetlands are surrounded by a fire-maintained second-growth longleaf pine (*Pinus palustris*) forest.

Wetland		Wetland Area (m^2)	% Agriculture*	% Pasture**	% Row Crop	% Other***
Agricultural	Westside	12900	45	94	0	55
	Eastside	15300	49	88	0	51
	W12	4400	22	78	0	88
	W16	22600	100	36	64	0
	W20	73800	49	0	49	51
	W2	22700	51	0	51	49
	W6	20500	98	0	98	2
	W5	24800	86	0	86	14
	Striplings	2900	74	74	0	26
	Skanky	37500	31	0	31	69
Reference	P2	60400	0	0	0	0
	P3	46900	0	0	0	0
	P4	172000	0	0	0	0
	P15	254000	0	0	0	0
	P21	84700	0	0	0	0
	P42	25900	0	0	0	0
	P46	113000	0	0	0	0
	P52	69500	0	0	0	0

P53	47500	0	0	0	0
P58	81800	0	0	0	0

* all agricultural activities; includes row crop and pasture

** includes improved and unimproved pasture

*** includes silviculture development, and unimproved woodlands

Table 2: Results from the AIC model selection. The best three models from each sampling period and all dates combined are shown. K describes the number of variables in the model. The Δi is the difference between the AIC of the best fitting model and that of model *i*. The w_m is the normalized relative likelihood values known as the model weights.

Date	**Parameters in Model**	**K**	**F-value**	**R^2**	**AIC**	**Δi**	**w_m**
Feb.	DM, AFDM, NO_3, PO_4	4	156.6	0.980	410.9	0.000	0.214
2009	DM, AFDM, PO_4	3	196.4	0.977	411.3	0.380	0.177
	DM. NO_3, PO_4	3	183.4	0.975	412.5	1.583	0.097
April	DM, AFDM, IC, NO_3, PO_4	5	31.0	0.923	492.8	0.000	0.452
2009	pH, DM, AFDM, IC, NO_3, PO_4	6	24.3	0.924	494.5	1.682	0.195
	AFDM, IC, PO_4	3	36.6	0.880	497.2	4.393	0.050
June	DM	1	79.4	0.850	417.0	0.000	0.139
2009	DM, IC	2	39.4	0.858	418.1	1.095	0.080
	DM, NO_3	3	38.5	0.856	418.4	1.426	0.068
All	DM, AFDM, IC, NO_3, Rain	5	48.8	0.839	1372.6	0.000	0.255
dates	DM, AFDM, IC, NO_3	4	57.0	0.826	1374.5	1.908	0.098
	pH, DM, AFDM, IC, NO_3, Rain	6	39.9	0.839	1374.6	1.953	0.096

Figure Legends

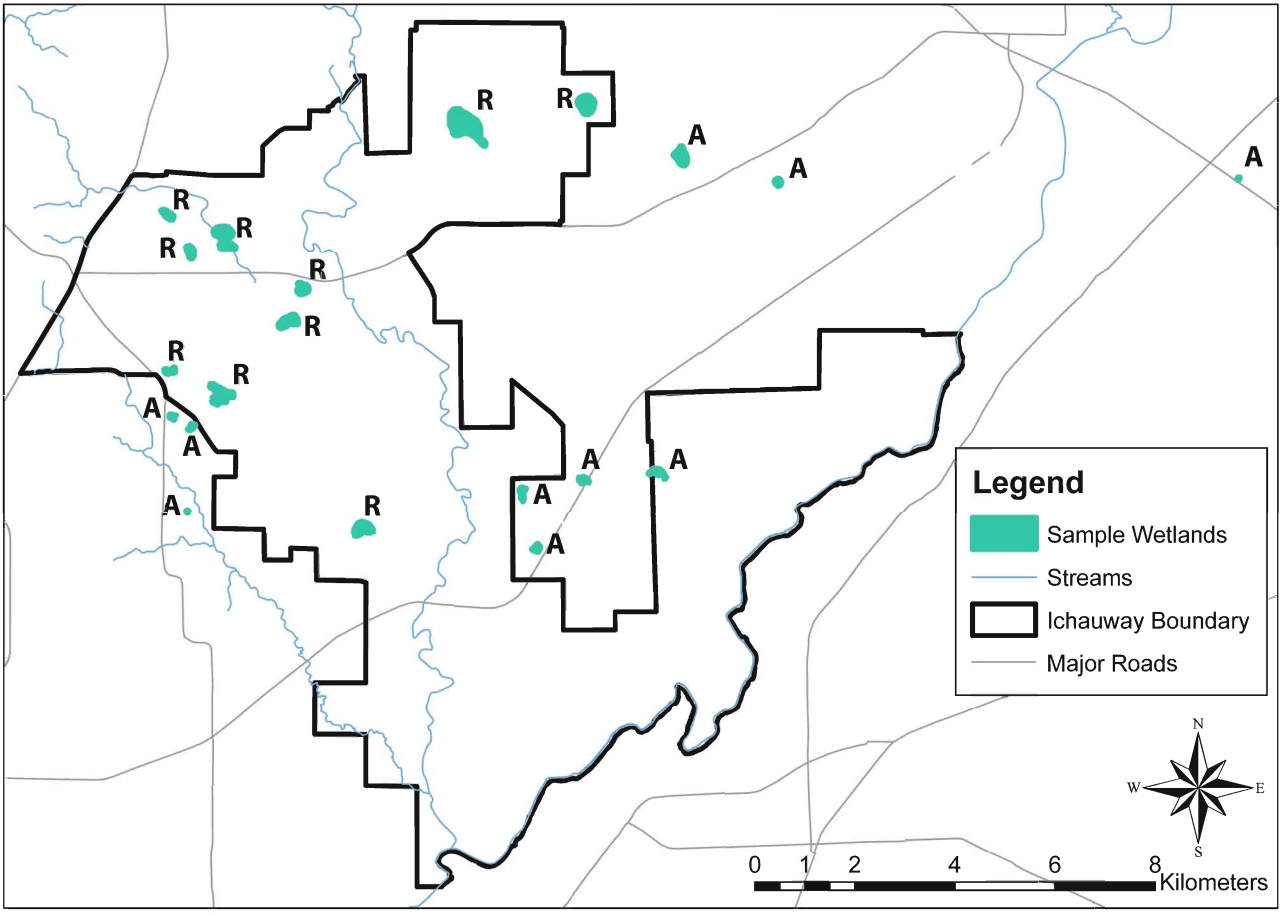
Fig 1 Sample sites were located within and near Ichauway. The sites (R - reference; A - agricultural) used in this study are labeled.

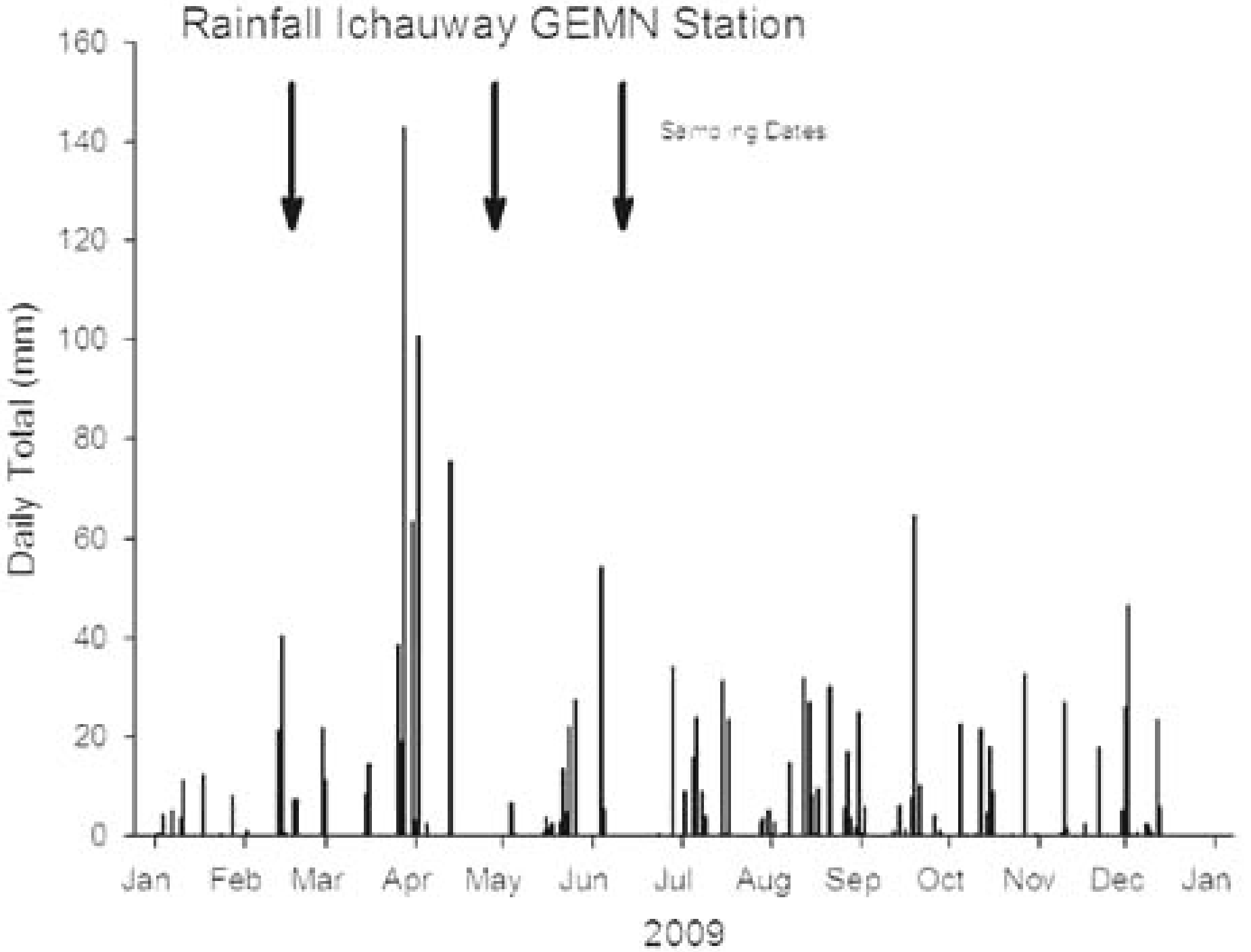
Fig 2 Daily rainfall totals for the sampling area. Arrows depict the sampling periods (February 2009, April 2009, and June 2009).

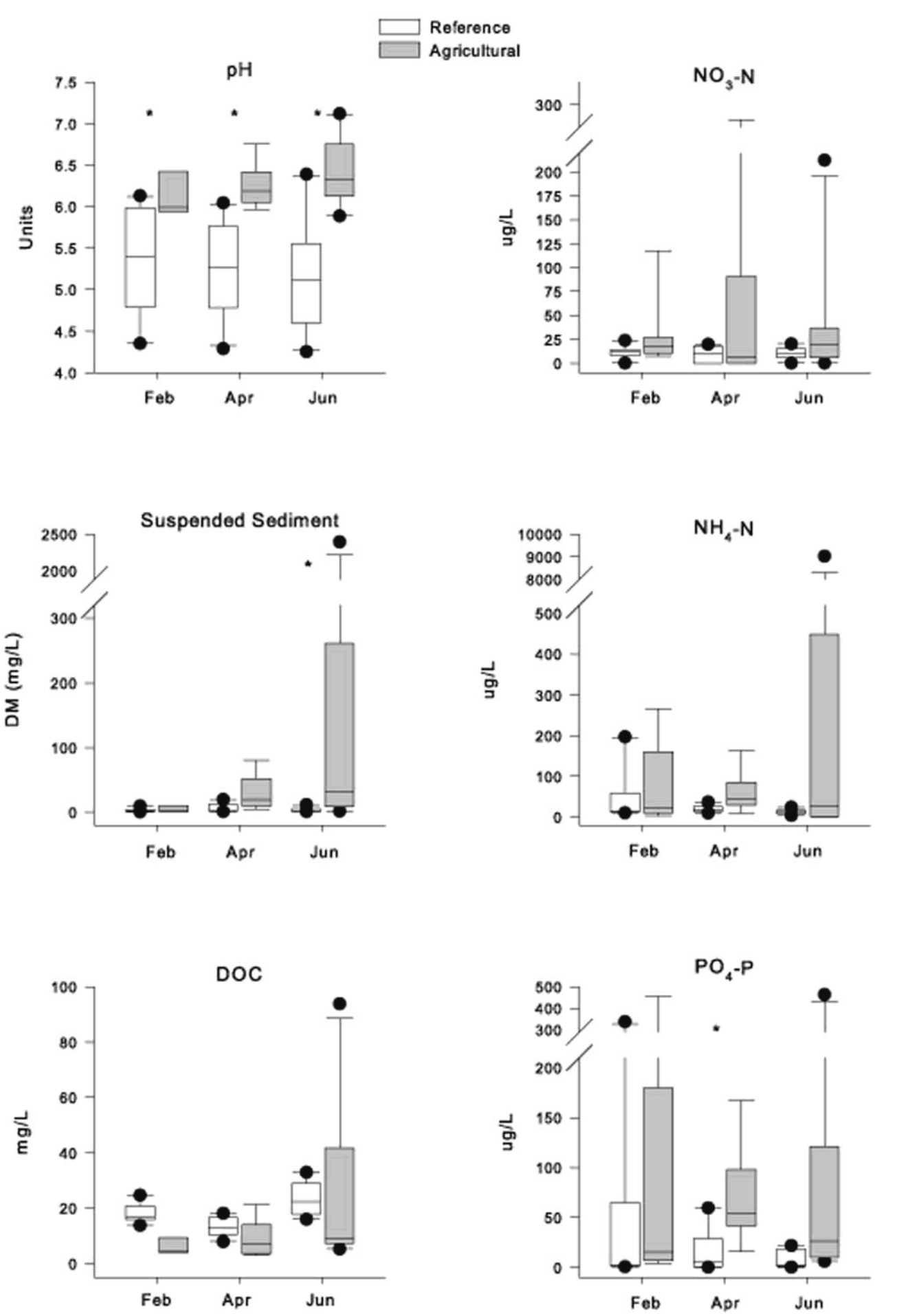
Fig 3 Averaged water chemistry results for agricultural and reference wetlands. Asterisks denote significant differences.

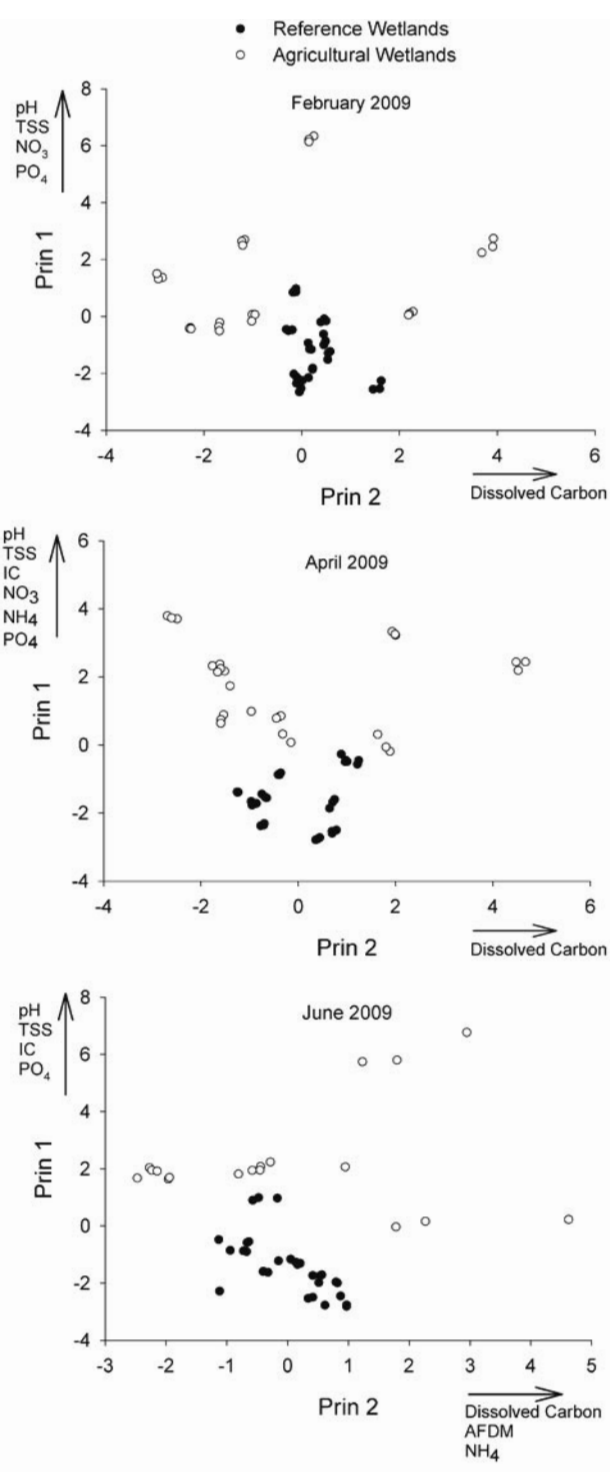
Fig 4 Results from the PCA illustrating how reference and agricultural wetlands group out in multivariate space. Arrow along the bottom of the axes indicate the variables that were important for the axis score.

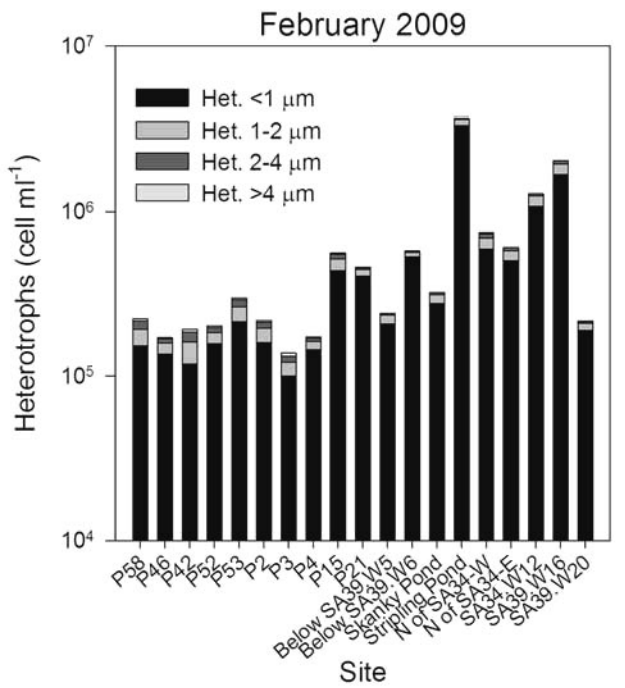
Fig 5 The total heterotroph concentration in all the ponds during the February (a), April (b), June (c) sampling periods, and the mean concentration (±SE) across all sampling periods (d). The total numbers of heterotrophs are categorized as size classes in A-C.

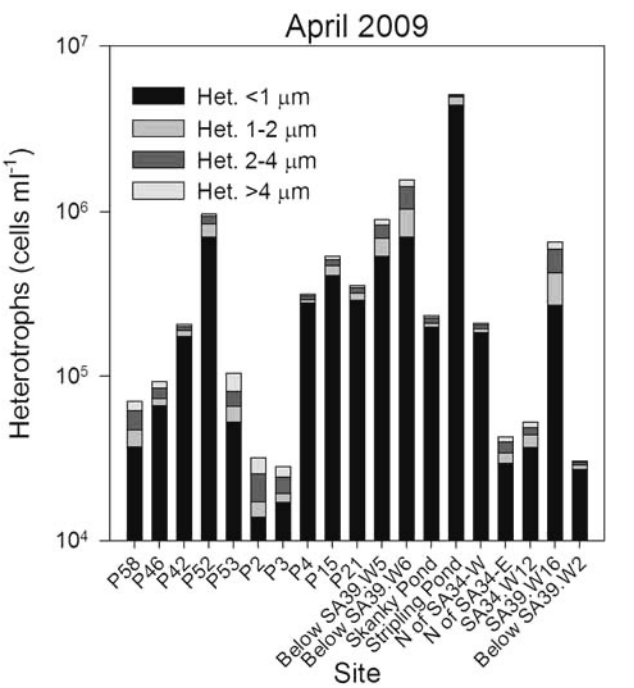

R
R
R
R
R
R
R
R
R
R
A
A
A
A
A
A
A
A
A
A
Legend
Sample Wetlands
Streams
Ichauway Boundary
Major Roads
N
W
E
S
0
1
2
4
6
8
Kilometers

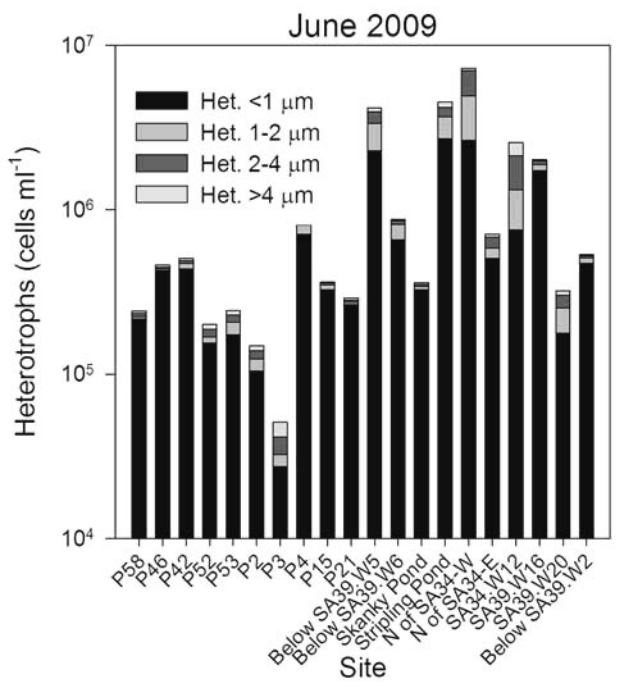

Rainfall Ichauway GEMN Station
Sampling Dates
Daily Total (mm)
160
140
120
100
80
60
40
20
0
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Jan
2009

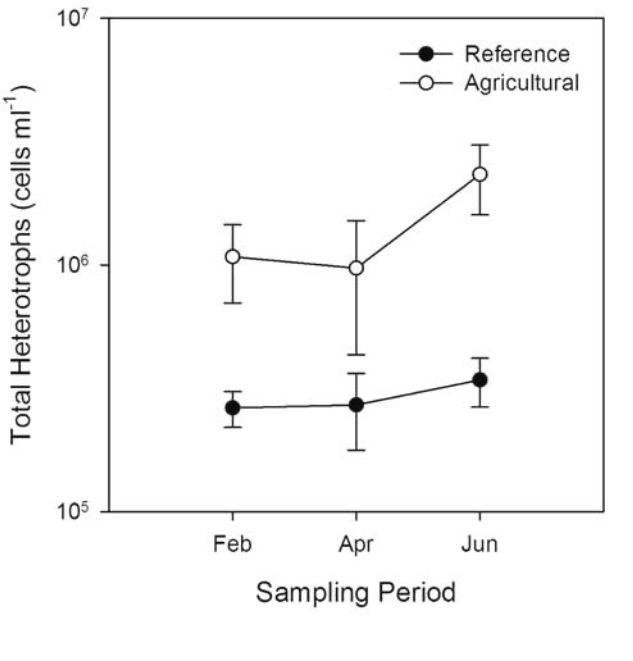

Reference
Agricultural
pH
Units
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
Feb
Apr
Jun
NO3-N
ug/L
300
200
175
150
125
100
75
50
25
0
Feb
Apr
Jun
Suspended Sediment
DM (mg/L)
2500
2000
300
200
100
0
Feb
Apr
Jun
NH4-N
ug/L
10000
9000
8000
500
400
300
200
100
0
Feb
Apr
Jun
DOC
mg/L
100
80
60
40
20
0
Feb
Apr
Jun
PO4-P
ug/L
500
400
300
200
150
100
50
0
Feb
Apr
Jun


Reference Wetlands
Agricultural Wetlands
February 2009
pH
TSS
NO3
PO4
Prin 1
Prin 2
Dissolved Carbon
April 2009
pH
TSS
IC
NO3
NH4
PO4
Prin 1
Prin 2
Dissolved Carbon
June 2009
pH
TSS
IC
PO4
Prin 1
Prin 2
Dissolved Carbon
AFDM
NH4


February 2009
Het. <1 μm
Het. 1-2 μm
Het. 2-4 μm
Het. >4 μm
Heterotrophs (cell ml-1)
10^7
10^6
10^5
10^4
P58
P46
P42
P52
P53
P2
P3
P4
P15
P21
Below SA39.W5
Below SA39.W6
Skanky Pond
Stripling Pond
N of SA34-W
N of SA34-E
SA34.W12
SA39.W16
SA39.W20
Site


April 2009
Het. <1 μm
Het. 1-2 μm
Het. 2-4 μm
Het. >4 μm
Heterotrophs (cells ml-1)
10^7
10^6
10^5
10^4
P58
P46
P42
P52
P53
P2
P3
P4
P15
P21
Below SA39.W5
Below SA39.W6
Skanky Pond
Stripling Pond
N of SA34-W
N of SA34-E
SA34.W12
SA39.W16
Below SA39.W2
Site


June 2009
Het. <1 μm
Het. 1-2 μm
Het. 2-4 μm
Het. >4 μm
Heterotrophs (cells ml-1)
10^7
10^6
10^5
10^4
P58
P46
P42
P52
P53
P2
P3
P4
P15
P21
Below SA39.W5
Below SA39.W6
Skanky Pond
Stripling Pond
N of SA34-W
N of SA34-E
SA34.W12
SA39.W16
SA39.W20
Below SA39.W2
Site


Reference
Agricultural
Total Heterotrophs (cells ml-1)
10^7
10^6
10^5
Feb
Apr
Jun
Sampling Period