JOURNAL

OF THE PENNSYLVANIA ACADEMY OF SCIENCE

Founded on April 18, 1924

ISSN: 1044-6753

August 1996
Volume 70, Number 1

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A Publication of
The Pennsylvania Academy of Science

GROUNDWATER-SURFACE WATER INTERACTION IN AN AGRICULTURAL WATERSHED[1]

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ABSTRACT

The effects of the interaction between soil characteristics and land use on ground and surface water quality was studied for 62 months, beginning in June 1988. Fourteen lysimeters located on different soil types and subjected to different land uses and 9 stream locations were sampled monthly for 9 different water quality parameters. Similar seasonal variations occurred among these parameters in the different lysimeters and the receiving stream. Fecal coliform and fecal strep concentrations were significantly higher in hydric Frenchtown soils in pastures than in the other soil types or land uses. There appears to be a migration of bacteria along the clay fragipan to the receiving stream. Fencing cattle out of the stream and restoring the riparian wetland reduced fecal coliform and phosphate, 30.2 and 60.3% respectively, below the wetland. Although agriculture had an adverse impact on water quality, these impacts can be mediated by maintaining and/or enhancing of riparian buffer zones.

[J PA Acad Sci 70(1):3-8, 1996]

INTRODUCTION

Over the last decade, agriculture has been documented as a major contributor to the degradation of surface and groundwater systems in the United States and elsewhere in the world (Brenner et al. 1991; Clark et al. 1985; Diebel et al. 1992; Hill 1987; Macharis 1985; Reay et al. 1992; Tim et al. 1992). Clark et al. (1985) estimated that non-point pollutants, primarily from agricultural runoff, account for 73% of the total biochemical oxygen demand, 83% of the bacterial loads, and 92% of the suspended sediments in waterways in the United States. Moreover, the EPA (1987) estimated that 75% of the lakes, 64% of the rivers, and 19 estuaries were impacted adversely by discharge from agricultural lands.

Likewise, groundwater discharges and wells have also been shown to be affected adversely by agricultural drainage (Brenner and Mondok 1995; Reay et al. 1992). In Mercer County, Pennsylvania for example, 22 or 32.2%, of the 68 residential wells sampled that were adjacent to agricultural areas contained fecal coliforms; in addition, nitrate was detected at concentrations of 1.0 mg/L or greater in some of these water supplies. Moreover, analysis of land use practices revealed that agriculture had a direct impact on nutrient and bacterial concentrations, both in overland flows and groundwater discharges of streams from 11 different watersheds in northwestern Pennsylvania (Brenner and Mondok 1995). Reay et al. (1992) found that bacteria and nutrient concentrations in surface waters were up to 20 times greater in areas where ground water discharges were directly linked to agricultural activities within the watershed. One objective of the current study was to investigate the impact of land use and soil characteristics on ground water quality and its relationship in Mercer County, Pennsylvania. A second objective was to determine the effects of riparian wetland restoration and stream fencing on water quality.

[1]Received for publication 20 March 1995; accepted 21 September 1995.

STUDY AREA

The study area was situated within the Munnell Run watershed in Mercer County, Pennsylvania. The entire drainage basin is located in the Glaciated Allegheny Plateau in northwestern Pennsylvania (Grice et al. 1971). All the soils are derived from glacial till and are poorly drained with seasonally perched water tables (Grice et al. 1971).

Dominant soils within the watershed are hydric Frenchtown and Wayland, along with Ravenna, which often has hydric Frenchtown inclusions (Figure 1). Frenchtown is a poorly drained hydric soil developed on thick deposits of firm glacial material. It is characterized by a low permeable fragipan at depths of 25-40 cm, with a water table within 15 cm of the surface for at least six months a year (Grice et al. 1971). Frenchtown is a major wetland soil within the Munnell Run watershed and other regions of Pennsylvania, which generally accumulates water from the surrounding soils. Wayland soils are poorly drained with a silt loam in the upper 45 cm underlined by a gravely sandy-loam. According to the Mercer County Soil Survey (Grice et al. 1971), this soil is classified as hydric, but the underlying sand and gravels result in a greater permeability than occurs in the clay-rich Frenchtown soil. Wayland soils occur along the flood plain of Munnell Run. Ravenna soils also tend to be poorly drained with a low permeable fragipan at depths between 30 and 45 cm. Ravenna soils typically have a seasonal water table within 15-45 cm of the surface. Although these soils are not classified as hydric, they often have hydric inclusions, especially when associated with Frenchtown soils adjacent to wetlands.

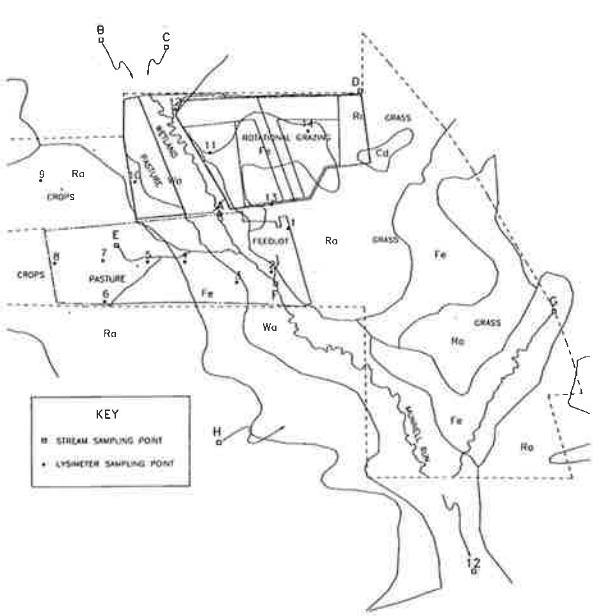

FIGURE 1. Location of lysimeters in relation to soil types, land use and Munnel Run in Mercer County, Pennsylvania. Lysimeter locations are number (1-14), and stream sampling stations re designed by letters (A-H).

The entire watershed is agricultural with pastures and row crops being the principal land use. All private homes within the watershed have on-lot (septic) sewage systems of varying ages and operational efficiencies. During the spring of 1992, cattle were fenced out of a portion of the stream and adjacent wetland areas and placed in a rotational grazing system. In the spring of 1992, this portion of the riparian zone was restored as a palustrine scrub and shrub wetland with the planting of 1000 black alder (*Alnus glutinosa*), also with a mixture of 15 other wetland shrubs, including silky (*Cornus amomum*) and red-osier (*C. stolonifera*) dogwoods and buttonbush (*Cephalanthus occidentalis*).

METHODS

This 62-month study was initiated in June 1988 with the installation of 14 lysimeters in the saturation zone of the three soil types occurring on the pastures and croplands within the watershed (Figure 1). Monthly samples were obtained from each lysimeter and at an additional nine stream locations. Samples were collected in 250 ml sterile polyethylene bottles, placed in an ice chest and returned to the laboratory for analysis of fecal coliforms, fecal strep, and seven chemical parameters (pH, alkalinity, calcium, magnesium, orthophosphate, ammonia, and conductivity). Fecal coliform and fecal strep concentrations were determined by multiple tube fermentation procedures and reported as most probable number (MPN/100 ml) (Greenberg et al. 1992). All bacteriological analyses were completed within two hours of sampling and all chemical parameters were analyzed in accordance with the 18th edition of Standard Methods for Water and Wastewater Analysis (Greenberg et al. 1992). Data were analyzed using a variety of statistical procedures as described by Neter and Wasserman (1974), and an alpha level of $P<0.05$ or less was accepted as significant.

RESULTS AND DISCUSSION

According to an analysis of variance (ANOVA) using a log transformation univariate analysis, seasonal variations in the chemical and biological characteristics occurred among the 14 lysimeters, as well as among the different soil types and land uses (Table 1), but there was not a significant interaction between either soil type or land use for any of the water quality parameters (Table 2). Significant seasonal variations occurred in pH among the 14 lysimeters and land uses ($P<0.001$), but did not occur among the different soil types ($P>0.12$). However, significant difference in $CaCO_3$ and conductivity, as well as in Ca and Mg concentrations, did occur among the different soil types. For example, samples obtained from Wayland soils had higher alkalinity, as well as calcium and magnesium, than either Frenchtown or Ravenna soils (Table 3).

The chemical characteristics of groundwater, as indicated by the differences among the different lysimeters, also varied according to land use (Tables 1 and 3). The pH and $CaCO_3$ alkalinity, Ca, Mg concentrations, along with conductivity, varied among the different land use categories ($P<0.001$) (Table 1), with these values being significantly lower in croplands than in either pastures or wetlands ($P<0.001$) (Table 3). This was also reflected in lower conductivity in croplands when compared to groundwater samples from other land use categories ($P<0.001$).

Alkalinity varied seasonally, with higher concentrations occurring during summer than in the other seasons of the year ($P<0.001$) (Table 3). Calcium was significantly higher during the spring (March, April, May) and fall (Sept., Oct., Nov.) than during other seasons of the year ($P<0.001$), while conductivity was lower during these months than during the summer (June, July, August) or winter (Dec., Jan., Feb.) ($P<0.001$). Magnesium was the only parameter that did not exhibit a significant seasonal variation ($P<0.296$) (Tables 1 and 3).

Ammonia varied significantly among lysimeters ($P<0.001$), as well as seasonally ($P<0.05$), but did not vary significantly among the different soil types ($P>0.614$) or land use categories ($P>0.193$). Phosphate concentrations were significantly higher during the summer months ($P<0.001$), while no significant differences occurred among the different lysimeters ($P>0.361$), soil types ($P>0.361$) or land use categories ($P>0.908$) (Table 1).

Based on a Bonferroni's Multiple Comparison Analysis, fecal coliform and fecal strep varied significantly among the different lysimeters ($P<0.001$). Fecal coliform concentrations also varied among the different soil types ($P<0.001$) with highest concentrations occurring in hydric Frenchtown soils followed by Wayland and Ravenna soils (Table 4). The greater concentrations of coliforms found in the Frenchtown soils was probably due to the poor drainage characteristics of this clay-based soil. The lower concentrations of fecal coliforms in Wayland flood plain soils may be due to the sandy-gravely loam subsoil, resulting in better drainage. Fecal

TABLE 1. Comparison of the effects of season, soil type, land use and among the different lysimeters using ANOVA.

Parameter	Mean Square	F value	P>F
Season (3 df)			
pH	4.511	13.58	0.0001
Total Alkalinity	1.283	14.41	0.0001
Conductivity	0.649	5.92	0.0006
Calcium	0.715	6.79	0.0002
Magnesium	0.029	0.23	0.8774
Phosphate	0.660	9.47	0.0001
Ammonia	0.026	2.10	0.0995
Fecal Coliform	23.177	22.31	0.0001
Fecal Strep.	1.312	17.20	0.0001
Soil Type (3 df)			
pH	0.640	1.93	0.1239
Total Alkalinity	1.804	20.27	0.0001
Conductivity	2.174	19.80	0.0001
Calcium	3.117	29.62	0.0001
Magnesium	0.939	7.34	0.0001
Phosphate	0.133	1.91	0.1267
Ammonia	0.006	0.55	0.6481
Fecal Coliform	2.387	2.30	0.0764
Fecal Strep	0.124	1.63	0.1813
Land Use (5 df)			
pH	1.275	3.92	0.0017
Total Alkalinity	0.659	7.08	0.0010
Conductivity	0.628	5.43	0.0010
Calcium	1.112	9.93	0.0001
Magnesium	0.679	5.27	0.0001
Phosphate	0.050	0.71	0.6182
Ammonia	0.019	1.51	0.1860
Fecal Coliform	3.293	3.16	0.0080
Fecal Strep	0.206	2.73	0.0189
Lysimeters (13 df)			
pH	1.901	6.15	0.0001
Total Alkalinity	2.423	49.39	0.0001
Conductivity	1.755	20.55	0.0001
Calcium	3.075	52.29	0.0001
Magnesium	0.753	6.20	0.0001
Phosphate	0.064	0.89	0.5621
Ammonia	0.026	2.17	0.0096
Fecal Coliform	5.724	5.97	0.0010
Fecal Strep	0.266	3.62	0.0001

TABLE 2. Comparison of the effects of season on water quality parameters in different soil types, land uses, and lysimeters.

	Mean Square	F value		
Parameter	Interaction	Error	Interaction	P>F
Soil Type (9 df)				
pH	0.060	0.332	0.19	0.9952
Total Alkalinity	0.095	0.049	1.07	0.3817
Conductivity	0.135	0.110	1.23	0.2710
Calcium	0.034	0.105	0.33	0.9660
Magnesium	0.133	0.128	1.05	0.4007
Phosphate	0.381	0.069	0.55	0.8409
Ammonia	0.021	0.012	1.64	0.1015
Fecal Coliform	1.587	1.039	1.53	0.1339
Fecal Strep	0.106	1.076	1.39	0.1898
Land Use (15 df)				
pH	0.284	0.326	0.87	0.5946
Total Alkalinity	0.061	0.093	0.66	0.8240
Conductivity	0.045	0.116	0.40	0.9803
Calcium	0.055	0.112	0.49	0.9470
Magnesium	0.082	0.129	0.64	0.8464
Phosphate	0.045	0.070	0.64	0.8409
Ammonia	0.009	0.013	0.72	0.7629
Fecal Coliform	0.795	1.043	0.76	0.7198
Fecal Strep	0.113	0.075	1.50	0.0987
Lysimeters (39 df)				
pH	0.162	0.309	0.52	0.9929
Total Alkalinity	0.038	0.049	0.78	0.8253
Conductivity	0.074	0.085	0.87	0.7035
Calcium	0.061	0.059	1.03	0.4179
Magnesium	0.091	0.121	0.75	0.8640
Phosphate	0.043	0.713	0.60	0.9737
Ammonia	0.012	0.012	0.99	0.4855
Fecal Coliform	0.836	0.959	0.87	0.6933
Fecal Strep	0.068	0.034	0.93	0.5991

coliform and fecal strep also exhibited significant seasonal variations with the highest concentrations occurring during the summer months ($P<0.001$). There was not, however, a significant interaction between season and soil characteristics for fecal coliforms ($P>0.13$), or between season and land use ($P>0.71$). Likewise, fecal strep concentrations did not vary among the different soil types ($P>0.18$), nor did they exhibit significant seasonal interaction with land use ($P>0.09$) or soil type ($P>0.18$) (Table 2).

In general, the chemical characteristics of Munnell Run parallel those of the lysimeters with seasonal variations in pH ($P<0.001$), alkalinity ($P<0.001$), and calcium ($P<0.001$) (Table 4). The lowest alkalinity ($\bar{X}=36.1\pm3.14$) and calcium values ($\bar{X}=70.7\pm9.61$) occurred at those stations being impacted by agriculture ($P<0.001$), compared to means of 48.4 ± 8.66 and 78.2 ± 6.94 for alkalinity and calcium, respectively, at non-agricultural stations. Likewise, those stations being impacted by agriculture also had mean conductivities of 188 ± 33.6 compared to 225 ± 32.8 occurring at non-agricultural stations (Table 5).

Phosphorus and ammonia concentrations varied seasonally in Munnell Run ($P<0.001$) with the highest concentrations occurring during the summer months ($P<0.001$) (Table 3). Based on a t-distribution analysis, the highest concentrations of both phosphorus ($\bar{X}$ 4.1 ± 2.0) and ammonia ($\bar{X}$ 0.92 ± 0.37) occurred at stations receiving drainage from on-lot sewage systems. However, phosphorus concentrations at stations impacted by agriculture ($\bar{X}$ 2.4 ± 0.19) were also significantly higher than non-agriculture stations ($\bar{X}$ 1.9 ± 0.05) ($P<0.001$). There was no significant difference in ammonia concentrations among the different stations (Table 5).

Fecal coliform concentrations were generally higher in the stream than in samples obtained from the lysimeters, but both exhibited seasonal variations with the highest concentrations occurring during the summer months. Fecal coliforms averaged 474/100 ml in the three lysimeters located in the flood plain, significantly lower ($P<0.01$) than the mean of 1600/100 ml occurring in the adjacent stream. Land use, including drainage from adjacent pastures and on-lot sewage systems, also significantly impacted fecal coliform and fecal strep concentrations in Munnell Run. Both fecal coliforms ($\bar{X}$ 1840 ± 98.8) and fecal strep ($\bar{X}$ 987 ± 126.3) were

TABLE 3. Mean (SE) Chemical Characteristics of Water Samples Collected from 14 Lysimeters in Different Soil Types and Land Use categories Over a 62 month period.

Lysimeter	Soil Type	Land Use	pH	$CaCO_3$ mg/L	Uohms/ cm	Ca mg/l	Mg mg/l
1	Ra	Wet	7.32	124.4	394	183.8	93.8
N=60		Pasture	(0.80)	(13.0)	(14.3)	(12.7)	(11.6)
2	Wa	Wet	7.04	86.0	480	172.7	38.0
N=61		Pasture	(0.07)	(8.5)	(23.6)	(9.9)	(9.3)
3	Fe	Wet	6.68	22.5	213	33.2	65.2
N=37		Pasture	(0.11)	(2.7)	(36.3)	(4.2)	(7.0)
4	Fe	Wet	6.79	26.0	140	35.0	57.5
N=37		Pasture	(0.08)	(2.2)	(25.5)	(3.5)	(7.1)
5	Ra	Wet	7.16	62.0	227	81.1	53.6
N=52		Pasture	(0.06)	(5.2)	(28.3)	(6.0)	(4.3)
6	Ra	Wet	6.89	24.4	117	37.5	47.7
N=62		Pasture	(0.08)	(1.9)	(43.4)	(3.6)	(4.4)
7	Ra	Wet	6.58	23.5	157	42.7	45.8
N=62		Pasture	(0.11)	(4.8)	(52.3)	(5.6)	(5.5)
8	Ra	Below	6.58	18.6	105	24.4	41.6
N=40		Cropland	(0.11)	(1.2)	(85.3)	(5.1)	(6.8)
9	Ra	Cropland	6.84	29.3	118	43.6	46.0
N=23			(0.12)	(2.8)	(63.3)	(4.7)	(7.8)
10	Ra	Rotational	6.83	38.4	195	59.5	42.3
N=59		Pasture	(0.07)	(3.5)	(27.9)	(4.3)	(3.8)
11	Fe	Rotational	6.99	62.5	251	86.7	59.0
N=59		Pasture	(0.06)	(4.8)	(27.6)	(7.1)	(5.0)
12	Wa	Restored	6.90	35.5	215	63.8	43.9
N=62		Wetland	(0.06)	(3.1)	(27.4)	(5.0)	(5.2)
13	Fe	Pasture	6.93	39.0	184	57.5	43.2
N=60			(0.06)	(2.7)	(34.7)	(7.9)	(7.9)
14	Fe	Pasture	6.96	110.0	197	63.6	49.8
N=61			(0.07)	(2.8)	(28.9)	(3.2)	(4.3)

Fe - Frenchtown
Ra - Revenna
Wa - Wayland

TABLE 4. Mean (SE) Bacteriological and Nutrient Characteristics of Water Samples Collected from 14 Lysimeters Located in Different Soil Types and Land Uses Over a 62 month period.

Lysimeter	N	Soil Type	Land Use	Fecal Coliform/ 100ml	Fecal Strep/ 100ml	PO_4 mg/l	NH_3-N mg/l
1	60	Ra	Wet	472	80	3.0	0.27
			Pasture	(14)	(13)	(0.27)	(0.12)
2	61	Wa	Wet	184	57	1.9	0.19
			Pasture	(9)	(3)	(0.04)	(0.06)
3	37	Fe	Wet	896	186	2.8	0.83
			Pasture	(28)	(20)	(0.16)	(0.05)
4	52	Fe	Wet	681	106	1.6	0.20
			Pasture	(19)	(15)	(0.06)	(0.01)
5	62	Ra	Wet	333	77	2.1	0.10
			Pasture	(12)	(15)	(0.06)	(0.01)
6	62	Ra	Wet	590	147	2.0	0.04
			Pasture	(16)	(20)	(0.05)	(0.01)
7	40	Ra	Wet	678	122	2.0	0.21
			Pasture	(25)	(11)	(0.20)	(0.02)
8	37	Ra	Below	684	129	0.2	0.27
			Cropland	(28)	(19)	(0.01)	(0.01)
9	23	Ra	Cropland	197	75	0.06	0.06
				(27)	(22)	(0.01)	(0.01)
10	59	Fe	Rotational	824	140	1.5	0.08
			Pasture	(18)	(18)	(0.02)	(0.01)
11	59	Wa	Rotational	757	178	1.8	0.06
			Pasture	(16)	(22)	(0.10)	(0.01)
12	62	Wa	Restored	481	126	1.9	0.12
			Wetland	(13)	(20)	(0.10)	(0.01)
13	60	Fe	Cropland	406	141	1.8	0.09
				(14)	(28)	(0.01)	(0.01)
14	61	Fe	Cropland	536	172	2.4	0.46
				(15)	(34)	(0.10)	(0.04)

Fe - Frenchtown
Ra - Revenna
Wa - Wayland

significantly higher at those stations receiving septic drainage, as well as those receiving runoff from agricultural lands (fecal coliform $\bar{X}$ 1302 ± 84.4, fecal strep $\bar{X}$ 497 ± 143.3) than in non-agricultural areas (fecal coliform $\bar{X}$ 541 ± 79.1, fecal strep $\bar{X}$ 137 ± 38.3) ($P<0.001$). It appears, therefore, that the fecal coliform and fecal strep concentrations in Munnell Run are a result of agricultural runoff in conjunction with septic drainage into two tributary streams. An additional source of fecal coliforms and fecal strep in Munnell Run may be from groundwater discharges from the surrounding hydric soils. There also appears to be a possible movement of fecal bacteria between the non-hydric Ravenna ($\bar{X}$ 551/100 ml) and the hydric Frenchtown ($\bar{X}\pm669$/100 ml) soils. Frenchtown soils accumulate water from the surrounding Ravenna so that fecal coliforms could be transported laterally along the clay fragipan, acting as a conduit into the receiving stream (Figure 2). It is possible that nutrients and other chemicals may travel into streams via this same pathway.

Fencing cattle out of the stream and the restoration of a riparian wetland had a significant impact on water quality. There as a 30.2 percent reduction in fecal coliforms and a 60.3 percent reduction in phosphorus directly below the restored wetland during the first 13 months (Table 6). Moreover, there were a 21.9% and 44.2% reduction in these parameters at stream sampling stations located 1-3 km downstream from the restored wetland, respectively (Table 6).

The results obtained from this study indicate that

TABLE 5. Mean (SE) Seasonal variation between Lysimeters and the Receiving Stream.

	Spring N=196		Summer N=162		Fall N=169		Winter N=193	
	Lysimeter	Stream	Lysimeter	Stream	Lysimeter	Stream	Lysimeter	Stream
pH	7.08	7.46	6.89	7.30	7.52	7.52	6.71	7.21
	(0.04)	(0.03)	(0.05)	(0.04)	(0.03)	(0.04)	(0.04)	(0.04)
$CaCO_3$	41.2	35.9	62.7	54.7	59.9	49.9	33.5	30.7
mg/l	(6.2)	(12.6)	(4.6)	(2.7)	(5.2)	(3.2)	(1.9)	(1.4)
uohoms/	159	390	414	232	232	238	169	184
cm	(26.6)	(15.8)	(12.7)	(22.0)	(17.5)	(74.9)	(27.0)	(4.9)
Ca	57.0	55.0	60.0	71.7	95.0	85.9	67.7	57.7
mg/l	(9.7)	(2.7)	(12.4)	(3.0)	(4.2)	(4.0)	(4.6)	(2.4)
Mg	51.9	52.9	42.0	49.6	66.9	59.9	60.4	59.9
mg/l	(12.5)	(3.1)	(12.3)	(2.6)	(3.9)	(2.3)	(4.2)	(3.7)
PO_4	2.6	2.4	2.7	3.6	1.5	2.4	1.5	1.8
mg/l	(0.32)	(0.21)	(0.31)	(0.49)	(0.14)	(0.37)	(0.21)	(0.17)
NH_3-N	0.29	0.16	0.19	0.82	0.21	0.11	0.09	0.16
mg/l	(0.09)	(0.07)	(0.09)	(0.25)	(0.13)	(0.03)	(0.01)	(0.02)
Fecal Coliform/	229	851	932	875	617	1054	458	983
100ml	(44.0)	(75.4)	(77.0)	(10.6)	(70.0)	(89.9)	(59.0)	(87.3)
Fecal Strep/	77	281	233	584	118	217	77	604
100ml	(8.0)	(3.1)	(24.0)	(14.9)	(12.7)	(15.1)	(9.0)	(36.6)

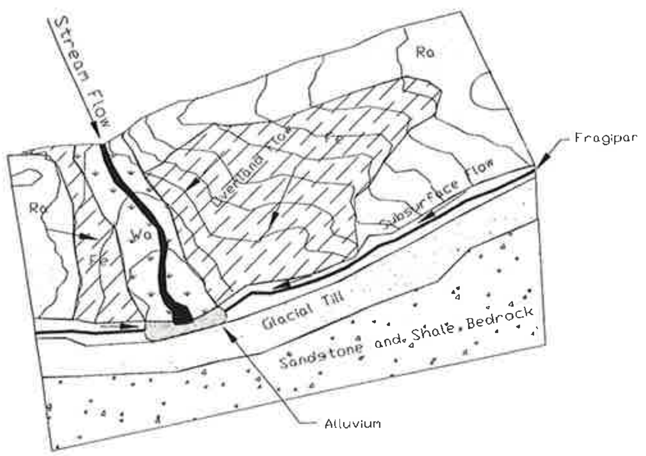

FIGURE 2. Model of the possible pathway of bacteria migration in different soil types found in the Munnell Run Watershed, Mercer County, Pennsylvania.

TABLE 6. Comparison of Stream Water Quality Parameters in Agricultural and Non-Agricultural Areas and the Effect of Fencing and Wetland Restoration on Water Quality.

	AGRICULTURE		Non Agriculture		SEPTIC Drainage		BELOW RESTORED WETLAND Before		After		3KM DOWNSTREAM Before		After	
No. Stations	4		3		2		1		1		3		3	
No. Samples	268		199		134		58		13		161		39	
Parameter	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
pH	7.49	0.03	7.34	0.15	7.32	0.09	7.42	0.08	7.62	0.09	7.40	0.04	7.57	0.05
Alkalinity mg/l	36.10	3.10	48.10	2.40	32.40	2.20	32.40	2.20	27.70	2.00	39.60	2.20	32.40	1.80
Conductivity uohmns	181.00	29.70	225.00	30.40	182.00	31.40	195.80	30.10	196.00	18.30	196.00	18.30	214.00	22.60
Calcium mg/l	70.40	9.60	78.20	6.90	65.80	3.60	65.80	3.60	29.80	1.70	72.50	3.10	33.40	1.60
Magnesium mg/l	60.10	18.10	58.60	2.50	47.10	2.50	72.40	6.50	25.10	3.10	68.50	3.70	25.90	2.10
Phosphate mg/l	2.38	0.19	4.08	2.00	4.08	2.00	2.26	0.14	1.04	0.29	2.74	0.24	1.53	0.53
Ammonium mg/l	0.18	0.02	0.28	0.05	0.92	0.37	0.12	0.04	0.07	0.04	0.19	0.06	0.09	0.03
Fecal Coliform /100ml	1302.00	84.40	542.00	79.10	1840.00	98.80	1432.00	137.00	1000.00	279.80	1340.00	78.20	1047.00	151.70
Fecal Strep /100ml	479.00	143.40	137.00	38.20	987.00	126.30	267.00	26.00	310.00	71.10	329.00	25.30	329.00	33.10

extensive pasturing of cattle on hydric soils has an adverse impact on the water quality of surface and sub-surface drainages, as well as on the receiving stream. However, fencing cattle out of the stream, establishing rotational grazing systems, and the restoration of riparian wetlands ameliorates adverse impacts on water quality. Additional fencing and wetland restoration is being planned within the Munnell Run Watershed and studies will continue to determine the effectiveness of these projects.

ACKNOWLEDGEMENTS

This study was supported by funding provided by the USGS Annual Allotment to the Environmental Research Institute, Pennsylvania State University and the Mercer County Conservation District.

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Journal of the Pennsylvania Academy of Science 70(1):9-14, 1996

ANALYSIS OF THE WOODY VEGETATION AND AVIFAUNA OF THE REIMERT MEMORIAL BIRD HAVEN[1]

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ABSTRACT

Woody vegetation and avifauna were studied at the Reimert Memorial Bird Haven - a 4.04 ha preserve located in Lower Macungie Township, PA and owned by the Wildlands Conservancy. The study was conducted to assemble baseline data for future research in order to promote the site's secondary function as an environmental education center.

Trees greater than 2cm dbh were evaluated on ten randomly selected 100m² sites throughout the sanctuary. Within each of the ten sites 16m² quadrats were randomly marked off and the woody shrubs species were identified and tallied.

Analysis determined that the vegetation of the Reimert Sanctuary is somewhat diverse. The dominant tree species were mixed oaks (black, red and scarlet), sweet birch and tulip-tree. While the Tulip-tree is the most valuable species on the property, the mixed oaks provide a majority of the cover. The understory is comprised predominantly of spicebush and red-panicled dogwood.

A winter bird survey determined that mostly passerines and woodpeckers were either residing at or visiting the sanctuary.
[J PA Acad Sci 70(1):9-14, 1996]

INTRODUCTION

The Reimert Memorial Bird Haven (RMBH) is a tract of land approximately 4.04 hectares. This property is owned by the Wildlands Conservancy and has served primarily as a bird sanctuary. Future plans lean toward developing an environmental educational facility on that site. Our analysis of the woody vegetation and avifaunal composition provides baseline data for the Conservancy to develop a management program to enhance the site's functions.

The bird sanctuary can be characterized as being an interior of a fragmented forest. Fragmented forests are the remains of natural forests and mid-successional woodlots that are fragmented into progressively smaller patches of woodlots isolated by plantations, agriculture, industrial and urban development (Harris, 1984). Considerable research has concentrated on fragmented forest dynamics with a view toward predicting the species composition of selected flora and fauna. Wilcox and Murphy (1983) discussed the effects of fragmentation on extinction as a "conservation strategy" for extinction that promotes habitat loss and insularization contributing to a decline in biological diversity. The species diversity of biological communities balances regional processes of species formation and geographic dispersal that add species to communities, against local processes of predation, competitive exclusion and other factors which may promote local extinction of thousands of species as a result of pollution and habitat destruction (Ricklefs, 1987).

An analysis of the woody vegetation at RMBH reveals a woodland that is approximately 80 years old with a composition to be that of mixed oak, white ash and tulip-tree. Using standardized random sampling methods, the present day secondary growth canopy species are mixed oaks (black, red and scarlet), white ash, and tulip-tree. This type of forest occurs at elevations between 150-1500 meters from southern New York and southwestern New England through the Blue Mountain Ridge, the Ridge and Valley provinces, and throughout the Cumberland and the Allegheny mountains and plateaus (Braun, 1950). In this region mixed oaks, sweet birch and tulip-trees are characteristic of mountain coves ("cove hardwoods") and are more extensive on moist north and east facing slopes and on well drained flats. These species require ample soil depth, moisture and loose soil types. The tulip-trees are much less tolerant of shade than the other species identified.

[1] Submitted for publication consideration 18 June 1995; accepted 5 October 1995.

This long-term subclimax type will eventually be replaced by an upland oak climax providing there is no major stand disturbance (Eyre, 1980). Secondary groves of tulip-tree dot many of the more mesic disturbed slopes where mixed mesophytic forest formerly prevailed (Braun, 1950).

The Audubon Winter-Bird Population study was employed in determining species present on the property. The avifauna analysis enabled us to construct a list of birds inhabiting the sanctuary during the late winter through mid-April. A total of 17 species of migrant and non-migrant birds were counted, eight species of which are considered either neotropical or short distance migrants. According to Cody (1981), the vegetation structure of habitat is a good predictor of avifaunal diversity; canopy height cover distinguishes habitats for woodland birds. Studies have shown that species numbers increase with patch size and species have different threshold levels of habitat area, below which they occur only sporadically and above which they occur regularly (Galli, 1976). Species diversity is reduced in more isolated habitats because of higher extinction rates and thresholds (Cody, 1985). Whitcomb, et al. (1974) found that different avifaunal assemblages occurred in small isolated forests in comparison with extensive forests; these differences were characteristic of deciduous forests throughout eastern North America. These studies also suggest that isolation, as well as size, is an important determinant of avifaunal composition.

The purpose of this study was to determine the composition and diversity of this tract of land and supply a baseline survey of resident and non-resident bird species (for a narrow seasonal period) that may inhabit or utilize the sanctuary.

STUDY AREA

The study was conducted in Lower Macungie Township, Lehigh County (approximately 1.61 km southwest of Macungie, off Mountain Road). This area can be described as a mosaic of closely adjoining forests. The study site (RMBH) of 4.04 ha is well contained within the forest fragment. Elevation at the study site was determined to be between 550 and 750 feet (167.6 and 228.8 m), slope was calculated between 10° and 20° with a north-northwest aspect.

Soil types are in the Fleetwood-Chester Very Stony Association. The Chester series is made up of deep soils that are well drained and silty (USDA, 1963). The Fleetwood series are moderately deep to deep, well drained, unglaciated, gravely and stony soils (Soil Survey, Lehigh County).

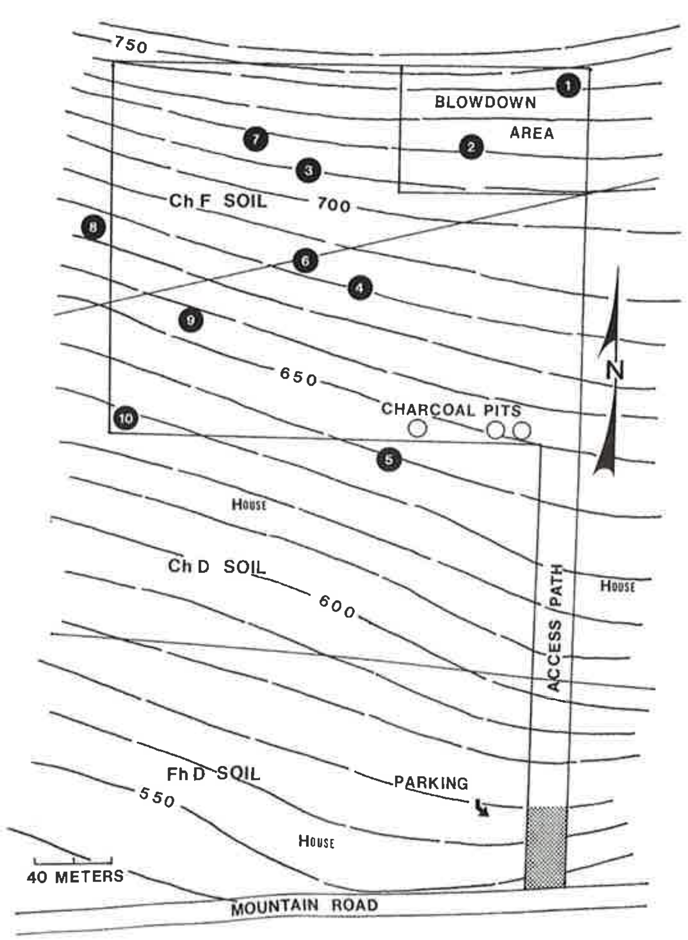
The forest has been moderately disturbed by construction on the perimeters and by winds and storms. A heavy blowdown area consisting of uprooted trees is present in a 1.46 ha quadrant located in the northeast corner. Three of the four sides of RMBH are surrounded by forest, where the fourth is bordered by road and field.

Three charcoal pits are found along the southern boundary of the property line. The presence of oak species, which are fire tolerant, are often associated with recurring fire relating to logging and burning, land clearing, chestnut blight in the mid-Atlantic region and the burning associated with the charcoal iron industry (Abrams, 1992). These pits may be remnants of the charcoal industry.

METHODS AND MATERIALS

Woody Vegetation

Ten 100m² quadrats, measuring 10m by 10m were randomly established (McCann and Brewer, 1982) throughout the study site (see map). All trees greater than 2cm diameter breast height (dbh) were counted and identified, while within each of these quadrats, a 16m² quadrat was established for analysis of all woody shrubs and vines. When the cumulative number of qudrats exceeded eight, no new species were found indicating that the number of quadrats researched were representative of the study site (Brewer, 1982). The slope of the property was measured using an inclinometer. Triangulation methods were employed to determine tree height (Myers, 1980).



MAP. Diagram of the Reimert Memorial Bird Haven depicting the soil types and quadrat locations.

A list of the herbaceous vegetation was established in order to determine the species present.

Avifauna

A winter-bird study using the Audubon Winter-Bird Population Study method was conducted in order to develop a list of birds that may inhabit the study site during the mid-winter through early spring (January-April, 1993) months. Identifications were made on the basis of field marks and calls.

RESULTS

Seventeen species of trees were recorded. The most important species at the sanctuary are the tulip-tree, black oak and the white ash, though only tulip-tree and white ash are present to any great extent (Table 1). The relative dominance calculations determined that the sanctuary is a mixed oak (black, red, scarlet and chestnut), sweet birch, tulip-tree and white ash forest. The importance values derived provide information about aerial coverage by conveying how much crown cover a tree will occupy in a forest (Oplinger, 1977). The diversity indices calculated for each quadrat (Table 2) fell within 0 (no diversity) and 2.646 (fairly diverse) with a mean diversity index of 1.69.

A stand table was constructed to show the number of trees per hectare for a particular diameter class and species, thus describing the composition of the forest by species and size (Table 3).

Nine species of woody shrubs were identified and the results are presented in Table 4. *Cornus racemosa* was the most common species in the understory, while spicebush and blackberry were of secondary importance.

A list of herbaceous vegetation was compiled during the limited time frame of September through January 1992-93 and is presented in Table 5.

The presence of 17 species of birds was documented of which eight were neo-tropical or short distance migrants (Table 6).

DISCUSSION

All life forms have some value, economic or ecological, realized or potential, and by managing for diversity we manage for all life forms. According to the Simpson Diversity Index (maximum value = 1.0) the woody vegetation of the Reimert Memorial Bird Haven has a value of 0.842 implying the sanctuary is quite diverse. The Shannon Diversity index is 3.231 compared to a D-Max of 4.087, the most diversity possible for this site given the number of species present. The species diversity as determined by Monk (1967) for northeastern Pennsylvania is approximately 2.34. Therefore, the RMBH is more diverse than typical forests of the region if size is not a consideration. Naturally diversity increases as habitat size increases.

The dominant tree species, as determined by importance values, were found to be the tulip-tree (*Liriodendron tulipifera*), the oaks (*Quercus velutina, Q. Rubra, Q. Coccinea, Q. Pinus*) and white ash (*Fraxinus*

TABLE 1. Quantitative Values of Trees - RMBH.

Species	Importance Value	Frequency	Relative Frequency	Density	Relative Density	Basal Area	Number	Relative Dominance
Liriodendron tulipifera	113.90	0.9	21.95	0.026	30.20	0.0783	26	10.30
Quercus velutina	37.18	0.2	4.87	0.002	2.32	0.2517	2	33.25
Faxinus americana	28.68	0.5	12.19	0.021	24.40	0.0034	21	0.45
Quercus rubra	21.20	0.2	4.87	0.004	4.65	0.1156	4	15.27
Quercus coccinea	17.83	0.2	4.87	0.002	2.32	0.1055	2	13.90
Betula lenta	16.77	0.2	4.87	0.004	4.65	0.0798	4	10.54
Quercus prinus	15.55	0.2	4.87	0.002	2.32	0.0880	2	11.62
Acer saccharinum	13.80	0.2	4.87	0.003	3.49	0.0066	3	8.72
Carya cordiformis	8.96	0.4	9.76	0.004	4.65	0.0083	4	1.09
Crataequs	6.43	0.1	2.44	0.004	4.65	0.0077	4	1.02
Carya ovata	6.11	0.3	7.32	0.003	3.49	0.0016	3	0.21
*Vt**	5.48	0.1	2.44	0.004	4.65	0.0005	4	0.07
Fagus grandifolia	5.45	0.2	4.87	0.003	3.49	0.0028	3	0.37
*Pt**	2.50	0.1	2.44	0.001	1.16	0.0040	1	0.53
Cornus florida	2.23	0.1	2.44	0.001	1.16	0.0020	1	0.26
Castanea dentata	2.06	0.1	2.44	0.001	1.16	0.0007	1	0.09
Sassafras albidum	2.04	0.1	2.44	0.001	1.16	0.0005	1	0.07

*Denotes unidentified species.

TABLE 2. Diversity Indices of the Trees of The Reimert Memorial Bird Haven.

#Taxa	17.0
#Individuals	86.0
Simpson Diversity	0.842
Simpson Dominance	0.158
Shannon Diversity*	3.231
D Max*	4.087

* = logs to base 2

TABLE 3. Stand Table Depicting Numbers of Tree Species per Hectare According to Size Class.

Species	Small (2.0-2.99cm)	Medium (3.0-12.9cm)	Large (>13.0cm)
Tulip-tree	20	80	160
White ash	110	80	20
Red oak	0	0	40
Sweet birch	0	10	30
Bitternut hickory	10	20	10
Hawthorn	0	30	10
Vt*	40	0	0
Silver maple	0	20	10
Shagbark hickory	0	20	10
Beech	0	30	0
Black oak	0	0	20
Scarlet oak	0	0	20
American chestnut	0	10	10
Flowering dogwood	10	10	0
Chestnut oak	0	0	10
Sassafras	10	0	0
Pt*	0	10	0

*Denotes unidentified species.
Stand table formula: # trees/total area sampled x 10,000.
1 ha = 10,000 m^2

americana). According to the average importance values IV the tulip-tree is of most value even though the oaks are providing most of the cover. The maximum importance value attainable is 300. The tulip-tree importance value on the study site was 113.9, with the mixed oaks accounting for a value of 104.9. The white ash was second in terms of frequency and relative density, yet had a lower IV (28.68) due to the very low relative dominance. The low dominance values were due to the small basal area of the sampled trees. The combined values (oaks and tulip-tree) account for over half of the maximum index value, therefore stressing the ecological importance of these species to the site. Tulip-trees are a shade intolerant species which explains that while they are common within the study site they are not a dominant species due to the shade provided by the oaks. According to Hunter (1990), the role of different tree species in forest succession is the basis for an important distinction that foresters make between shade tolerant and shade intolerant trees. Shade intolerant trees are easily dispersed species that soon invade a disturbed site and grow rapidly, forming the first forest community in the successional sequence. Because they cannot reproduce in their own shade, they do not persist on a site and are gradually overtaken and replaced by slow growing shade tolerant trees (oaks). This would appear to be the case for the tulip-tree and sweet birch community at the sanctuary. Oaks have the ability to play the role of "takeover" artists in the forest. They spend years growing beneath the tulip-tree and other sunloving trees and, when the overlapping canopy finally dies, oaks are well established and ready to dominate (Benyus, 1989).

The blowdown area, in the northeastern corner which encompasses 1.46 ha., creates an opening that may allow the shade intolerant species to thrive (tulip-tree and birch). This area may contribute an enormous amount of nutrients to the vegetation community. Logs are major sites for tree regeneration because these are particularly good sites for forming mutualistic relationships with fungi. The primary importance of logs may lie beyond the welfare of the individual species and involve a major ecosystem process of nutrient recycling on which all species ultimately depend (Brewer, 1982).

TABLE 4. Quantitative Analysis of Woody Shrubs and Vines of the Reimert Memorial Bird Haven.

Species	Freq.	R.F.	Dens.	R.D.	No.
Red-Panicle Dogwood (*Cornus racemosa*)	0.6	22.2	0.487	53.06	78
Spice Bush (*Lindera benzoin*)	0.6	22.2	0.156	17.00	25
Blackberry (*Rubus occidentalis*)	0.4	14.8	0.081	8.84	13
Multiflora Rose (*Rosa multiflora*)	0.3	11.1	0.037	4.08	6
Virginia Creeper (*Parthenocissus quinquefolia*)	0.1	3.7	0.006	0.68	1
Unidentified species					
R	0.1	3.7	0.031	3.40	5
W	0.3	11.1	0.037	4.08	6
X	0.2	7.4	0.050	5.44	8
Z	0.1	3.7	0.031	3.40	5

TABLE 5. List of Herbaceous Species of the Reimert Memorial Bird Haven, Observed September 1992-January 1993.

Species (Common name)	(Scientific name)
Round-lobed Hepatica	*Hepatica americana*
Spleenwort	*Asplenium*
Lady Fern	*Athyrium filix-femina*
Maidenhair Fern	*Adiantum pedatum*
Mayapple	*Podophyllum peltatum*
Bloodroot	*Sanguinaria canadensis*

TABLE 6. List of Resident and Non-Resident Bird Species of the Reimert Memorial Bird Haven. (Jan.-Apr. 1993).

Species (Common name)	(Scientific name)
PERMANENT RESIDENTS	
White-Breasted nuthatch	*Sitta carolinensis*
Red-Bellied woodpecker	*Centurus carolinus*
Downy woodpecker	*Dendrocopus pubescens*
Hairy woodpecker	*Dendrocopus villosus*
Black-capped chickadee	*Parus atricapillus*
Tufted titmouse	*Parus bicolor*
Northern cardinal	*Cardinalis cardinalis*
House finch	*Carpodacus mexicanus*
Blue jay	*Cyanocitta cristata*
SHORT DISTANCE MIGRANTS	
Dark-eyed junco	*Junco hyemalis*
American robin	*Turdus migratorius*
Song sparrow	*Melospiva melodia*
Mourning dove	*Zenaida macroura*
American goldfinch	*Spinus tristis*
Yellow-shafted northern flicker	*Colaptes auratus*
Purple finch	*Carpodacus purpureus*
NEOTROPICAL MIGRANT	
Hermit thrush	*Hylocichla guttata*

The soils are of the Fleetwood-Chester Very Stony Association (USDA Soil Survey, 1963). This association is well suited for the species of trees found at the site such as tulip-tree, white ash and the various oaks. These soils developed from weathered quartzite, granitic gneiss and gneiss. Chester F and D soil types dominate the tract of land studied with Chester F found predominantly in the blowdown area. This type of soil is stony and deep in most locations but shallow over bedrock. This may explain the heavy destruction of trees in the blowdown region as the root systems are unable to establish secure deep anchorage thus making them more vulnerable to high winds.

The understory cover is comprised of associates expected of a mixed oak-birch-tulip-tree forest (Eyre, 1980). Spicebush (*Lindera benzoin*) was codominant with the red-panicle dogwood (*Cornus racemosa*) which are primarily found on the moist soils of this type of woodland. The blowdown area provides a good opportunity for growth of the woody shrubs as the opening provided allows for better colonization by these species.

The winter bird survey provides a baseline list of mostly passerines residing at or visiting the sanctuary. According to a study conducted by Galli, Leck and Forman (1976), forest size has a significant effect on bird species. Insectivorous species are mostly small tract dependent while many other specialized feeders are size-independent. White-breasted nuthatches commonly found in leafy trees in the east were identified as overwintering residents. They are considered a size-dependent species. Snags and logs have attracted woodpeckers. Northern flicker, red-bellied, downy and hairy woodpeckers were all counted as size-dependent residents. This particular site affords these birds numerous cavities for shelter and nesting sites. These species of birds prefer oaks and ashes for their choice of foraging sites (Wilson, 1970). Tufted tit-mice were heard throughout the site as they are quite common in deciduous forests and are known to flock with nuthatches, small woodpeckers and chickadees. Titmice are size-independent. Black-capped chickadees, a size-dependent species, were noted frequently. Northern cardinals, usually considered an edge species, were seen frequently and are a size-dependent species. Dark-eyed juncos, which are found in a wide variety of habitats, were observed as were American robins which are usually found in moist woodlands and are considered a size-independent species. Song sparrows, which are normally found in brush areas were seen and heard as they inhabited the understory cover provided by the woody shrubs. They too, are size-independent.

Neotropical migrants and short distance migrants were recorded because the surveying period overlapped to include the early spring migrants that use the sanctuary.

In summary, the RMBH forest is diverse. This study provides a baseline from which other more intensive research could evolve leading to future recommendations of management policies designed for the Reimert Memorial Bird Haven.

ACKNOWLEDGEMENTS

We thank Dr. Barbara Benson for her guidance in the bird study and the Wildlands Consevancy for access to the study site.

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Journal of the Pennsylvania Academy of Science 70(1):15-21, 1996

THE FINE STRUCTURE AND SELECTED CYTOCHEMISTRY OF UNGERMINATED BASIDIOSPORES OF PLUTEUS[1,2]

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ABSTRACT

The fine structure of the basidiospores of *Pluteus cervinus* is described using TEM and light microscopy. The basidiospore wall is composed of two distinct and continuous layers. No surface ornamentation or germ pore is present. The protoplasm is surrounded by a typical membrane which lacks distinct invaginations. Basidiospores contain much stored lipid which is centrally located. Basidiospores are uninucleate with the nucleus closely appressed to the cell membrane located at the base of the basidiospore near the hilar appendage. Mitochondria are present with few, well-delineated plate-like cristae. Endoplasmic reticulum is scant. Ribosomes occur regularly attached to the ER and outer mitochondrial membrane and are also densely packed throughout the cytoplasm. Variously sized, single membrane-bound vacuoles containing an electron dense material are present and exhibit acid phosphatase activity. Assays of malate synthase, a marker enzyme of the glyoxylate cycle, are positive, suggesting that microbody-like organelles are probably glyoxysomes.

[J PA Acad Sci 70(1):15-21, 1996]

[1]Submitted for publication consideration 1 April 1995; accepted 28 October 1995.

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INTRODUCTION

Taxonomic description becomes more useful when based on exact anatomical observation and when homologies become more evident (McLaughlin 1977; Singer 1986; Miller 1988). Detailed morphological studies of the ultrastructure of basidiospores, the dynamics of basidiospore germination, and mitosis and meiosis are especially important as correlatives to traditional taxonomic methods. The basidiospore has become one of the most important characters in the taxonomy of the Agaricales (Singer 1986). In recent years, the importance of the fine structure of the basidiospore has increased as an aid to classification of the agarics.

Despite the growing importance of the fine structure of spores in fungal taxonomy, some difficulties have been encountered concerning their preparation for examination. Ultrastructural studies of mature basidiospores have been quite difficult due primarily to the fact that dormant basidiospores with complex or thick walls are impermeable to embedding material (Bracker 1967; Stock and Hess 1970; Hess 1973; Greuter and Rast 1975; Gardner et al. 1975; Hawker and Madelin 1976; Alexopoulos and Mims 1979; Arita 1979; Ruch and North 1988). Consequently, many of the early studies concerning the ultrastructure of basidiospores were based on photomicrographs which had poor definition and lacked much fine internal detail. These difficulties have been overcome by using small diameter glass beads to fracture the wall of spores previously fixed with glutaraldehyde, thus allowing embedding resins to penetrate the spore (Olson and Eden 1977; Olson 1978; Ruch and Motta 1987). As a result several studies on fine structure and cytochemical aspects of ungerminated basidiospores have been published (Ruch and Motta 1987). The present investigation was undertaken to determine the fine structure of the basidiospores of *Pluteus cervinus* (Fr.) Kummer (Order Agaricales, Family Pluteaceae) since virtually no information was available. Ultrastructural analysis was aided by cytochemical studies to determine the distribution of lipids, the number and distribution of nuclei and the distribution of acid phosphatase within basidiospores. In addition assays to determine the presence of malate synthase were conducted. Results obtained from the cytochemical studies and enzyme assay assisted the interpretation of the structure and function of basidiospores. Ultrastructural analysis of the basidiospore wall will also aid our understanding of the taxonomy of *Pluteus cervinus.*

MATERIALS AND METHODS

Collecting basidiospores. Basidiospores were harvested by placing the caps of freshly collected mushrooms, gill side down, in sterile disposable petri dishes in a moist chamber. Freshly collected discharged basidiospores were used in all experiments comprising this study.

Fluorescent Light microscopy. For rapid staining of nuclei, basidiospores were fixed in 95% ethanol and glacial acetic acid (3:1) for 30 minutes. The samples then were washed in 25% ethanol for 5 minutes and suspended with 0.7 ml mithramycin in 25% ethanol (Slater 1976). After 24 hours, basidiospores were examined and photomicrographs were made using an Olympus Vanox Fluorescent microscope equipped with a 515 barrier filter and an IF 490 excitation filter.

Lipid distribution. The distribution of lipids was revealed by staining basidiospores, previously fixed in 50% ethanol, with a saturated solution of Sudan Black B in 70% ethanol (Jensen 1962). Photomicrographs were made using a BH2 Olympus light microscope.

Transmission electron microscopy. Basidiospores were fixed in 3% glutaraldehyde plus 1% paraformaldehyde in 0.05 M cacodylate buffer at pH 7.4, containing 5 mM calcium chloride, for 6 hours at 25°C. Following three 10-minute washes in the fixative buffer, basidiospore walls were fractured as described by Ruch and Motta (1987). Basidiospores were mixed with glass beads (0.45-0.50 mm diam.) in a ratio of 2:5 (v:v, spore suspension: glass beads) in a 15 x 100 mm conical centrifuge tube and agitated rapidly on a vortex mixer at the highest speed for 15 seconds. Basidiospores were then separated from the glass beads and post fixed in 1% osmium tetroxide for two hours at 25°C. Following several washes in cacodylate buffer, basidiospores were pelleted by centrifugation and mixed with a drop of barely molten 2% agar. After solidifying, the agar basidiospore mixture was cut into 1 mm^3 pieces, dehydrated in graded series of ethanol and embedded in Epon. Sections were cut with a diamond knife on a Sorvall Porter-Blum MT2-B Ultramicrotome and were stained with uranyl acetate followed by lead citrate. Electron micrographs were made with a Hitachi H-600 transmission electron microscope.

Localization of acid phosphatase. The distribution of acid phosphatase was demonstrated following the protocol of Ruch and Motta (1987). Freshly collected basidiospores were fixed for 1 hour at 15°C in 1% glutaraldehyde in 0.05 M cacodylate buffer at pH 7.4. After fracturing the walls, basidiospores were washed three times for 15 minutes each in 0.05 M citrate buffer at pH 5, followed by three 10 minute washes in 0.05 M acetate buffer at pH 5. Following pre-incubation for 60 minutes in the reaction medium minus the substrate, the fractured basidiospores were incubated in the complete reaction medium for 2 hours at 37°C. The reaction medium consisted of 0.3 ml distilled water and 4.7 ml of solution containing the following components: 10 ml of 0.2 M acetate buffer (pH 5.0), 10 ml of 1.25% sodium β-glycerophosphate, and 20 ml of 0.22% lead nitrate. The controls consisted of (a) basidiospores incubated in the reaction medium minus the substrate, sodium β-glycerophosphate, or (b) basidiospores treated with the enzyme inhibitor, sodium fluoride (0.01 M). After sequential washes in the reaction medium buffer and fixative buffer (3 times each), basidiospores were post-fixed in 1% OsO_4 and processed for TEM.

Preparation of enzyme extract. Basidiospores were collected by centrifugation and then suspended in 5 ml cold grinding medium. The grinding medium, modified from Cooper and Beevers (1969), consisted of 1.0 mM EDTA, 0.15 mM Tricine buffer {N-tris(hydroxymethyl)methylglycine} at pH 7.5, 10 mM KCl, 1.0 mM $MgCl_2$, 1 mg/ml dithioerythritol, and 0.1% Triton X-100. Basidiospores, mixed with glass beads (0.45-0.5 mm diam) in a ratio of 2:5 (v:v, glass beads:cell suspension), were homogenized in a Braun Cell Homogenizer MSK for 60 seconds in the cold. The homogenate was centrifuged at 500 x g for 10 min at 4°C and the supernatant used for enzyme assays. An enzyme extract, prepared from the endosperm of 4-day-old castor bean seedlings, was assayed simultaneously. To prepare the extract, the endosperm was ground with a cold mortar and pestle in the grinding medium (1 g wet weight endosperm per 4 ml grinding medium) until a smooth paste was obtained. Following centrifugation at 500 x g for 10 min. at 4°C, lipids, which collected at the top of the supernatant, were discarded. The supernatant was used for assays.

Malate synthase enzyme assay. Malate synthase activity was assayed by measuring the formation of a yellow complex between 5,5'-dithiobisnitrobenzoic acid (DTNB) and the sulfhydryl group of CoA at 412 nm at 25°C with a Beckman DU-64 spectrophotometer (Cooper and Beevers 1969). The reaction mixture consisted of 0.6 ml of 0.5 M Tris at pH 8.0, 0.3 ml of 19.5 mM DTNB, 0.3 ml of 0.1 M $MgCl_2$, 0.3 ml of 2.34 mM acetyl coenzyme A, 0.3 ml of 0.26 M sodium glyoxylate, 2.0 ml double distilled water, and 0.02 ml basidiospore enzyme extract or 0.01 ml of enzyme extract of castor bean. The assay was initiated by the addition of the extract. Total protein was measured by the method of Lowry et al. (1951) using bovine serum albumin as the standard. Specific activity of malate synthase is defined as the units of enzyme activity per milligram protein. One enzyme unit is defined as the amount of enzyme that produced 1 µmol of product per minute.

RESULTS

Basidiospores of *Pluteus cervinus* have a relatively thin wall (about 0.35 - 0.45µm in median section) composed of two distinct; layers, both being continuous around the spore (Figure 1-4). The outer layer or episporium was a thin, electron-opaque layer of nearly uniform thickness (0.15 - 0.2 µm) except near the hilar appendage where it formed a rounded protuberance into the endosporium. The inner layer or endosporium was electron-transparent and was of nearly uniform thickness (0.2 - 0.25 µm) except in the region of the hilar appendage where it became thinner (Figures 1-4). The wall of the basidiospore apex was rounded and no germ pore was present.

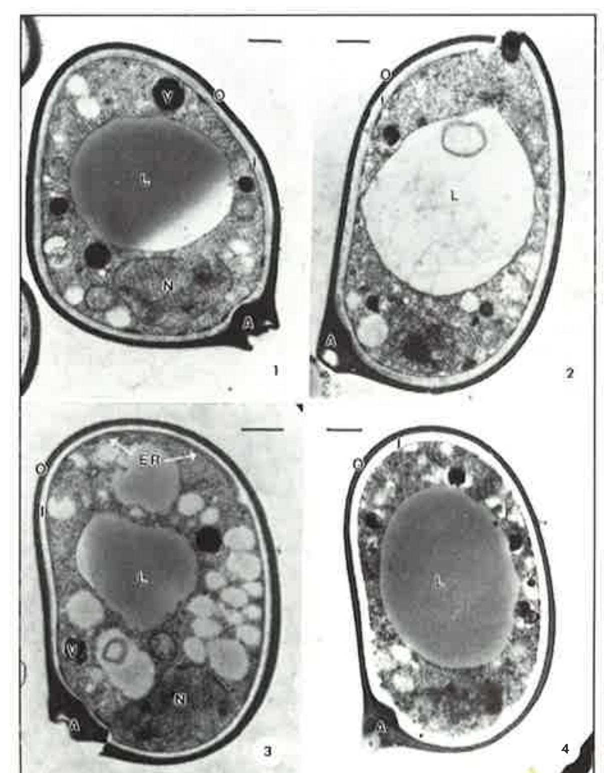
Freshly discharged basidiosores of *P. cervinus* have a distinct morphology (Figures 7-12), although its organelles were similar to those typically found in basidiospores. The plasma membrane was appressed to the endosporium and lacked any apparent, distinct invaginations. The most conspicuous feature of the basidiospore was the large, centrally located, nonmembrane-bound lipid (Figures 1-4, 6-7). Figure 6 illustrates a basidiospore stained with Sudan Black B, a lipid soluble stain. Due to the quantity of lipid, organelles were pushed to the periphery of the cytoplasm in close proximity to the cell wall.

Each basidiospore was uninucleate (Figures 1, 3, 5, 7, 8). The nucleus appeared elongated rather than spherical and was located at the base of the basidiospore closely appressed to the plasma membrane near the hilar appendage. In ultrathin section the nucleus was enveloped by a double membrane. Internally, the nucleoplasm appeared homogenous except for a prominent nucleolus.

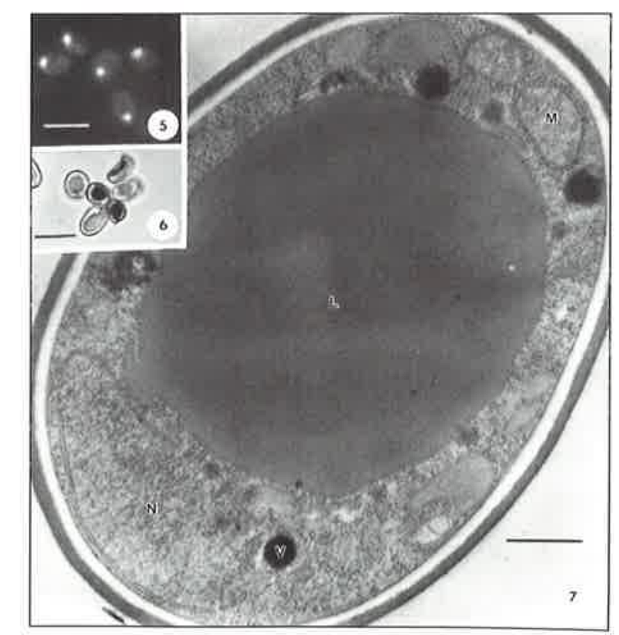
Well-defined mitochondria were widely distributed throughout the cytoplasm (Figures 7, 9-12). In section, each mitochondrion was bound by a well-defined outer membrane which was associated with small granules, probably ribosomes or glycogen. Mitochondria were typically circular in section, and only a few well delineated, plate-like cristae were observed.

Endoplasmic reticulum consisting of poorly developed short cisternae occurred near the plasma membrane (Figure 3). Golgi apparatus, rosettes of glycogen (α-particles), and microtubules were not observed, but ribosomes were packed densely throughout the cytoplasm.

Each basidiospore contained numerous single membrane-bound vacuoles of various sizes, each containing differing amounts of an amorphous dense material



FIGURES 1-4. Longitudinal sections of ungerminated basidiospores of *Pluteus cervinus.* O, episporium; I, endosporium; V, vacuole; L, lipid; N, nucleus; A, apiculus (hilar appendage); ER, endoplasmic reticulum. Note the bipartite wall and the rounded protuberance of the episporium into the endosporium in the region of the apiculus. Bars = 1 µm.



FIGURES 5-6. Spores of *Pluteus cervinus.* Figures. 5. Fluorescent mithramycin staining illustrating the uninucleate condition of the basidiospores. Figure 6. Basidiospore stained with Sudan Black B showing the centrally located lipids. Figure 7. Thin section through a dormant spore showing distribution of organelles. V, vacuole; L, lipid; N, nucleus; M, mitochondria. Figures 5-6: Bars = 10 µm; Figure 7: Bar = 0.5 µm.

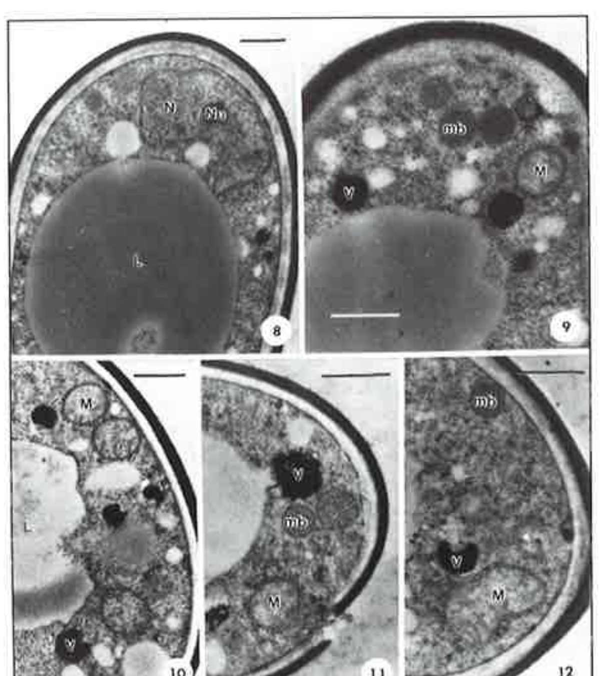
(Figures 1-4, 9-12). These vacuoles were shown to contain acid phosphatase using the Gamori method (Ruch and Motta 1987). The reaction product appeared as a heavy deposit of electron-opaque material (Figures 13, 14). No reaction product was seen in the nucleus or other organelles, nor in the NaF control (Figure 17). Small amounts of reaction product were seen in basidiospores incubated in the reaction mixture minus the substrate due to endogenous reserves of substrate (Figures 15, 16).

Single membrane-bound microbodies were also observed in basidiospores (Figures 9, 11, 12). These structures occurred in close proximity with lipids and mitochondria. Microbodies were circular in cross section and the outer membrane was not associated with small granules. The matrix had a homogeneous, fine powdered appearance.

The in vitro experiment to detect malate synthase activity is summarized in Table 1. Malate synthase, a marker enzyme of the glyoxylate cycle, was present.

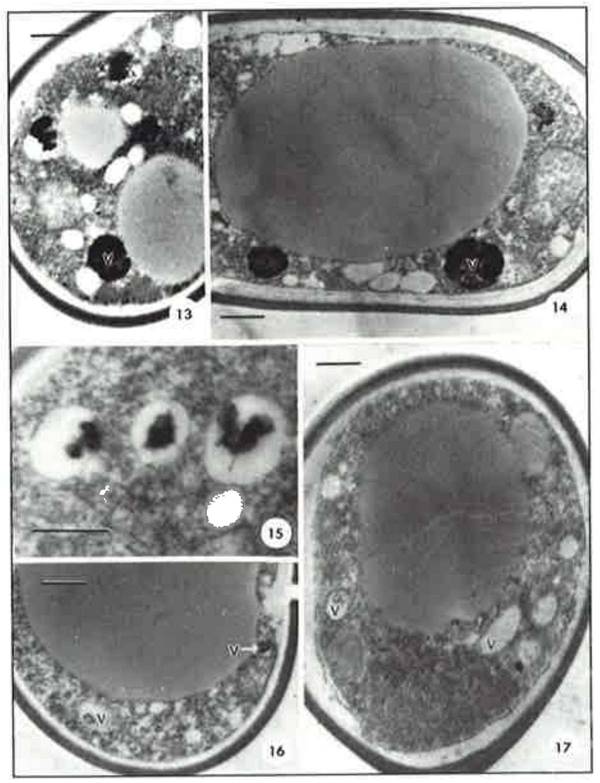
DISCUSSION

The most conspicuous internal feature in the basidiospores of *Pluteus cervinus* is the large centrally located lipid mass. High lipid content is a feature of most mushroom spores which have been studied ultrastructurally (Voelz and Niederpruem 1964; Manocha 1965; Hyde and Walkinshaw 1966; Stock and Hess 1970; Heintz and Niederpruem 1971; Oláh 1973; Nakai and Ushiyama 1974; Greuter and Rast 1975; Hess and Weber 1976; Arita 1979; Longo et al. 1979; Madelin 1981; Fineran and Fineran 1984; Ruch and Motta 1987; Ruch and North 1988).



FIGURES 8-12. Thin sections of *Pluteus cervinus* basidiospores showing the distribution of various organelles. V, vacuole; L, lipid; N, nucleus; Nu, nucleolus; M, mitochondria; mb, microbody. Bars = 0.5 μm.

Basidiospores, like those of *P. cervinus,* rely on this endogenous supply of lipids for germination (Reisener 1976; Ruch et al. 1991; Ruch and Samuel 1992). Much of the stored lipid is converted to carbohydrate during germination in part by the glyoxylate cycle (Smith and Berry 1976; Griffin 1994). That the glyoxylate cycle occurs in the basidiospores of *P. cervinus* was shown by the presence of malate synthase, a marker enzyme of the cycle. The presence of malate synthase also suggests that the single membrane-bound microbodies are glyoxysomes. However, no one has demonstrated cytochemically that the microbodies found in the basidiospores of homobasidiomycetes are glyoxysomes.



FIGURES 13-17. Cytochemical localization of acid phosphatase activity in dormant basidiospores of *Pluteus cervinus*. Spores stained only with uranyl acetate. Figures 13-14. Basidiospores treated with complete reaction mixture (Gamori method) demonstrating the localization of acid phosphatase activity in the single membrane bound vacuoles. Figures 15-16. Control basidiospores treated with the complete reaction mixture minus substrate, sodium β-glycerophosphate. Note the light deposition of lead phosphate in the vacuoles resulting from endogenous substrate reserves. Figure 17. Sodium fluoride control. Note the lack of lead phosphate deposit in the vacuoles. Bars = 0.5 μm.

TABLE 1.
Malate Synthase Specific Activity of ***Pluteus cervinus.***

	SA[a]
Pluteus cervinus	3.80
Castor bean	4.77

[a]Specific activity (SA) equals units of enzyme activity per mg protein. One enzyme unit is defined as the amount of enzyme that produces 1 μmol of product per minute.

Microbodies are associated with mitochondria and lipids in basidiospores of *P. cervinus.* Lipids are converted to carbohydrates by the following pathway during germination: β-oxidation oxidizes the fatty reserves to acetyl-CoA. Although some of the acetyl-CoA is used for ATP production, most enters the glyoxylate cycle to form succinate. The succinate, which is transported to the mitochondria, enters the Krebs cycle and is converted to malic acid or oxaloacetic acid. In the cytoplasm the malic acid is further oxidized to pyruvic acid by the malate enzyme, and oxaloacetic acid is converted to PEP by PEP carboxykinase. Finally, the pyruvate or PEP is reduced to phosphorylated sugars via gluconeogenesis. The sugar phosphates are then used for cell wall synthesis during germination (Bachofen and Rast 1968; Smith and Berry 1976; Griffin 1994).

Distinct invaginations in plasma membrane of fungal spores have been reported by several investigators (Hyde and Walkinshaw 1966; Stock and Hess 1970; Heintz and Niederpruem 1971; Beckett 1976; Arita 1979; Ruch and Motta 1987; Benhamou et al. 1990; Traquair et al. 1991). However, none were seen in ultrathin sections of the plasma membrane of dormant basidiospores of *P. cervinus.* The function of these structures is unknown, but it seems likely that these structures serve as a membrane reserve since they disappear during the initial increase in spore volume during germination (Stock and Hess 1970; Heintz and Niederpruem 1971).

The lack of a well developed endoplasmic reticulum in the basidiospores of *P. cervinus* is characteristic of most dormant fungal spores and reflects the greatly reduced metabolic activity in these spores (Bracker 1967; Sussman 1969; Hawker and Madelin 1976; Smith et al. 1976; Ruch and North 1988). Mitochondria in basidiospores of *P. cervinus* were expected to have only a few well delineated plate-like cristae for a similar reason (Ruch and Motta 1987).

Basidiospores of *P. cervinus* are uninucleate, similar to the dormant, ungerminated basidiospores of *Lentinus edodes* (Berk.) Sing. (Nakai and Ushiyama 1974) and *Coprinus psychromorbidus* Redhead & Traquair (Traquair et al. 1991). Most basidiospores are, however, binucleate. Ultrastructural studies of binucleate mushroom spores include *Lenzites saepiaria* Fr. (Hyde and Walkinshaw 1966), *Schizophyllum commune* Fr. (Aitken and Niederpruem 1970), *Coprinus lagopus* (Fr.) Fr. (Heintz and Niederpruem 1971), *Agaricus bisporus* (Lange) Imbach (Greuter and Rast 1975), *Pholiota nameko* (T. Ito) S. Ito & Imai (Arita 1979), *Psilocybe cubensis* (Earle) Sing. (Ruch and Motta 1987), *Agaricus campestris L.* ex Fr. (Ruch and North 1988). The occurrence of two nuclei in mushroom spores is probably the result of one nucleus migrating from the basidium and undergoing a subsequent mitotic division in the immature basidiospore (Akai et al. 1976).

Acid phosphatase activity, similar to that demonstrated in the single membrane-bound vacuoles of *P. cervinus,* have been documented in many fungi (Matile and Wiemken 1967; Pitt and Walker 1967; Pitt 1968; Iten and Matile 1970; Wilson et al. 1970, 1978; Matile 1971; Holley and Kidby 1973; Wilson 1973; Nehemiah 1973; Hislop et al. 1974; Hänssler et al. 1975; Maxwell et al. 1978; Ho and Zak 1979; Mason and Wilson 1979; Rosing 1984; Ruch and Motta 1987). Although it is relatively easy to demonstrate the lysosomal activity of these vacuoles, their function is still questionable. One proposed function has been the compartmentalization of acid hydrolases which are involved in mobilization of storage reserves and digestion of macromolecules within the cell, including lipids (Hänssler et al. 1975). Mason and Wilson (1979) reported that lipid bodies were closely associated with fungal cell vacuoles, and Wilson et al. (1970, 1978) noted that such lipids fuse with the vacuolar membrane and discharge their content into the vacuoles.

Of the basidiospore features described above, only the number and position of nuclei and the distribution of lipids are of possible taxonomic value. The most important ultrastructural feature of taxonomic use is the structure of the basidiospore wall. The morphology of the walls of dormant, ungerminated basidiospores which have been studied appears to be quite diverse. Despite this diversity, basidiospores are either thin-walled or thick-walled.

The most common wall structure appears to consist of two or three layers, although as many as six layers have been reported (Manocha 1965; Perreau 1967; Stock and Hess 1970; Heintz and Niederpruem 1971; McLaughlin 1973, 1977; Arita 1979; Oláh 1973; Clémençon 1974; Grueter and Rast 1975; Keller 1977; Burge 1979; Ruch and Motta 1987; Ruch and North 1988; Traquair et al. 1991). Among thin-walled basidiospores, *Schizophyllum commune* (Voelz and Niederpruem 1964), *Exidia nucleatea* (Schweinitz) Burt (Wells 1964), and *Lenzites saepiaria* (Hyde and Walkinshaw 1964) possess a single layered wall, while *Lentinus edodes* (Nakai and Ushiyama 1974) has a bipartite wall. *Pluteus cervinus* has a bipartite basidiospore wall, the episporium and endosporium following the terminology of Singer (1986).

ACKNOWLEDGEMENTS

The results reported herein were made possible by a grant from the Asia Foundation, Partners for

International Education and Training (PIET). We also thank Beth Verhoestra for technical assistance with electron microscopy, and Dr. J. Hill Hamon for his critical evaluation of the manuscript.

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Journal of the Pennsylvania Academy of Science 70(1):22-29, 1996

MONTANDON MARSH: A VEGETATION DESCRIPTION OF A POTENTIALLY ENDANGERED WETLAND[1]

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ABSTRACT

A descriptive analysis of the vegetation and soils of Montandon Wetlands in Northumberland County, Pennsylvania was conducted to provide baseline data against which to compare future vegetation dynamics. Compositional and abundance changes within the vegetation communities may be linked to ongoing successional patterns and/or natural fluctuations in the hydrologic regime, or to nearby sand and gravel mining scheduled to begin within the next year or two. Montandon Wetlands provide habitat for notable populations of six Plants Of Special Concern In Pennsylvania: *Carex bullata, Leptoloma (Digitaria) cognatum, Juncus scirpoides, Ludwigia polycarpa, Rotala ramosior,* and *Schoenoplectus (Scirpus) fluviatilis.* Aerial photographs and extensive ground reconnaissance provided the basis for description of five vegetation associations: marsh, hummocks and hollows, wet meadows, woods, and communities that have established in the few sites that were mined during the 1950's and 60's. To quantitatively assess community composition, a series of parallel transects was placed at each of three sites within the 50-hectare (ha) wetland complex; each series was bracketed by transects in the uplands. The vegetation assemblages catalogued along these transects were correlated with underlying soils and topography. Vegetation assemblages and soil chemistry varied with topographical position and soil types. Dominant and characteristic plant species at each transect differed, apparently due to topographic position (i.e., inundation levels), and soil type and chemistry. The percentage organic matter in the soil generally increased from uplands to wetlands, while phosphorus generally decreased with distance from the cultivated fields that border the western edge of the marsh. Recommendations for restoration of the area to be mined and for management of Montandon Wetlands include: maintaining the current hydrologic patterns in the naturally occurring wetlands, buffer zones, continued monitoring, creation of viable native wetlands in the mined area. Suggestions for the area to be mined consider creation of self-perpetuating wetlands. A conservation easement should be an integral component to preserving this valuable natural area.
[J PA Acad Sci 70(1):22-29, 1996]

INTRODUCTION

Forty-eight percent of all species of special concern (those listed as threatened, endangered or rare by state or federal government) in the United States are associated with wetlands. Unfortunately, their number continues to grow. The United States is losing over 182,000 ha of wetlands per year (Kusler and Kentula 1990). Pennsylvania experienced a net loss of 11,330 ha of wetlands between 1956 and 1979. This level of wetlands destruction (on average 485 ha lost per year) continued through the 1980's (Davis 1989). If conservation efforts continue to be undermined by economic and political objectives, the loss of these valuable natural areas will continue.

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The Montandon Wetlands complex (the wetlands) consists of the most significant graminoid marsh within central Pennsylvania (U.S. Fish and Wildlife Service 1990). The wetlands are interspersed with upland habitats and sand dunes. Six extant Plants Of Special Concern In Pennsylvania (POSCIP, PA Code Title 25 1987) can be found within this complex of xeric to hydric habitats. Three of these species are listed as endangered: *Carex bullata* Schkuhr ex Willd. (bull sedge), *Juncus scirpoides* Lam. (scirpus-like rush), and *Ludwigia polycarpa* Short & Peter (false loosestrife). *Leptoloma (Digitaria) cognatum* (Schultes) Chase (fall witchgrass), is listed as threatened; and two POSCIP species are listed as rare: *Rotala ramosior* (L.) Koehne (tooth-cup) and *Schoenoplectus (Scirpus) fluviatilis* (river bulrush). The *Carex bullata* population is one of only two remaining populations in the state, the *Juncus scirpoides* stand is the only extant population within Pennsylvania (Pennsylvania Science Office, The Nature Conservancy, pers. comm.), the *Ludwigia polycarpa* population is one of only three known in Pennsylvania (the other two occurring downstream along the Susquehanna River), and the *Schoenoplectus fluviatilis* population is the only extensive stand in Pennsylvania. Two Pennsylvania threatened bird species, the American and least bittern, have been observed here occasionally and suspected of nesting in the marsh as well as several of birds of special concern (Pennsylvania Game Commission 1985, Schweinsberg 1988, Brauning et al. 1994). These unusual plant populations and the rarity of this habitat type have caused the marsh to be recognized as a valuable natural area by a number of state and national agencies (e.g., The Nature Conservancy, Pennsylvania Department of Environmental Resources-Bureau of Forestry, Environmental Protection Agency and the U.S. Fish and Wildlife Service).

This important wetland ecosystem is threatened by the impacts of a sand and gravel mining project scheduled to begin within the next year or two. Even though the wetlands themselves will not be mined, nearby perturbations have the potential to affect the status of the ecosystem. Landscape modifications made during mining could alter the wetlands' hydrology and might increase sediment loading thus disturbing crucial habitat for species of special concern. In order to understand the vegetation dynamics following disturbance, it is crucial that we have an account of the vegetation patterns that are present before mining. The baseline data collected during 1990 through 1991 and reported here will allow us to observe future vegetation dynamics, help us distinguish between natural and mining-induced changes, and provide a rationale for monitoring and management.

STUDY SITE

Montandon Wetlands are located west of the West Branch of the Susquehanna River directly east of Lewisburg on the western edge of the village of Montandon in the central Susquehanna Valley of central Pennsylvania (40°58′N lat., 76°52′W long.). The wetlands are bordered by farmlands on the ridges to the east, west, and south. The wetlands complex is bisected by Route 45 on an east-west axis. The area north of Route 45 is mainly emergent marsh and wet meadow surrounded by wet woods. Weak fens are scattered within both sections. The main focus of this study was the southern portion of the wetlands locally known as Montandon Marsh. The study site (marsh and surrounding upland) is approximately 50 ha.

Climate

The central Susquehanna Valley has a temperate climate with warm, humid summers and moderately cold, humid winters. The year 1991 was an unusually dry year with precipitation for March 1991-Dec. 1991 totaling 378 mm less than the long-term mean for these months (Bucknell University Geology Department). Low precipitation caused drought conditions in the marsh before and during the growing season with much of the marsh drying to mud-flat conditions by mid-July.

Geology and Hydrology

The wetlands complex is situated on a relict floodplain (the Binghamton Terrace) of the West Branch of the Susquehanna River (Peltier 1949). This terrace was part of the braided channel system that constituted the Susquehanna River during the mid- to late-Wisconsinian glacial period (prior to 10,000 years ago) (Clark et al. 1992). It is likely that the wetlands are located in one or several of the abandoned channels of this braided system (Richard Nickelsen pers. comm.). The bedrock underlying the marsh is Helderberg-Keyser and Tonoloway limestones, which may provide a minor contribution of calcium and magnesium to the nutrient supply of the wetlands, due to upwelling (Craig Kochel pers. comm.). The limestone bedrock is overlain by 4-8 m of alluvial gravel deposits, which are covered by silts and clay deposited as overbank sediments after the Susquehanna River abandoned these braided channels. These silts and clays laid the ground work for establishment of ponding and wetlands vegetation. Wind-laid sand dunes, 3-4 m high are found scattered throughout the wetlands and surrounding upland (Peltier 1949). These alluvial and eolian deposits create varied microtopography, soil textures, and ponding/drainage systems.

On-going studies suggest that there are three main sources for the water in Montandon Marsh: surface runoff, and both a deep and shallow aquifer in the gravel underlying the marsh (Kochel 1994). Recharge to this gravel aquifer is mainly due to regional flow from east to west (towards the river). There are two primary controls on the water level in the marsh: a set of east-west dikes (running perpendicular to the long axis of the marsh) that act as dams to slow water flow from north to south, and a set of herring-bone channels that direct flow southward through the wet meadow, allowing little ponding in that area (Kochel 1994).

Soils

Soils within and surrounding the wetlands are primarily alluvial deposits of the Susquehanna River. The soil series encountered in the study area is representative of an association repeated throughout the Montandon Wetlands complex. Well-drained Barbour-Linden soils are found on the ridge to the west of the wetlands and on the peninsula, the highest topographical position. Basher soils are somewhat poorly drained and found at lower elevations than Barbour-Linden soils. On site, Basher soils are found in the wet woods bordering the eastern edge of the wetland. Holly soils, usually flooded, occupy the lowest relief position and underlie the wetland. Sand decreases while silt increases from Barbour to Basher to Holly soils. There are corresponding decreases in permeability and increases in nutrient-holding capacity (USDA, SCS 1985). Within the undisturbed wetlands, Holly soils include deep decomposed peat.

METHODS AND ANALYSIS

Vegetation Map

Major vegetation associations of the marsh and surrounding upland were determined during ground survey. These associations were delineated with the aid of aerial photographs (due to color differences on infra-red film), and traced onto a topographical map at a scale of 1:1200 with 60-cm contours provided by Survey Services, Inc. Approximately 200 plant specimens from the study site have been deposited in The Wayne E. Manning Herbarium (BUPL) at Bucknell University, Lewisburg, Pennsylvania (Hochman 1991). Nomenclature follows Rhoads and Klein (1993).

Vegetation, Soils, and Topography

Three permanently marked sampling areas were chosen to observe the distribution of vegetation types, soil types and chemistry along topographical gradients (Figure 1). At each site, a series of permanently marked transects was placed with the length running parallel with the long axis of the marsh, perpendicular to the relief gradient from upland through bottomland woods. The transects were 10 or 20 m long. They were numbered from west to east at each site beginning with number 1, thus site **a** consists of transects 1-7, site **b** consists of transects 1-9 and site **c** consists of transects 1-5. A line-intercept method (Brower et al. 1989) was used to record percentage cover of species along transects within ecotonal areas and the wetlands. Upland wooded transects were sampled by point-quarter technique (Brower et al. 1989). Data recorded included diameter at breast height for trees, crown diameter for shrubs, number of stems per individual for both trees and shrubs, and percentages cover for herbs within 1 x 0.5 meter quadrats. All point-quarter samples consist of 20 points spaced 10 m apart. Herbaceous cover was sampled by placing the upper corner of a 1 x 0.5 m frame one meter to the east of each point. All point-quarter transects consist of one line except for transect 5 at site 1 (southern woods). This

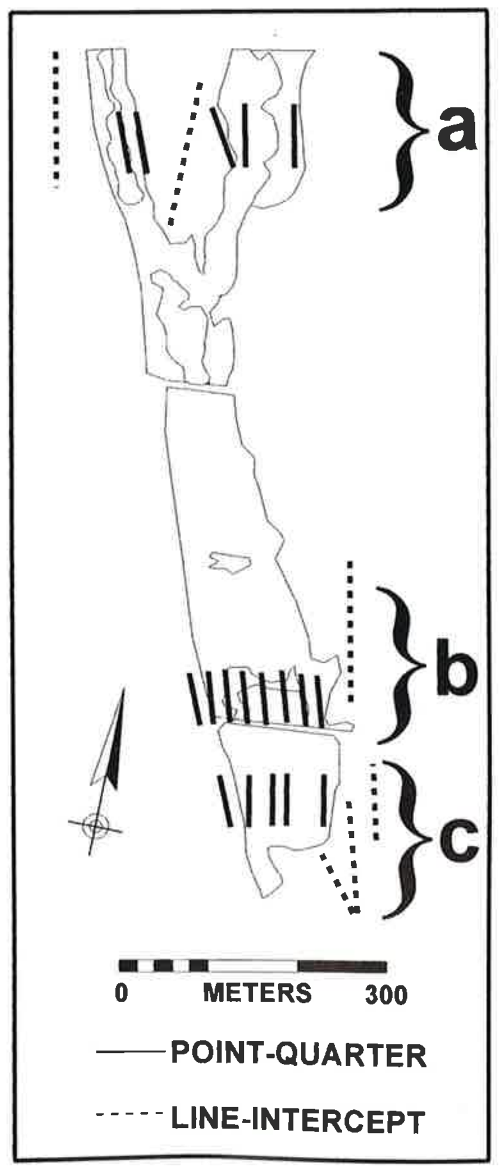

FIGURE 1. Map of Montandon Marsh. Locations of permanently marked sampling areas chosen to survey vegetation, soils, and topography are shown. Sampling was carried out using both line-intercept technique and point-quarter technique (Brower et al., 1989). Transects at each site are numbered beginning with number one on the west and proceeding east.

transect was composed of three separate lines of point-quarters. This was necessary due to paths cutting through the study area. Construction Engineering, Inc. (Millville, Pennsylvania) surveyed the sampling areas. Elevation data taken at each transect were used to determine water levels.

Data from point-quarter sampling were used to generate absolute and relative values of frequency, density, dominance, and importance value for woody species from the number of individuals per point, and diameter at breast height for trees or crown diameter for shrubs. The program Ecological Analysis, Vol. 3 (Eckblad 1989) was used to determine species diversity as the Shannon-Weaver index (H′). To minimize the difficulties of measuring plant species diversity in communities composed of vegetatively propagating individuals (e.g., sedges, grasses), percentage cover data were used with the Shannon-Weaver index (Abrahamson et al. 1984).

Soil samples were taken at each transect within the three sites. Five to eight cores were taken to a depth of 20 cm along each transect and were then pooled into one sample. This depth is within the rooting zone of most trees in forested wetlands and constitutes the rhizosphere of most shrubs and herbs (Dunn and Stearns 1987). Samples were air dried on newspaper for three days. A&L Eastern Agricultural Laboratories Inc. (7621 Whitepine Road, Richmond, Virginia 23237) performed the following analyses on each sample: soil pH (1 to 1 soil to water solution), buffer pH (Shoemaker, McLean, and Pratt ISPM), organic matter (Walker-Blackman), available phosphorus in ppm (Weak Bray), Cation Exchange Capacity (meq/l), and exchangeable potassium, calcium, hydrogen, sodium and magnesium. A&L Agricultural Laboratories Inc. used the Walker-Blackman method to determine the percentage organic matter in these soils. This test is limited to organic matter levels of 9.9% and below so consequently values of 9.9% represent 9.9% and above.

RESULTS AND DISCUSSION

Vegetation Map

Five vegetation associations were described and delineated qualitatively: marsh, wet meadow, hummock/hollow, woods, and communities that developed in the few areas mined during the 1950's and 1960's (Figure 2). Bold letters and numbers refer to Figure 2.

The marsh association was recognized to have three phases. **A**—Patchy polydominant marsh, i.e., distinct patches with differing sets of dominants including: *Schoenoplectus fluviatilis, Impatiens capensis* (jewelweed), *Cicuta bulbifera* (water hemlock), *Sparganium eurycarpum* (burr-reed), *Sagittaria latifolia* (broad-leaved arrowhead), *Typha latifolia* (cattail), and *Decodon verticillatus* (swamp loosestrife). **B**—In the western arm and to the south of the peninsula is a polydominant community of *Cephalanthus occidentalis* (button bush), *Decodon verticillatus,* and *Cicuta maculata*. This area does not exhibit distinct patches. **C**—A monodominant stand of *Schoenoplectus fluviatilis* is present in this area.

The three phases of wet meadow communities are less

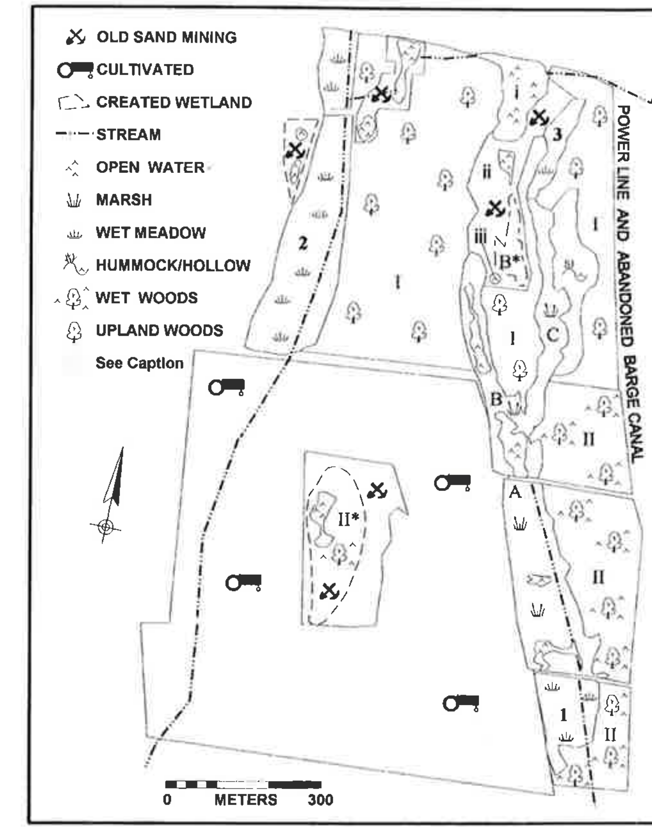

FIGURE 2. Vegetation map of Montandon Marsh. The wetlands and surrounding uplands have been divided into seven habitat types. The shaded circles represent phases of these habitat types.

Three phases of marsh.
A—Patchy polydominant marsh.
B—Polydominant community of *Cephalanthus occidentalis* (button bush), *Decodon verticillatus,* and *Cicuta maculata.*
C—Monodominant stand of *Schoenoplectus fluviatilis.*

Three phases of wet meadow.
1—*Phalaris arundinacea* (reed canary grass) meadow.
2—Graminoid meadow.
3—Most inundated of the meadow phases: *Bidens cernua* (beggars ticks), *Scirpus cyperinus* (wool-grass), and *P. arundinacea.*

Three phases of woodlands.
I—Upland woods *A. rubrum, Ulmus rubra* (slippery elm), *Quercus rubra* (red oak), *Q. velutina* (black oak), and *Sassafras albidum* (sassafras).
II—Swamp woods dominated by *A. rubrum* and *A. saccharinum.*
II*—Swamp woods dominated by *Acer* spp. but interspersed with *B. nigra.*

Four communities developed following sand and gravel mining.
i—Pond with steeply sloped sides and contains little aquatic vegetation.
ii—Pond with *Potomogeton* sp. (pondweed), *Utricularia macrorhiza* (bladderwort).
iii—Pond contains an unidentified *Utricularia* sp.
B*—Severe scraping of topsoil resulted in composition similar to the marsh at **B**.

inundated than the marsh areas. **1**—*Phalaris arundinacea* (reed canary grass) meadow with pockets of *T. latifolia, Solidago gigantea* (late goldenrod), and one large hummock on which *Acer rubrum* (red maple), *Toxicodendron vernix* (poison sumac) as well as other shrubs and trees grow. **2**—Graminoid meadow, species include: *Carex lurida* (sallow sedge), *C. lupulina* (hop-like sedge), *Juncus effusus* (soft rush), *P. arundinacea*, and *Polygonum* spp. (smartweeds). **3**—This is the wettest of the three wet meadow sites; *Bidens cernua* (beggars ticks), *Scirpus cyperinus* (wool-grass), and *P. arundinacea* are present.

The hummock/hollow association consists of a patchwork of communities whose composition is dependent upon microtopography. Hollows consist of emergents such as *Schoenoplectus fluviatilis* and *Decodon verticillatus*. Hummocks are home to species such as: *Betula nigra* (river birch), *Vaccinium corymbosum* (high-bush blueberry), *Toxicodendron vernix*, and *Salix* spp. (willow), *Sphagnum squarossum* (moss), *Osmunda cinnamomea* (cinnamon fern), and *Carex* spp. (sedges).

Woodland associations surround the wetlands on three sides. *Acer rubrum, Prunus serotina* (black cherry), *Lindera benzoin* (spice bush), *Viburnum dentatum* (northern arrow-wood), and *Toxicodendron radicans* (poison ivy) are typical in all the wooded areas. **I**—Upland woods dominated by *A. rubrum* with *Ulmus rubra* (slippery elm), *Quercus rubra* (red oak), *Q. velutina* (black oak), and *Sassafras albidum* (sassafras) scattered through the woodland. **II**—Swamp community dominated by *A. rubrum* and *A. saccharinum*. Wetter areas within this swamp contain *Quercus palustris* (pin oak), *Betula nigra*, and *Ilex verticillata* (winterberry). Swamp woods dominated by *Acer* spp. but interspersed with *B. nigra* have developed at **II***.

During the 1950's and 60's a few small areas were mined for sand and gravel. Three small ponds and two depressions were created. Communities that have established in these mined areas colonized the bare sands and gravels directly as these disturbed areas were never mitigated. A few of these areas continue to be disturbed by all-terrain vehicle traffic. Areas on Figure 2 outside the dotted lines and inside the mined region represent these highly disturbed open, sandy flats. Pond **i** has very steeply sloping sides and contains little aquatic vegetation. Pond **ii** contains *Potomogeton* sp. (pondweed), *Utricularia macrorhiza* (bladderwort), and *Polygonum* spp. Pond **iii** contains an unidentified *Utricularia* sp. Severe scraping of topsoil during mining at **B*** has resulted in a transitional polydominant community with species composition similar to the marsh at site **B** and to the hummock and hollow association. This small community includes: *Vaccinium macrocarpon* (large cranberry), *Typha latifolia, Scirpus cyperinus, Juncus* spp., and seedlings and saplings of *Betula nigra*, and *Salix* spp.

Vegetation, Soils, and Topography

Site **a** is emergent marsh, and in the western arm there is a polydominant community that includes *Decodon verticillatus, Cicuta maculata* (water hemlock), and *Cuscuta gronovii* (dodder). In the eastern arm, there is a monodominant community of *Schoenoplectus fluviatilis*. Disturbed woods characterized by *Acer saccharinum* and *Robinia pseudoacacia* (black locust) border on the west. The wooded peninsula is characterized by *Acer rubrum*. Site **b** is marsh characterized by a patchy distribution of plants such as *Sagittaria latifolia, Typha latifolia, Lemna minor* (duckweed), and *Sparganium eurycarpum*, bordered by hedgerow and wet deciduous woods. Site **c** in the southernmost wet meadow is dominated by *Phalaris arundinacea*. This meadow is bordered on the west by a hedgerow between the wetlands and cultivated fields to the east are *Acer rubrum* and *A. saccharinum* bottomland woods.

The western woods at site **a** (transect 1) are dominated by *Acer saccharinum* and *Robinia pseudoacacia* (Figure 3). The woods are characterized by a very tangled understory of *Rubus* spp. (blackberries) and *Rosa multiflora* (multiflora rose). Transect 2 is located within open marsh; this polydominant community includes *Decodon verticillatus, Cephalanthus occidentalis, Cuscuta gronovii*, and *Cicuta maculata*. Because of variable microtopography this area is the most diverse site within the wetlands, H′=2.8 (Figure 3). Woody swamp species such as *Nyssa sylvatica* (black gum) and *Ilex verticillata* (winterberry) characterize the ecotone between the wetlands and uplands at transect 3. The well-drained soils of the upland (transect 4) provide habitat for *Sassafras albidum, Quercus velutina*, and *Q. rubra* within the *Acer rubrum* woods. Transect 6 is located in a 1.6-ha monodominant stand of *Schoenoplectus fluviatilis*. Transect 7 crosses a series of hummocks and hollows. Because of variable microtopography this is a more diverse site within the wetland, H′ = 2.3 (Figure 3).

Organic matter is higher in wetlands than uplands at site **a** (Figure 4). This is primarily a result of slow decomposition under varying levels of anoxia, but also may be due to the higher productivity found in wetlands than uplands. Phosphorus levels are abnormally high at site **a** relative to sites **c** and **b**, perhaps due to upwelling of water and unusual flow patterns from surrounding areas (Craig Kochel, pers. comm.).

The hedgerow at site **b** (transect 1) is dominated by *Acer negundo* (Figure 3). The wetlands consist of a series of polydominant patches whose vegetation composition is dependent upon elevation and hydroperiod. Characteristic species at each transect include: *Phalaris arundinacea* at highest elevation within the wetlands; *Cicuta bulbifera, Carex lacustris, Sagittaria latifolia*, and *Typha latifolia* occupy lower elevations; *Sparganium eurycarpum* and *Lemna minor* occupy the most inundated area. Transect 5 was the most inundated area which may account for it being the least species rich of transects

sampled at site **b** (# taxa = 8). Transect 8 which is located in the ecotone between wetlands and upland, is both the most species rich (# taxa = 31) and diverse H′ = 2.2. The wetlands are bordered on the east by wet deciduous woods, dominated by *Acer rubrum, Lindera benzoin*, and *Prunus avium* (sweet cherry). *Betula nigra, Juglans nigra* (black walnut), and *Quercus palustris* are scattered through the canopy.

As at site **a**, organic matter at site **b** generally increases from upland to wetlands (Figure 4). Phosphorus generally declines with distance from the cultivated fields. These changes in phosphorus levels are most likely related to runoff and sedimentation from the cultivated fields because higher levels were measured in the hedgerow. Most of the sediment eroded from the fields is caught here. At transect 7, phosphorus is relatively high compared to levels at the surrounding transects, this may be due to flow patterns adjacent this transect.

At site **c**, *Acer negundo* (boxelder) and *Viburnum dentatum* are co-dominant in the tree and shrub layer of the hedgerow (transect 1) (Figure 3). *Phalaris arundinacea* exhibits dominance throughout the wet meadow (transects 2, 3, 4). It is co-dominant at transects 2 and 3 with *Polygonum amphibium* var. *emerseum* (water smartweed). The predominance of a thick stand of *Phalaris arundinacea* appears to be excluding other species from colonizing this area. Consequently, it is the least diverse and least species rich of all sites sampled within the

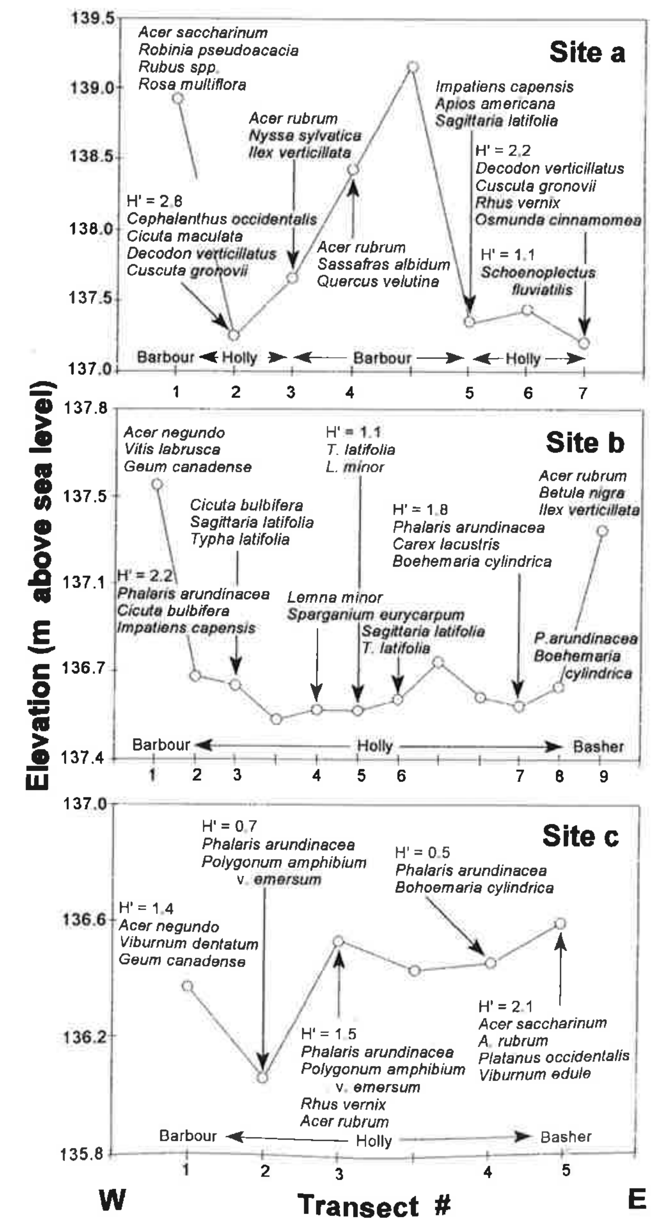

FIGURE 3. Characteristic species at sites **a**, **b**, and **c** superimposed onto plots of the relief gradient in meters. Where a transect number is not shown only elevation data were obtained. X-axis is not to scale.

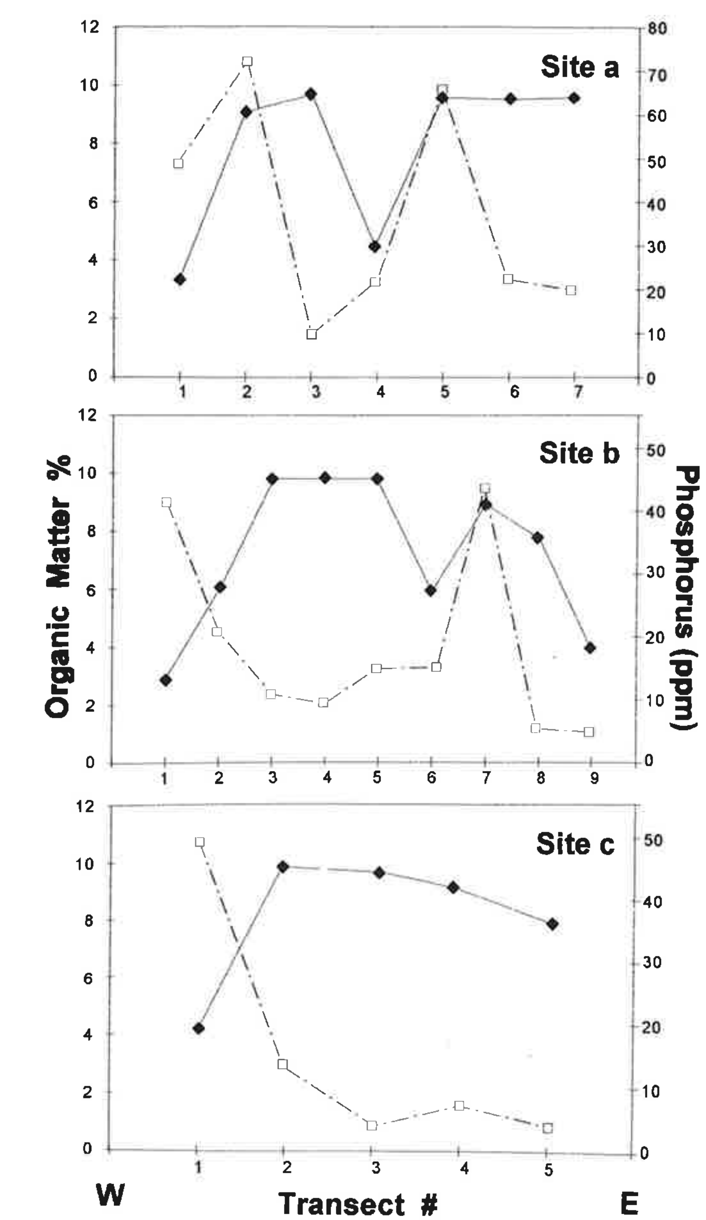

FIGURE 4. Soil chemistry at sites **a**, **b**, and **c**. Percentage organic matter (filled symbols) and available phosphorus in ppm (open symbols) are plotted, for each transect (transect 1 being the closest to cultivated fields). X-axis is not to scale.

wetlands (# taxa = 2, H′ = 0.7) (Figure 3). Transect 3 is located on a large hummock within the wet meadow. The hummock has an appreciable tree and shrub layer consisting mainly of *Acer rubrum* and *Toxicodendron vernix*. The wet woods at transect 5 are dominated by *A. rubrum, A. saccharinum, Platanus occidentalis* (sycamore), and *Fraxinus nigra* (black ash). These woods are the most diverse and species rich woods sampled within the wetland (# taxa = 35, H′ = 2.2) (Figure 3). This diversity is probably due to varied microtopography and soil permeability within this area. See (Hochman 1991) for more detailed listing of diversity indices.

Organic matter at site **c** is higher in the wetlands than in the hedgerow and bottomland woods, and higher in the woods than in the hedgerow (Figure 4). Phosphorus generally declines with distance from the cultivated fields.

Organic matter and available phosphorus at sites 1, 2 and 3 show the most consistent patterns of all soil chemistry parameters measured. Values for other soil constituents are cited in Hochman (1991).

Management and Mitigation

Gravel mining near the wetlands is scheduled to begin within the next year or two. It is proposed that a series of smaller ponds or a lake totaling approximately 30-ha in surface area and up to 9 m deep will be created by mining. These water bodies will stretch in a north to south direction along the ridge just west of the wetlands. To preserve the unique natural wetlands, mining activities must include: (1) Minimizing disruption of the existing hydrologic regime, (2) Creation of a buffer zone surrounding the wetlands so that the system is not negatively affected by sediment loading or agricultural chemicals, (3) Creation of a self-perpetuating wetland system within the mined area that is similar in species composition to Montandon Wetlands, (4) Design of haul roads, overburden dumps, pond and drainage systems (for the mined area) with goals 1 through 3 in mind, (5) Design of a long-term monitoring scheme for the created wetlands, and (6) Continued long-term monitoring of the naturally occurring Montandon Wetlands.

CONCLUSIONS

The plant communities and soil chemistry of the Montandon Wetlands vary with topography and soil types even though there are additional factors that likely contribute to the location of plant assemblages. These additional factors include water-flow patterns, inundation levels and periodicity, and micro-variation in the permeability of soils. Organic matter and available phosphorus at sites **a**, **b**, and **c** showed the most consistent patterns of all soil chemistry parameters measured. Organic matter is higher in wetter areas than in upland or well-drained sites. This was primarily due to varying levels of anoxia in wetlands/bottomland areas. At sites **b** and **c**, phosphorus levels generally declined with distance from the cultivated fields, but it is difficult to explain phosphorus level at site **a** without a better understanding of water flow. There may be upwelling and/or unusual flow patterns that cause the high phosphorus levels found at site **a**.

It is likely that the drought of the summer 1991 initiated a dry-marsh phase in the vegetation of Montandon Wetlands. The high species diversity we measured was due to the presence of a large number of seedlings of annual and opportunistic species that took advantage of the high organic matter and low-water table to germinate in the wetlands. It is unlikely that this diversity is due to phenological changes because censusing took place in mid-June to the end of July, after the major spring and early summer growth-spurt had occurred. It will be important for future studies to compare diversity measures, species composition, and overall life-history strategies from data collected during this drought year to data collected during wetter years, to assess vegetation changes due to natural hydrological cycles, on going successional patterns, or to the nearby sand and gravel mining.

Montandon Wetlands is a significant natural area, providing crucial habitat for species of special concern in Pennsylvania. Its stewardship is being addressed by the local community, the Merrill Linn Land and Waterways Conservancy (a land trust operating in the central Susquehanna Valley), the gravel mining company, and Bucknell University students of both hydrology and biology. A conservation easement through the local Merrill W. Linn Land and Waterways Conservancy would provide the integral component for the preservation of this natural area. This paper represents a summary of a larger study that surveyed rare plant stands and habitats, and presented detailed recommendations for management and long-term monitoring (Hochmann, 1991). These baseline data will provide a foundation for future investigation, conservation, and partnership between mining interests and preservation.

ACKNOWLEDGEMENTS

Funding was provided by the Katherine Mabus McKenna Foundation, Bucknell University, and Central Builders Supply. We thank Drs. Craig Kochel and Richard Nickelsen for help with the geology and hydrology, Dr. Allen Schweinsberg for information on breeding bird populations, John Kunsman for information on rare plants, and Dr. Larry Heppner and Norm Conrad for insights on soil results. Field assistance was provided by Jonathan Brown, Christine Hawkes, Matt Hess, and Becky Packer. We appreciate the mapping and surveying support of Survey Services, Inc. and Construction Engineering, Inc. Access to the site was kindly provided by Tony Markunas of Central Builders Supply.

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DIATOMS AND WATER QUALITY IN LANCASTER COUNTY (PA) STREAMS: A 45-YEAR PERSPECTIVE[1]

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ABSTRACT

Diatom communities colonizing glass substrates in selected streams of Lancaster County, PA in the summer of 1993 were consistently dominated by members of a small suite of taxa, including *Cocconeis placentula* v. *euglypta, Navicula cryptocephala, Nitzschia palea* and *Melosira varians.* We suspect that these, together with several common co-occurring species, constitute a guild, the members of which are generally favored by agricultural enrichment. The dominance of guild members in nearly all our samples, together with generally high nitrate values and moderate to high silt loads, leads us to conclude, as Patrick and Roberts (1949) did more than 40 years ago, that agricultural enrichment is the dominant ecological characteristic of streams in Lancaster County - even in basins outside the most fertile limestone farmlands. Despite the ubiquity of this "agricultural guild" of dominant diatoms, Renkonen community similarities between stations and between streams were not especially high. This was due to variability among sites in the relative abundances of species comprising the guild. We conclude that Federal clean streams legislation has had minimal effect on generalized, non-point-source agricultural pollution within the county. However, we also review previously unpublished evidence which indicates that clean streams legislation has had salutary impacts on the point-source urban and industrial pollution that Patrick's team recorded in some county streams in 1948.
[J PA Acad Sci 70(1):30-39, 1996]

[1]Received for publication consideration 27 April 1995; accepted 16 January 1996.

INTRODUCTION

Ecological studies of diatoms in Lancaster County streams began in 1948 when Ruth Patrick, of the Philadelphia Academy of Natural Sciences, chose the Conestoga River system as the watershed in which to develop a generally applicable "Biological Measure of Stream Conditions" (Patrick and Roberts 1949; Patrick 1949). Patrick's team of aquatic scientists conducted a 6-month summer and autumn survey of the water chemistry and biota (including diatoms) of all the major streams of the Conestoga system, Lancaster County's largest drainage basin.

The present paper chiefly reports work we carried out in the summer of 1993, but also includes pertinent findings from several unpublished diatom studies carried out since the late 1960's, when J.L. Richardson and his students at Franklin and Marshall College re-initiated diatom studies within the county. Several undergraduate projects during the past 30 years have focused on streams or stream segments that Patrick and Roberts had identified with specific point-source pollution problems. In addition, a team of Franklin and Marshall College undergraduates was awarded an NSF-funded "Student-Originated Study" grant in the summer of 1972 to re-survey the condition of streams in the Conestoga basin, following Patrick's 1948 approach (Fenster et al. 1972). These student investigations provide useful information about Conestoga watershed diatoms and water quality in the years 1972-1993, and we report pertinent findings from them here. Together with the benchmark study by Patrick's team, this series of investigations provides an overview of stream health in Lancaster County during the 45-year period 1948-1993.

Unlike the earlier projects, our summer 1993 investigation included basins outside the Conestoga watershed, which occupies a large fraction of the county's intensively farmed central limestone region (Figure 1). In 1993 we examined two tributaries of the Conestoga and two streams lying south of the Conestoga watershed - one also traversing limestone and one wholly within the hilly schist belt that characterizes the southern third of the county. Like the earlier Franklin and Marshall College projects, our 1993 study sought to characterize diatom floras at the level of characteristic communities and common taxa; our counts of about 300 valves per sample were insufficiently large to develop exhaustive species lists.

One goal of this paper, beyond simply documenting then-and-now diatom floras in Lancaster County, is to provide a local diatom-inferred perspective on the achievements and shortcomings to date of our nation's clean water legislation. In a paper with a similar goal, Patrick and Palavage (1994) reported for three non-Pennsylvania watersheds difficulty in comparing and evaluating data obtained in different decades by different investigators. We encountered similar difficulties in our comparisons, and prudence dictates that our description of *non-point source* pollution trends over the years be relatively generalized. We can be more precise, however, regarding the changing status of certain *point-source* problems first identified by Patrick's team.

STUDY SITES

Our June-August 1993 survey included 17 stream sites within Lancaster County, and concentrated particularly but not exclusively on the Little Conestoga Creek, Pequea Creek and Fishing Creek (Figure 1). The Little Conestoga lies within the larger Conestoga basin and was included in Patrick's 1948 survey.

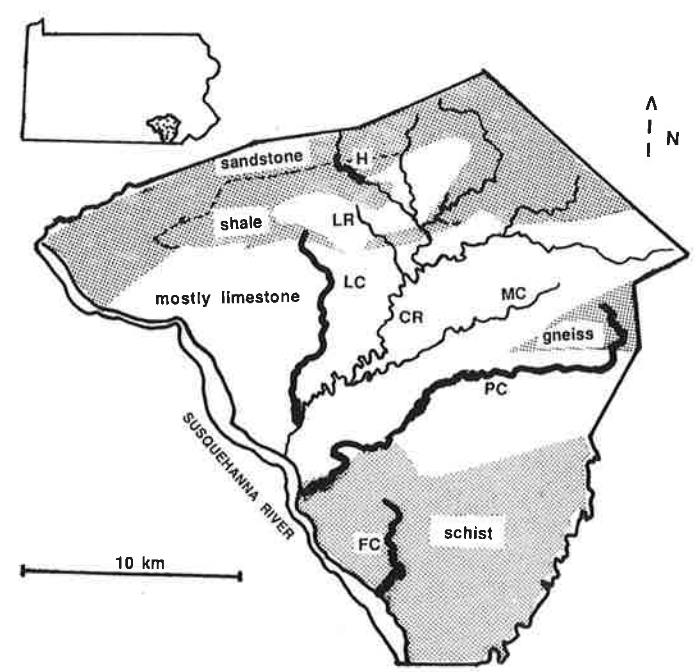

FIGURE 1. Site map of Lancaster County showing geologic features and streams that were studied. (For simplicity, stream systems and geologic intrusions that are not part of this investigation are omitted). Darkened streams and stream segments are those sampled in 1993. Abbreviations are as follows: CR=Conestoga River, FC=Fishing Creek, H=Hammer Creek, LC=Little Conestoga, LR=Lititz Run, MC=Mill Creek, PC=Pequea Creek.

Little Conestoga Creek, 32 km in length, rises in rolling meadows underlain by shale in the north-central part of Lancaster County. Except in its headwater reaches, however, this stream traverses the county's fertile limestone basin, flowing south through low-relief farmlands into suburbs west of Lancaster before passing into the less populated mosaic of woods and farmland that characterizes its lower reaches. Urbanization and suburbanization - housing developments, shopping centers, industry and a golf course - characterize this watershed's middle stretches far more today than in 1948. Of the several tributaries to the Little Conestoga, two that today are notably affected by humans are (1) the milky, pumped groundwater outflow from an active limestone quarry north of East Petersburg, and (2) a stream west of Lancaster city that carries somewhat elevated levels of heavy metals and anthropogenic hydrocarbons. Patrick and Roberts (1949) made no mention of industrial pollution entering the Little Conestoga in 1948, but noted evidence of sanitary wastes, together with wastes from chicken and duck farms, entering at several points.

Hammer Creek, also in the Conestoga basin, was sampled by Patrick's team in 1948 and also by ourselves in 1993. We sampled at sites above and below a 43-hectare reservoir which was not in existence at the time of Patrick's study. Rising in limestone farmlands in Lebanon County, Hammer Creek cuts through the sandstone, mostly wooded Furnance Hills that form Lancaster County's northern border, re-entering limestone croplands before it joins Cocalico Creek, the Conestoga River's largest tributary. The stations that we sampled on Hammer Creek lie in sandstone, but most of the water is derived from limestone farmlands upstream.

Pequea Creek, 82 km in length and lying south of the Conestoga basin, is the largest of the streams we surveyed in 1993. The headwaters of Pequea Creek drain gneiss-derived soils on the southern slopes of the Welsh Mountain ridge east of New Holland. The creek than traverses Lancaster County from east to west through the limestone heartland of the county's Amish farming community. In its final stretches Pequea Creek leaves the limestone basin and descends to the Susquehanna River through a wooded valley underlain by schist. Through this relatively steep lower stretch the creek's hard-water character, acquired in crossing the limestone farmlands, persists. Pequea Creek passes through several small towns but urban impacts are slight, and at the time of this study no part of the watershed received sewage plant inputs. Through much of its length, however, agricultural influences on Pequea Creek are high, most visibly in the form of suspended silt and clay.

Fishing Creek, 21 km long and approximating the Little Conestoga in discharge volume, lies completely within a basin of Wissahickon schist (Figure 1). This stream flows initially through a mixture of woods, cropland and pastureland that provides substantial silt inputs during storms. The lower half of Fishing Creek is deeply incised into the rolling Piedmont, and its valley is extensively forested.

In 1948 Patrick's team recorded severe point-source pollution problems in Lititz Run and Mill Creek, two tributaries in the central part of the Conestoga watershed. Subsequent investigations of these problems by Franklin and Marshall students are reported here. The point-source inputs to both these streams are concentrated in their headwater regions and are associated with the towns of Lititz and New Holland, respectively.

MATERIALS AND METHODS

In our summer 1993 investigations we visited each stream at least twice. On the first visit we chose sampling sites, made preliminary collections of rock scrapings and soft sediment samples, and introduced glass substrates to serve as diatom colonization surfaces. The glass substrates were long-necked, nonreturnable, 12-ounce beer bottles inserted neck-first into the substrate. Burn mounts (using Hyrax mounting medium) made from the initial samplings were used for practice identification of common species under 1000X magnification (oil immersion, bright field microscopy) and for initial qualitative characterization of communities. Three weeks after the first visit we returned to each stream and collected some or all of the bottle substrates. (At some sites, half the bottles were left in the water for another three weeks to provide records of diatom community development over a 6-week period). Scrapings from the upstream, downstream and lateral surfaces of each bottle were combined into a single sample. Notes were made on the condition of each bottle as it was collected - whether still standing erect, whether directly exposed to sunlight, and (particularly) whether coated with appreciable silt. Water samples of 500 ml were collected in polyethylene bottles and iced for transport to the laboratory; current speed was determined by floating a bottle over a measured distance close to the substrate incubation sites; and pH was measured on site using pH Hydrion paper.

In the laboratory, water samples were analyzed without delay for orthophosphate, nitrate, alkalinity and conductivity, using Hach, Inc. reagents and instrumentation (DR2000 spectrophotometer, digital titrator and conductivity meter). Burn mounts (in Hyrax) of the bottle scrapings were counted at 1000X to determine the composition of the diatom community that had developed on each bottle. Three hundred frustules were routinely counted per sample, no attempt being made to distinguish between frustules that were living and those that were dead at the time of collection.

Similar bottle-colonization methods (of 3 and 4 weeks, respectively) had been used in the studies by Dobbs (1975) and Curley (1977) that are reported herein; they cleaned their diatom samples by the peroxide-dichromate method of Van der Werff (1954) and counted 500 frustules per Hyrax-mounted preparation. Fenster et al. (1972) followed the field method of Patrick's team, collecting rock scrapings and preparing diatom slides from these by Van der Werff's cleaning technique. Fenster et al. provide species lists and indicate dominant species at each station, but do not report percentage counts. Hustedt (1930), Patrick and Reimer (1966, 1968), and Weber (1966) were the chief taxonomic references used by Dobbs, Curley, Fenster et al., and ourselves.

We compared diatom counts from different stations in our summer 1993 survey by means of Renkonen's (1938) percentage similarity index, expressed by Krebs (1989) as

$$P = \Sigma \text{ minimum } (p_{1i}, p_{2i})$$

where P = percentage similarity between communities 1 and 2

P_{1i} = percentage of species I in community 1

P_{2i} = percentage of species I in community 2

Using this index, the calculated similarity between two communities can range from 0% (when the communities have no species in common) to 100% (when all species occur in both communities, and in identical percentage abundances).

RESULTS

Physico-chemical data from our 1993 survey are presented in Table 1. Our field pH readings may be unreliable; both the narrow range and the slightly acid values recorded even at stations in the limestone watersheds are surprising. For other chemical parameters the ranges (and medians) were: Conductivity 48-690 micromhos/cm (510), Alkalinity 10-282 mg/l $CaCO_3$ (156), Nitrate 0.5-12.9 mg/l (7.0), Orthophosphate 0-1.04 mg/l (0.05). In contrast to the equivocal pH data, the high medians for conductivity and alkalinity clearly reflect the limestone geology of the majority of sites. At the headwater station of Pequea Creek (underlain by gneiss), conductivity and alkalinity values were well below those of any other station. Almost all nitrate values were quite high, reflecting the extensive manuring and chemical fertilization of croplands in these watersheds. Orthophosphate values, however, were generally low. This contrast with nitrate may reflect the summer 1993 sampling period: low rainfall (resulting in low surface runoff into streams), combined with high demand for dissolved P by algae and macrophytes, may have resulted in seasonal depression of dissolved P despite the potentiality for P enrichment. On the other hand, low rainfall during the sampling period may have favored relatively high nitrate levels. Most dissolved nitrate typically reaches a stream not via surface runoff but via groundwater, and in low-rainfall seasons N-enriched groundwater is a major component of streamflow in many parts of Lancaster County.

TABLE 1. Physico-Chemical Data

Sites are listed sequentially beginning with the headwater station of each stream.
Whenever a slash appears, it denotes which of the two sampling dates the data represent.

Stream	Sta.	Conductivity µmho/sec.	Alkalinity as mg/l $CaCO^3$	Nitrate mg/l	Phosphate mg/l	pH	Silt on bottle	Temp (C)	Site Notes	Current m/s
Little Conestoga	6	490/480	76/93	10.2/7.7	.02/.00	6.3/6.0	mod/high	/21.5	Headwater	v. slow/.05
	5	/690	/267	/12.9	.02/.17	/6.5	/mod	/19	Quarry	/.29
	4	650/670	174/169	11.3/10.2	.02/.05	6.5/6.5	high/high	/21	Rt. 72	.17/eddy
6-23-93/7-14-93	3	650/620	203/282	7.3/6.7	.11/.08	7.0/6.5	mod/low	/24	RR Bridge	.07/.17
	3	640/680	187/200	7.0/5.7	.01/.08	6.7/6.8	mod/mod	/21	Manor Park	.13/.13
	1	580/690	218/199	5.5/8.5	.13/.0	6.5/6.5	mod/high	/22	Boyer Pres.	.18/v. slow
Fishing Creek	3	400	74	4.9	1.04	6.0	low	23	headwater	v. slow
7-6-93	2	200	27	6.2	.08	6.0	hihg	20	in gorge	.15
	1	190	20	7.0	.06	6.0	mod	21	near mouth	.10
Pequea Creek	4	48	10	0.5	.05	5.5	low	19	headwater	slow
7-22-93	3	520	180	6.3	.13	6.5	high	25	Paradise	slow
	2	510	150	7.0	.32	6.8	high?	26	Rt. 272	fast
	1	490	149	6.2	.14	6.3	low	26	Cov. Bridge	fast
Hammer Creek	2	490/510	158/156	6.8/7.9	.03/.03	6.5/6.0	high/mod	17/16	above lake	.03/slow
6-28-93/7-19-93	1	380/380	87/109	4.3/3.4	.05/.04	6.3/6.5	high/mod	27/25	below lake	.17/slow

Table 2 lists the most common diatom taxa we encountered. With few exceptions, dominance on our glass substrates–here defined as 10% or more of a diatom assemblage–was shared by a small suite of species in which *Cocconeis placentula* v. *euglypta, Nitzschia palea* and *Navicula cryptocephala* were recurringly prominent. *Melosira varians* was found at fewer sites, but where it occurred it often shared dominance with the above species. The site having the least diverse community was a cool, turbid tributary to Little Conestoga Creek that drained an active limestone quarry. Here, after 3 weeks of community development, *Diatoma vulgare*–dominant at no other sites–comprised 62% of the sample and *Gomphonema parvulum* 31%. Although found at many sites, *G. parvulum* exceeded 10% of the count only in headwater sites and small tributaries such as this one.

TABLE 2. Most Common Taxa in Lancaster County Streams

The first column represents the number of samples in which the taxon occurred, while the second column shows the number of samples in which it constituted > 10% (and, in parentheses, >15%) of the sample count. 24 samples are represented, from 17 sites, the majority being 3-week incubations and seven being 6-week incubations.

	Samples containing taxon (N=24 samples)	Samples in which taxon exceeded 10% (15%)
Cocconeis placentula v euglypta	22	16 (7)
Nitzschia palea	17	16 (8)
Navicula cryptocephala	21	7 (2)
Melosira varians	12	6 (1)
Gomphonema parvulum	20	3 (1)
Amphora perpusilla	18	3
Achnanthes lanceolata	19	2
Rhoicosphenia curvata	15	2
Navicula radiosa	20	1
Diatoma vulgare	15	1 (1)

Three-week communities developed on glass substrates in headwater and downstream stations of Little Conestoga, Pequea and Fishing Creeks are compared in Table 3. The headwater communities of these streams were rather dissimilar and reflected the different geologies and water chemistries of these sites. The shaded headwater station in Pequea Creek, chemically distinguished by its low values for alkalinity, conductivity and nitrate, was the only station at which *Eunotia* (a genus especially characteristic of soft, often acidic waters) occurred in any but trace amounts. Downstream stretches of these three streams developed more similar diatom communities (Table 3); here, the same small suite of species provided the dominants regardless of whether the water was schist-derived and relatively soft, or limestone-derived and relatively hard.

(In Pequea Creek, our bottle substrates were lost from sites P1 and P2, the two lowest stations. At these stations, smooth non-glass surfaces–a metal tub and a metal rod found at the time of collection–were scraped to provide the diatom samples. These submerged surfaces had probably been available for colonization considerably longer than the 3- and 6-week periods represented by our bottle substrates. At P2 the metal tub substrate had developed a somewhat distinctive community dominated by *Rhoicosphenia curvata* and *Gomphonema olivaceum*. At P1 the dominants on the metal rod substrate belonged to the suite of species that dominated most of our glass substrates.)

TABLE 3. Represenative Diatom Communities of Lancaster County Streams

Each number is that taxon's percent of the total count fo that station. The communities developed over 3 weeks on glass bottle substrates. (LC=Little Conestoga, PC=Pequea, FC=Fishing, HC=Hammer).

	Headwaters			Downstream			Reservoir	
							(Above)	(Below)
	LC6	PC4	FC3	LC2	PC3	FC1	HC2	HC1
Achnanthes								
lanceolata	8.6	23.2	2.2	3.6	0.3	4.1	2.6	0.6
minutissima	7.6	3.6	2.4	5.5	2.9	?2.0	15.4	33.5
Amphora perpusilla	-	-	-	13.1	0.3	0.3	5.5	2.2
Cocconeis placentula v euglypta	10.1	-	12.7	24.3	24.3	10.9	4.8	17.5
Cyclotella spp	0.3	-	1.5	0.8	2.3	-	-	3.1
Cymatopleura solea	-	-	-	-	0.6	0.3	-	-
Cymbella spp	-	6.7	3.2	-	0.3	2.7	0.7	9.8
Diatoma								
anceps	-	3.0	-	-	-	-	-	-
vulgare	-	3.0	-	1.4	-	1.4	-	-
Eunotia spp	-	18.9	-	-	-	-	-	-
Fragilaria								
capucina v mesolepta	-	-	13.2	-	-	-	-	-
other/unid	-	4.2	-	-	1.9	3.4	-	0.9
Gomphonema								
olivaceum	1.9	-	-	-	-	-	4.0	4.0
parvulum	32.4	12.8	4.1	1.4	5.2	7.5	3.7	3.7
other/unid	-	8.3	2.2	-	-	-	1.1	2.4
Melosira varians	-	-	8.3	-	12.0	13.3	4.4	1.8
Meridion circulare	1.3	-	-	-	-	-	-	-
Navicula								
cryptocephala	-	3.7	5.6	8.7	13.9	14.6	19.8	4.0
cf gothlandica	-	-	-	1.9	-	-	9.2	8.9
gracilis	0.9	-	20.7	-	0.6	-	2.2	-
radiosa	0.6	-	1.2	3.8	6.1	17.3	3.3	-
viridula	-	-	-	3.5	2.6	-	0.4	-
other/unid	-	2.4	0.2	1.1	0.6	1.4	-	-
Nitzschia								
acicularis	3.3	-	0.7	6.6	-	-	0.7	-
dubia	-	-	-	-	1.0	0.7	-	-
palea	30.7	-	19.1	15.0	16.8	13.9	16.5	2.5
thermalis v minor	-	-	-	1.1	-	-	-	-
other/unid	1.3	-	2.2	4.9	4.5	2.1	-	0.6
Pinnularia spp	-	5.5	-	-	-	0.3	-	-
Rhoicosphenia curvata	3.8	-	-	1.4	0.6	1.7	4.8	-
Stauroneis spp	-	-	-	0.8	-	-	-	-
Stephanodiscus rotula	-	0.6	1.5	-	-	-	-	4.3
Surirella ovata	0.3	-	0.7	1.1	1.6	2.0	-	-
Unid Pennate	-	5.5	-	-	-	-	-	-

Diatom communities from the above- and below-reservoir stations in Hammer Creek also are shown in Table 3. The communities developing at the above-reservoir station over 3 and 6 weeks were moderately similar to each other (55%), and those developing at the below-reservoir station were very similar to each other (72%), whereas the communities developing above and below the reservoir for equivalent periods were quite *dissimilar* (25% in the case of the 3-week samples, 26% in the case of the 6-week samples). In the below-reservoir community *Stephanodiscus rotula* was relatively prominent, presumably due to downstream seeding from the plankton community of the reservoir. Otherwise, the below-reservoir community in Hammer Creek was not unlike those of downstream stretches in the other creeks.

The sample series from Little Conestoga Creek provided our best opportunity to scrutinize longitudinal changes within one stream, and also to compare communities developed over 3- and 6-week time periods at the same station (Table 4). Barring strong local influences, one might expect diatom communities developing at adjacent stations to be more similar than those developing at more widely separated stations on the same stream. This expectation generally was not met in the Little Conestoga. Community similarities (as measured by Renkonen's Index) were on the whole surprising low – averaging 43% within both the 3- and 6-week sample series – *regardless of* whether adjacent or distant stations were being compared (Table 4). These calculated similarities were low not because there were major differences in species composition, but because within the common suite of dominant species relative abundances usually varied considerably from station to station.

How similar were the diatom communities developing at the same station for differing time periods? In the Little Conestoga, same-site similarities between 3-week and 6-week communities averaged only 48%; again, however, the same suite of dominant species typically characterized both 3- and 6-week substrates (Table 5). Patrick's (1976) widely quoted model of stream diatom succession would have predicted that adnate taxa such as *Cocconeis* and *Achnanthes* would be more important in the 3-week samples than in those incubated for 6 weeks, yielding some of their preeminence to non-adnate taxa as community development proceeded. However, comparison of our 3- and 6-week communities reveals no obvious successional patterns at all: there was no consistent trend of temporal increase or decrease in the adnate taxa or *any* of the common dominants.

TABLE 4. Community Similarities: Little Conestoga Creek

Three-week communities developed on glass substrates are compared against each other in a similarity matrix in Table 4A. The same comparisons for six-week communities are displayed in Table 4B. In each matrix, the sites are numbered in order from the most downstream station (Site 1) to the most upstream station (Site 6). Site 5, the quarry tributary, is omitted from these comparisons. Each number in the matrices represents the percent similarity between two stations.

4A — 3-WEEK COMMUNITIES

	Site 4	Site 3	Site 2	Site 1
Site 6	32	39	39	22
Site 4	-	44	58	65
Site 3	-	-	43	35
Site 2	-	-	-	53

4B — 6-WEEK COMMUNITIES

	Site 4	Site 3	Site 2	Site 1
Site 6	34	37	27	51
Site 4	-	42	43	57
Site 3	-	-	33	54
Site 2	-	-	-	56

TABLE 5. Little Conestoga Creek: Changes in Common Species Over Time

For each sampling site, the first number represents the percent of a given taxon in the 3-week sample, and the second number represents the percent of that taxon in the 6-week sample. The quarry tributary (site 5) was not sampled at 6 weeks, so is omitted from this comparison.

	Site 6	Site 4	Site 3	Site 2	Site 1
Achnanthes lanceolata	8.6-18.0	1.7-1.6	0.0-0.9	3.6-0.6	0.6-1.7
Amphora perpusilla	0.0-0.3	0.7-1.6	2.4-40.7	13.1-5.1	17.0-6.0
Cocconeis placentula v euglypta	10.1-29.9	43.0-10.0	4.9-19.7	24.3-8.3	48.6-32.4
Diatoma vulgare	0.0-0.0	0.7-0.6	0.6-0.6	1.4-1.1	0.3-0.9
Gomphonema parvulum	32.4-2.4	0.3-0.3	0.0-0.6	1.4-0.9	0.0-0.0
Melosira varians	0.0-0.0	0.0-0.1	14.4-0.0	0.0-14.3	0.0-11.4
Navicula cryptocephala	0.0-0.6	4.6-17.1	9.1-2.5	8.7-8.0	5.9-4.1
Navicula radiosa	0.6-0.9	2.6-3.5	8.8-0.3	3.8-0.8	3.7-3.8
Nitzschia palea	30.7-14.4	12.3-30.4	19.4-11.3	15.0-45.1	2.7-18.4
Rhoicosphenia curvata	3.8-31.4	4.3-4.2	6.0-3.1	1.4-0.0	1.5-1.7

Overall, the rather monotonous predominance of a recurring suite of diatom species – over time at individual stations, between stations in the same stream, and between streams – is the clearest finding of our summer 1993 study.

DISCUSSION

Agricultural Impacts

We suspect that most or all of the "dominant suite" of diatom species shown in Table 2 constitutes an *agricultural guild* – a subset of the local diatom flora that responds positively to agricultural enrichment. Relatively subtle niche differences, probably beyond the capacity of our physico-chemical data to specify, might cause varying dominance within this guild at different stations. These niche differences might involve differing concentration optima for individual nutrients or nutrient ratios, or differing tolerances and optima for the physical differences that we noted but did not quantify closely at each station – e.g. in silt accumulation, degree of shade, current speed, or incubation depth. Some members of our postulated agricultural guild also may be relatively common in non-agricultural situations, but where several of the member taxa prosper together they probably indicate nutrient enrichment, usually of an agricultural sort. The rare species that occurred in the communities of our 1993 survey must also tolerate agricultural enrichment to some degree, but we have no evidence that they thrive on it. Thus we include only the *recurring dominants* within our postulated agricultural guild.

It is hardly surprising, in the "garden spot of Pennsylvania", that we should implicate agriculture as a principal influence on stream diatom communities. Surpassed only by certain heavily-irrigated counties of the far west, Lancaster County exceeds all non-irrigated counties in the United States in agricultural production. Not only is cropland cultivated intensively, but densities of poultry, dairy cattle and hogs are very high. Particularly in the fertile limestone basin, fields are often tilled and/or cattle grazed right to the stream banks. The resulting nutrient enrichment might be expected to influence strongly the composition of stream biotas, including diatoms. Two aspects of the 1993 diatom data argue that this is so:

(1) *Autecology of the major species.* Within our suite of dominants, taxa such as *Nitzschia palea, Gomphonema parvulum,* and *Melosira varians* are well-known to favor organic enrichment; others – e.g. *Cocconeis placentula* v. *euglypta, Navicula cryptocephala, Achnanthes lanceolata* -

are known to be tolerant of such conditions and quite probably encouraged by them (cf. Hustedt 1930; Cholnoky 1968; Patrick and Reimer 1968, 1975). Although these taxa have not previously been grouped as elements of an agricultural guild, the literature confirms nutrient-loving commonalities among them.

(2) *Ubiquity of the guild.* Our summer 1993 study indicates that the recurring suite of dominant species is locally widespread, thriving both within and outside the county's central limestone basin. The Fishing Creek watershed, in the southern schist region, is less intensively farmed and considerably more forested than the predominantly limestone watersheds of Little Conestoga and Pequea Creeks. Nonetheless, from source to mouth, nitrate levels are high in Fishing Creek and the silt load is relatively heavy. Both these conditions are indicative of strong agricultural influence; and, we would argue, so is the guild of diatoms shared by Fishing Creek with the limestone streams. Indeed, in all the streams of our 1993 survey, even the headwater communities seem indicative of nutrient enrichment.

Given the amounts of silt and clay that accumulated on many of our bottle substrates, we were surprised by the abundance of the adnate taxa *Cocconeis placentula* v *euglypta* and *Achnanthes lanceolata.* Low-lying adnate diatoms such as these should be especially subject to smothering by silt. Perhaps sufficient erosional riffles and aquatic plants exist in most county streams so that populations of these adnate species – at least in seasons when the silt load is lowest – can build up on natural substrates and serve as major contributors to the immigrant populations of newly introduced hard substrates.

In another hard-water, heavily agricultural watershed - the upper Cedar River Basin of Iowa – Main (1977, 1988) recorded as prominent a number of the same taxa - e.g., *Diatoma vulgare, Nitzschia* spp., *Navicula cryptocephala, N. viridula* – that are prominent in Lancaster County streams. In the Cedar Basin streams, however, centric taxa are more prevalent than in ours, and the adnate genera *Cocconeis* and *Achnanthes* are less abundant. Might this difference reflect lower silt loads in our streams - at least during our relatively dry sampling season – than in the Iowa streams? This seems a possibility, especially since Main (1988) emphasizes silt as a major factor in the Cedar Basin. Additional studies certainly are desirable to establish whether the dominant diatom suites of agricultural watersheds are relatively similar wherever these watersheds occur, or whether the diatom communities of agricultural watersheds are sufficiently variable to undermine our concept of a distinct agricultural guild.

Geochemical Influences

In the streams we sampled, diatom community composition usually seemed less influenced by geologically-derived differences in water chemistry than by agriculture. The county's underlying bedrock clearly does influence overlying soils and the chemistry of the waters draining them; this was chiefly evidenced by differences we recorded within and between streams in conductivity and alkalinity. The sometimes distinctive diatom communities of our headwater stations do indeed seem to reflect these geochemical differences, though also bearing the stamp of agricultural enrichment. Downstream from the headwater stations, however, agriculture's influence seems to take precedence. For example, despite the comparatively low alkalinity of Fishing Creek's schist-draining waters, the dominant diatoms of this stream were – like those of the limestone streams – members of the agricultural guild.

Point-Source Impacts

In 1948, Patrick's team judged Lititz Run to be more seriously impacted by urban wastes than any other stream in the Conestoga River basin. Lititz Run was then "unfit for fish life" throughout its course, consequent on industrial and sewage effluents (Patrick and Roberts, 1949). Our summer 1993 survey did not include Lititz Run, but evidence of improvement since 1948 exists in unpublished reports by Dobbs (1975) and Mikulis (1993). A new sewage plant for the borough of Lititz was completed in 1951, and there have since been further treatment upgrades. Industrial as well as domestic wastes now pass through this facility before discharge into Lititz Run. Patrick herself resampled Lititz Run in November 1951, and at that time found the diatom flora to be heavily dominated by *Cocconeis placentula* v. *euglypta* (Patrick et al. 1954); other species of some prominence in the 1951 sample were *Achnanthes lanceolata, Gomphonmena parvulum, Nitzschia palea,* and *Navicula cryptocephala.* Patrick and Roberts (1979) consider this flora (which is strikingly similar in its dominant species to those we recognize within our agricultural guild) to be representative of rather harsh pollution. In a later study of Lititz Run, Dobbs (1975) found 3 of 5 winter sampling stations to be dominated by *Navicula radiosa,* the other two stations having *Achnanthes minutissima* as the dominant. Other prominent species in Dobbs' samples were *Diatoma vulgare, Navicula cryptocephala* and *Nitzschia sublinearis.* These communities seem indicative of at least slight improvement in water quality. At present, Lititz Run remains disproportionately burdened by large (but better treated) inputs from the Lititz sewage plant; to our knowledge there are no longer any direct effluents from industries. The return of fish to Lititz Run probably dates from the post-1951 improvements in waste treatment. The stream experienced a severe but temporary setback in August 1992, when a 43-gallon ammonia spill from the chocolate factory again eliminated fish and most invertebrate life from a 3-km reach. However, many fish and invertebrates had recolonized Lititz Run within a few months of the ammonia accident

(Unpublished PA Fish Commission data; also Mikulis 1993). Accidents excepted, Lititz Run is now much more hospitable to aquatic life than in 1948.

Conditions also are much improved in a stretch of Mill Creek that Patrick and Roberts (1949) described as seriously polluted by a cheese factory effluent. Between 1949 and the mid-1970's, however, Mill Creek experienced additional degradation as poorly treated discharges from the New Holland sewage plant and from a large poultry processing plant were added to those of the cheese factory. By 1972-1973 Mill Creek was being studied by Franklin and Marshall ecology classes as a classic case of massive, point-source organic pollution with partial downstream recovery. The summer diatom flora of Mill Creek at that time was dominated by *Nitzschia palea*, especially in its most polluted stretches, with *Navicula viridula* and *Cocconeis placentula* assuming prominence in the downstream recovery zone (Fenster et al., 1972). In subsequent years the cheese and poultry factories both built wastewater treatment plants, and New Holland's municipal plant also was upgraded. Curley 1977 resampled the diatom communities of Mill Creek at seven stations. Although he noticed clear effects of urban loading (including 80% dominance of *Navicula luzonensis*) at the station immediately downstream from New Holland's effluents, his assessment indicated considerable overall improvement from earlier years. In the years since Curley's study, occasional surveys by Franklin and Marshall ecology classes confirm the improved status of Mill Creek (see also McMorran 1986). This creek remains enriched by non-point-source agricultural inputs, but amelioration of New Holland's point-source inputs has been dramatic.

Urbanization and Little Conestoga Creek

Point-source urban and industrial effluents were not a focus of our 1993 survey, but data from several stations in the Little Conestoga watershed deserve comment in this regard. Station LC5, on the tributary carrying pumped flow from a limestone quarry, supported a notably low-diversity diatom community, perhaps due primarily to the constant high turbidity of this flow. Our nearest sampling site on the Little Conestoga itself was four km below the entry point of this tributary; here, at station LC4, turbidity was not noticeably high and the diatom community resembled those of other downstream stations far more than that of the quarry tributary. Five km below LC4 and circa 50 m above LC3, a second unnamed tributary introduced drainage to the Little Conestoga from several industrial sites and from an old city landfill. Cavanaugh (1992) studied the water and sediment chemistry, invertebrates and macroalgae of this tributary, and found it quite depauperate biologically. The impact of this degraded tributary on the larger stream appears to be slight. Our 3- and 6-week diatom communities at LC3 were as rich in species as those of other Little Conestoga stations, and the dominants were, as usual, members of the agricultural guild.

Station LC2 on the Little Conestoga lay less than one km downstream from a golf course where there is reason to suspect ample use of herbicides – but again, the species richness and composition of the diatom community at this station did not seem atypical of the stream as a whole. Perhaps one should not expect special impact from a golf course: herbicides are used widely not only on golf courses but on lawns and croplands, and today are a common non-point-source component of agricultural pollution in Lancaster County (cf. Durlin and Schaffstall, 1993). This is a major difference from the time of Patrick's survey. Although all diatoms (even those in our agricultural guild) may be averse to commercial herbicides, certain species have been reported to be more tolerant than others. *Cocconeis placentula* is one such comparatively tolerant species (Goldsborough and Robinson, 1986). In this regard, it may be significant that *C. placentula* v. *euglypta* was the most ubiquitous and dominant species in our 1993 samples from Lancaster County. While Patrick and Roberts reported this taxon to be common at many stations in 1948, it seems to be even more prominent today.

CONCLUSIONS

In 1948 Lancaster County's economy was predominantly agricultural, but with an appreciable industrial component located both in Lancaster City and in outlying communities. In the streams of the Conestoga basin, Ruth Patrick's team found pervasive agricultural influences; in addition, severe urban and industrial impacts – mostly in the form of heavy point-source inputs of organic wastes – fouled stretches of certain streams. Almost a half-century later, Lancaster County remains predominantly agricultural with an appreciable industrial component – but there have been many changes. The human population has nearly doubled – from 230,000 to about 420,000 in a county of 2156 km² (942 mi²). Industries are larger and more numerous, shopping centers and housing developments have proliferated, farmland acreage has declined. In light of these changes, we are impressed that the point-source pollution impacts documented by Patrick and Roberts (1949) have been so conspicuously reduced: environmental legislation at the state and federal levels, abetted by greater public sensitivity to pollution problems, has had major salutary effects. Indeed, Fenster et al. (1972) found many improvements already in place when they re-surveyed Patrick's stations two decades ago, before implementation of the Clean Water Act. Point-source pollution seemingly has continued to decline since that time.

On the other hand, the widely pervasive effects of agriculture documented by Patrick and Roberts (1949) seem unabated today. At most of their stations in 1948, Patrick's team recorded biotic associations that were deemed healthy in spite of a heavy silt load, but were also "characteristic of water containing organic enrichment; . . . the genus *Nitzschia,* particularly *Nitzschia palea,* and *Melosira varians* were most abundant in waters with high organic pollution" (Patrick and Roberts 1949). Our findings 45 years later echo theirs. Today we also would emphasize, more than those authors did, the frequent dominance of *Cocconeis placentula* v. *euglypta.*

Indeed, an agricultural guild of diatom species may be even more prominent today in Lancaster County's streams than in 1948. In recent decades agriculture has become more intensive as the rural population has grown and available farmland has decreased. Adapting to this situation, farmers have intensified their dairying and poultry production operations, in the process overfertilizing croplands with excess manure. As mentioned earlier, widespread herbicide use is another more recent component of today's "agriculture problem". Far more than in 1948, the public today recognizes agricultural pollution of our streams and groundwaters as a major county problem, with serious impacts beyond the county on the lower Susquehanna River and Chesapeake Bay.

Although it has had salutary effects on urban and point-source pollution, the federal clean water legislation of the 1970's seemingly has had negligible impact in abating the agricultural enrichment of Lancaster County streams. Recognizing that stream quality is a concern in all of the state's agricultural counties, the Pennsylvania legislature in 1993 passed a hotly contested nutrient management bill (Legislative Act 6) designed to reduce agricultural pollution statewide. If the efficacy of the new nutrient management legislation needs testing in ten or twenty years, useful information might come from another survey of the diatom communities of Lancaster County. Will the agricultural guild remain as dominant and widespread in the county's streams in the 21st century as it has been in the 20th?

ACKNOWLEDGMENT

The first author gratefully acknowledges a long-standing debt to Ruth Patrick, not only for her early studies in Lancaster County but for helpful mentoring early in his own career. We are grateful to the former Franklin and Marshall College students cited herein for access to their unpublished studies, and thank Michael Sullivan and unidentified reviewers for comments on earlier drafts of this paper.

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A STUDY OF THE ULTRASTRUCTURE AND SECRETIONS OF THE MALE ACCESSORY GLAND OF *ARTEMIA* (CRUSTACEA, BRANCHIOPODA)[1]

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ABSTRACT

The general structure and arrangement of the male accessory gland in *Artemia franciscana* has been studied extensively; however, the ultrastructure of the cells and the function of the secretions in reproduction have not been determined. A light and electron microscopic study was used to observe the histology and ultrastructure of the accessory gland. The results confirmed past observations of the accessory gland. In addition, microvilli and an exoskeletal lining were observed within the ducts of the accessory gland. Several different forms of the secretory products were also observed in the droplets within the cytoplasm of the gland cells. Histochemical results indicated that the secretions from the gland are a neutral mucopolysaccharide or mucoprotein. SDS-PAGE was used to separate proteins from the secretions of the accessory gland but was not successful. In summary, a more precise description of the histology and ultrastructure of the accessory gland in *Artemia* was achieved through the light and electron microscopic study while no progress in determining the function of the secretion of the accessory gland was made, primarily due to the inconsequential SDS-PAGE results.

[J PA Acad Sci 70(1):40-45, 1996]

INTRODUCTION

The male *Artemia* reproductive system consists of the paired structures typical of many crustaceans (Martin, 1992; Wolfe, 1971). Paired testes and vasa deferentia lead to the gonopores located at the tip of the eversible penes. These intromittent organs are capable of extending for copulation and are retracted during normal swimming behavior. The accessory glands are well-defined, paired structures situated near the tip of the eversible penes. The duct that drains each gland empties at the tip of the penis through a pore adjacent to the gonopore. The function of the accessory gland and its secretions in *Artemia* reproduction is as yet unknown. Wolfe (1971) speculated that the secretions may function as a lubricant for intromission of the penis, a sperm plug, or a sperm activator. The shell gland within the female *Artemia* reproductive system has a structure similar to that of the male accessory gland (Criel, 1980; Anderson, et al., 1970). The shell gland is a cluster of structures known as gland cell units, which are made up of gland, neck, and duct cells. The shell gland produces a lipoprotein secretion which forms the tertiary envelope in thick-shelled eggs. The similarity in structure between the accessory gland in the male and the shell gland in the female suggests that these structures may be homologous (Criel, 1980; Martin, 1992; Wolfe, 1971).

Accessory sex glands in crustaceans vary widely in function and in structure. The functions of these glands in both sexes include the safe transport of gametes prior to release and various diverse functions after the gametes are released (Adiyodi and Adiyodi, 1976; Adiyodi and Anilkumar, 1988). The general structure of the male accessory gland in most crustaceans is a modification of the epithelial lining of the reproductive tract and is not as well-defined and distinct as that observed in *Artemia* (Adiyodi and Adiyodi, 1976; Adiyodi and Anilkumar, 1988; Deecaraman and Subramoniam, 1980). Insects, by comparison, commonly have distinct, well-developed paired accessory glands, but, like their crustacean relatives, employ a diverse range of functional roles for the secretions (Adiyodi and Anilkumar, 1988; Leopold, 1975).

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), a technique used in determining the molecular weight of proteins and protein complexes, has been used extensively in the study of accessory gland secretions in insects and occasionally in Crustacea (Chen and Balmer, 1989; Deecaraman and Subramoniam, 1983; Leopold, et al., 1971; Monsma, et al., 1990; Terranova, et al., 1972; Weber and Osborn, 1969). This technique has provided researchers with a means to elucidate proteins unique to accessory gland secretions, label these proteins with immunofluorescent markers, and trace the passage of these moieties into mated females. By following a labeled molecule from the accessory gland of a male to a female which has recently mated, researchers have observed the role of the accessory gland secretion in some organisms.

The general morphology of the male accessory gland in *Artemia* has been described by Wolfe (1971) and reviewed by Martin (1992). Ultrastructural details of the gland, neck, and duct cells have not been observed and were the main interest of the first phase of this study. Electron microscope studies of these cells provided a more detailed description of the gland and the cells that make up its unique gland cell unit. Since the role of the secretory product has not been determined, the separation and identification of the proteins unique to the accessory gland secretions and the tracing of these proteins into the mated female should begin to elucidate these functions. Separation of the secretions with SDS-PAGE, the second phase of this study, was not successful in the identification of proteins unique to the accessory gland secretions.

MATERIALS AND METHODS

Adult male *Artemia franciscana* were fixed in Forssmann's glutaraldehyde-formaldehyde and embedded in paraffin (Forssmann, et al., 1977). The tissue was sectioned at 6 μm and stained using hematoxylin and eosin and observations of the overall structure and layout of the gland were made with the light microscope (Humason, 1972). Some tissue was stained with Alcian Blue/PAS, toluidine blue, and bromphenol blue to characterize the glandular secretions (Humason, 1972). Tissue prepared for electron microscopy was fixed in Forssmann's glutaraldehyde-formaldehyde and post-fixed in osmium tetroxide (Forssmann, et al., 1977). Following fixation, the tissue was dehydrated, embedded in Spurr's resin, and sectioned on a Sorvall ultramicrotome (Forssmann, et al., 1977). Silver or gold sections were collected on copper grids, stained with uranyl acetate and lead citrate, and examined with a Zeiss TEM 109 (Venable and Coggeshall, 1965).

Accessory gland, vas deferens, and clasper muscle tissue were dissected from large, male *Artemia franciscana* and used as samples for the SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), (Anonymous, 1988). For each sample type, the tissue from 10-20 *Artemia* was homogenized in 50μl of 2x sample buffer and heated at 100°C for one minute (Anonymous, 1988). Some stockpiling of samples in the 2x sample buffer also occurred over several days, during which the samples were frozen in liquid nitrogen and stored at 0°C between dissection sessions. The samples were loaded in 10 μl portions into the wells of a Zaxis 12% gradient SDS-polyacrylamide gel and run for approximately 1.5 hours at 150 mV. A Sigma molecular weight protein standard mixture (MW SDS-200 Kit) was also loaded in 10 μl portions into several of the wells to serve as a standard for molecular weight determinations. The molecular weights of the proteins in this mixture ranged from 29,000 to 205,000. After the completion of a run, gels were fixed in multiple changes of fixative solution overnight, followed by a staining period of at least 3 hours in Coomassie brilliant blue staining reagent (Anonymous, 1988). Destaining was accomplished by rinsing the gels in several changes of fixative solution until the gels were completely cleared (usually requiring 24 hours of rinsing.) Sample and standard banding patterns were observed and gels were stored in 7.0% glacial acetic acid.

RESULTS

Each male accessory gland consists of approximately 24-30 gland cell units arranged in a cluster, similar to a bunch of grapes. Each gland cell unit consists of a pair of gland cells, a neck cell, and a duct cell (Figures 1-3). The neck and duct cells of each gland cell unit form the duct that drains the secretions from the gland cell pair. All of the ducts arise from the individual gland cell units and combine to form one major collecting duct which runs

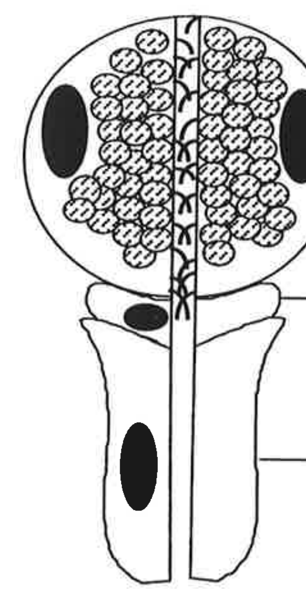

FIGURE 1. Diagram of a typical gland cell unit, the structural unit that makes up the accessory gland in *Artemia*. Three types of cells combine to form this structure: a pair of gland cells, a neck cell, a duct cell. The many microvilli that are located in the lumen of the gland cell pair are also illustrated.

[1]Submitted for publication consideration 15 September 1995; accepted 12 January 1996.

through the center of the gland (Figure 4). A lumen between the paired gland cells is present, into which the products from the cells are secreted (Figure 5 & 6). The cell membranes that form the walls of the lumen are lined with microvilli, and secretory products from the gland cells can be observed infrequently within the lumen (Figure 6). The cytoplasm of the gland cells contains large numbers of secretory droplets located throughout most of the cell and primarily along the luminal edge of the cell (Figure 5 & 6). Few other organelles are visible within the cell besides the numerous secretory droplets, the only exception being the large crescent-shaped nucleus (Figure 7). The nucleus of the gland cell is located at the opposite end of the cell from the luminal surface and contains

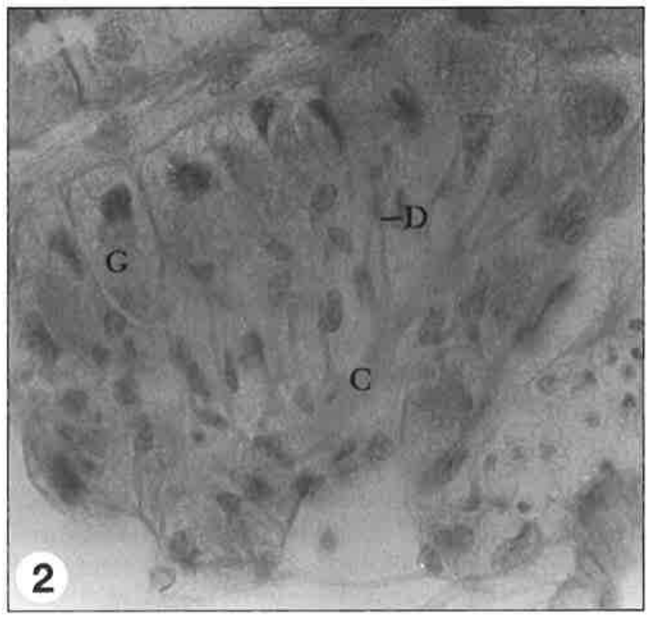

FIGURE 2. Light micrograph of the accessory gland showing the general layout of the gland including the gland cells (G), the duct which drain each gland cell pair (D), and the major collecting duct which runs through the center of the gland (C). 300x.

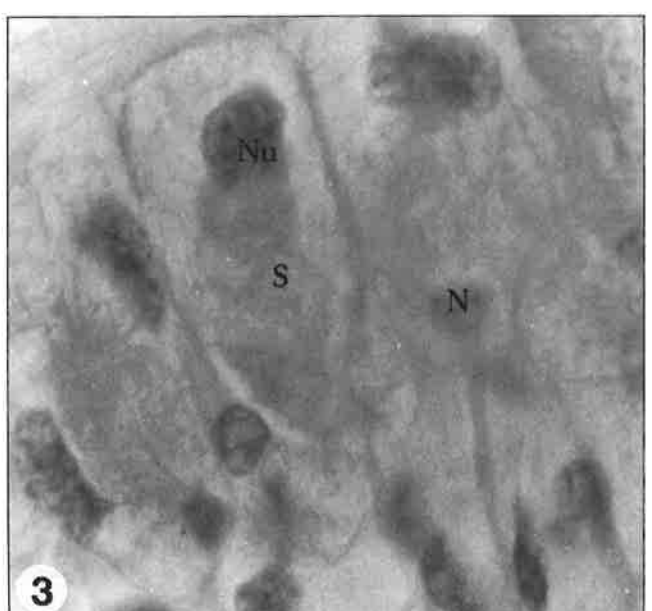

FIGURE 3. Light micrograph of several gland cells with the prominent nucleus (Nu) and secretory droplets (S). A neck cell (N) that forms the upper portion of the duct that drains the gland cells is visible. 1,200x.

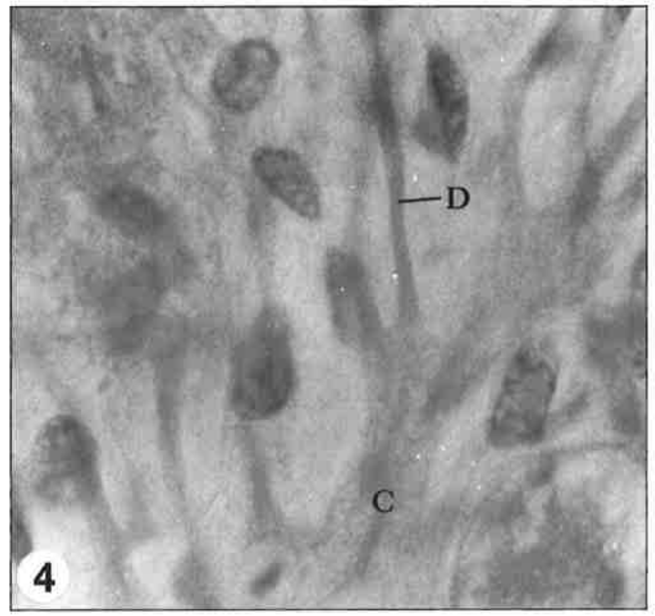

FIGURE 4. Light micrograph of the ducts (D) that drain the gland cells and the major collecting duct (C). 1,200x.

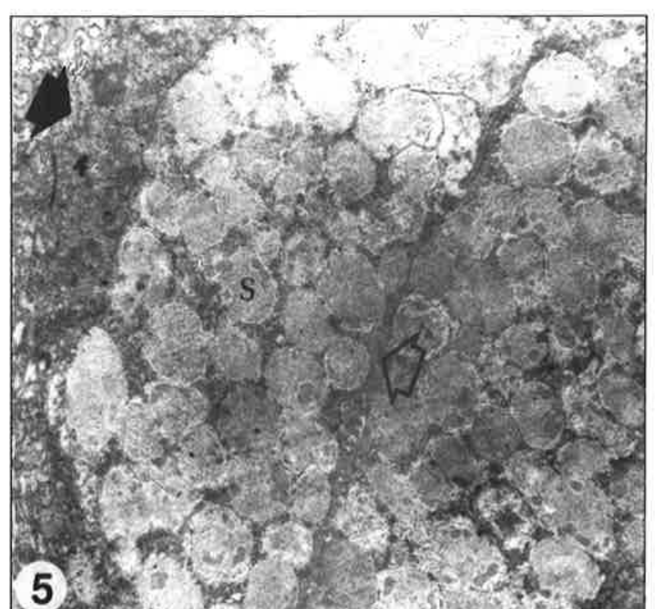

FIGURE 5. Electron micrograph of a gland cell pair and the lumen (open arrow) between the two cells. A large number of secretory droplets (S) and the prominent nucleus (solid arrow) of the cell are visible. 6,900x.

several conspicuous nucleoli. The secretory droplets occupy the majority of the interior space of the gland and were observed to contain more than one type or form of secretory product (Figure 8). Some secretory droplets contained a homogenous, granular product, while other droplets contained denser regions of secretion, or regions that appeared highly ordered. Neck and duct cells had no secretory droplets within the cytoplasm (Figure 9). The neck and duct cell lumina contained few microvilli in comparison to the gland cells and were lined with material resembling the exoskeleton. This lining was shed within the lumina of some neck and duct cells (Figure 10).

The histochemical staining of the accessory gland indicates that the secretions were neutral in mucopolysaccharide or mucoprotein reaction. Stainable secretory products were observed within the secretory droplets as well as within the cytoplasm of only the gland cells. The neck and duct cells did not stain appreciably for the carbohydrate/protein complex secretions.

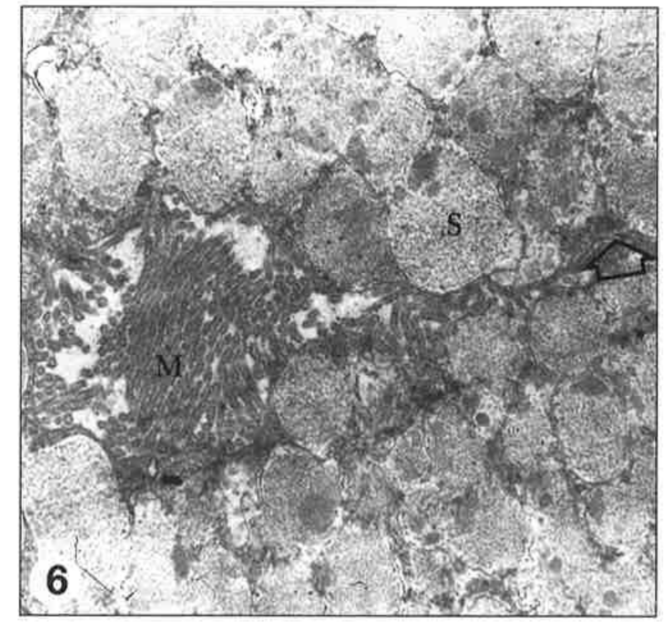

FIGURE 6. Electron micrograph of the lumen (open arrow) between two gland cells and the microvilli (M) extending from the luminal surface of the cells. A large number of secretory droplets (S) are in the cytoplasm of each cell. 6,900x.

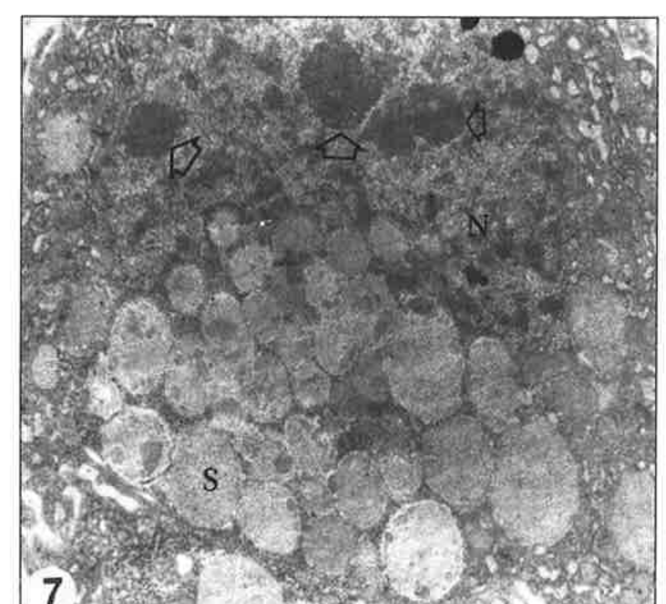

FIGURE 7. Electron micrograph of a gland cell with many secretory droplets (S) and a prominent nucleus (N). Within the nucleus are several dense nucleoli (open arrows). 6,900x.

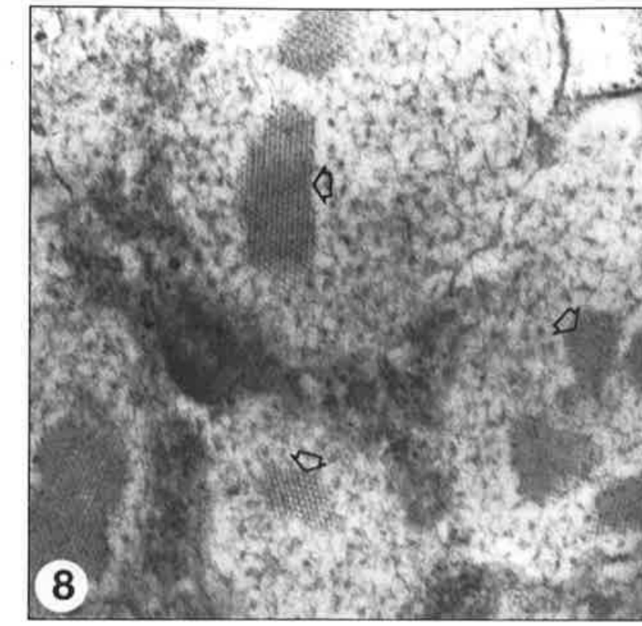

FIGURE 8. Electron micrograph of several secretory droplets within which granular secretory products visible as well as highly-ordered, crystalline-like secretory products (open arrows). 55,200x.

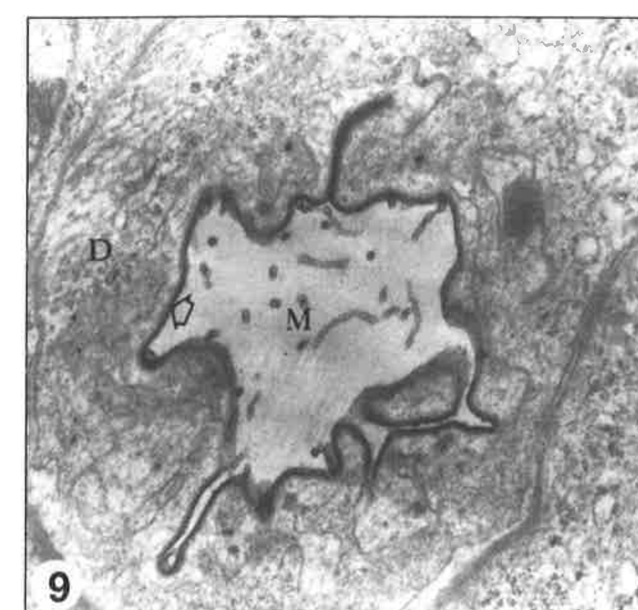

FIGURE 9. Electron micrograph of a cross section through a duct cell (D). Within the lumen, a few microvilli (M) are present and a dense chitinous lining (open arrow) covers the walls of the lumen. 27,600x.

The SDS-PAGE provided banding patterns similar to those diagrammed in Figure 11. The banding patterns for accessory gland, vas deferens, and clasper muscle were similar in band density and migration distance. No bands were observed that were unique to any one of the three samples.

DISCUSSION

The structure of the male accessory gland in *Artemia* has been described previously by Wolfe (1971). The present study confirms and adds new details concerning the ultrastructure of the gland to these descriptions. The general layout of the gland consists of a cluster of 24-30 gland cell units, each made up of a pair of gland cells and a duct comprised of a single neck cell and duct cell. The majority of the secretions which are produced by the gland are produced within the gland cell pair, suggesting that the neck and duct cells function only in the transport of the secretion to the outside. The lumina of the gland cells and the duct lumen were observed to contain microvilli. Many more microvilli were observed in the lumen of the gland cell pair, an observation that parallels the large number of microvilli seen in the lumen of the gland cell pair of the female shell gland (Anderson, et al., 1970). What role the microvilli play in the overall function of the accessory gland is unknown. They may increase the luminal surface area of the gland cells, which would aid in the secretion of the large number of secretory droplets. The material that lines the ducts of the accessory gland may be homologous to the cuticular lining of the ducts in the shell gland (Criel, 1991). This lining appears to be periodically shed into the lumen, perhaps coinciding with the molting of the exoskeleton. The absence of secretions within the neck and duct cells, as observed in many sections, would allow for a thick cuticular lining of the ducts to be present, since secretory materials do not need to pass across the neck and duct cell membranes.

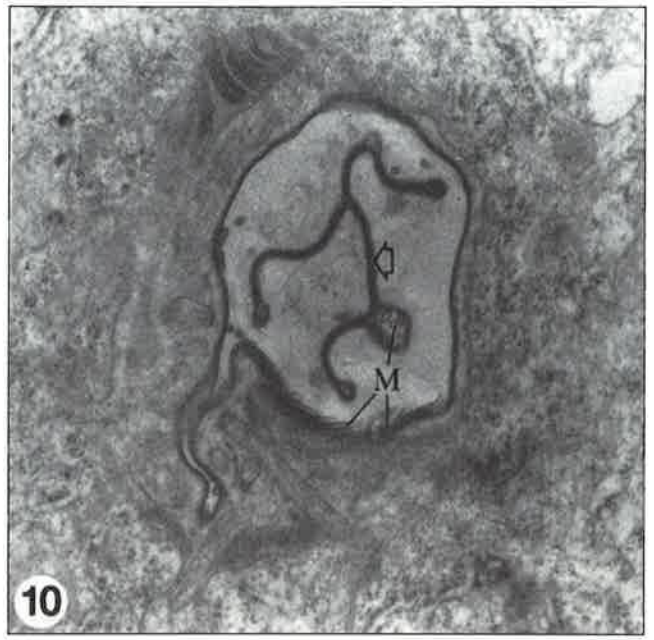

FIGURE 10. Electron micrograph of a cross section through a duct cell with its central lumen and microvilli (M). A shed exoskeleton lining (open arrow) is located within the lumen of the duct. 27,600x.

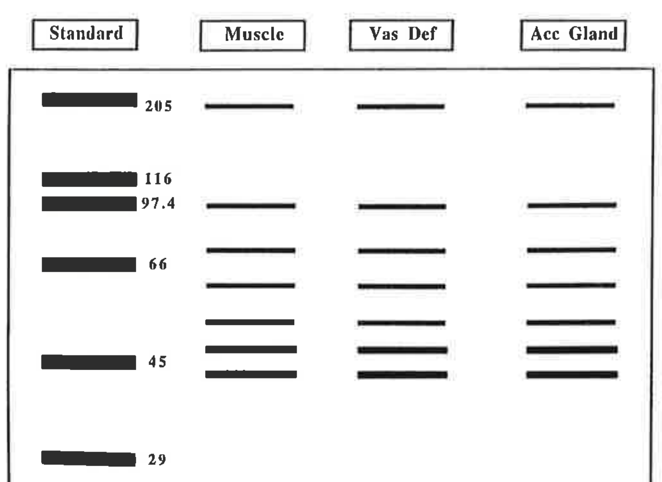

FIGURE 11. Diagram of the banding patterns from SDS-PAGE for accessory gland, vas deferens, muscle, and standard protein samples. Protein bands for the three tissue samples have similar densities and migration distances.

We think the secretions produced by the accessory gland undergo production stages that are coordinated with the release of the secretions. Observations of stainable secretory material within both the cytoplasm and secretory droplets support this hypothesis. When staining in the cytoplasm, the secretory material is thought to be undergoing production and is accompanied by considerable RNA activity and protein synthesis. When the secretory material is present within the droplets, the production of the secretion is completed and the products are stored in the droplets within the cytoplasm until release. During these later production stages, when the cytoplasm is filled with droplets and production of the secretions has slowed, few cytoplasmic organelles are visible within the cell. This suggests that the production of secretory products occurs only after the gland cells have released all of the stored secretions and the cytoplasm is empty. The observation of different types of secretory products within the droplets may suggest that the cells produce several distinct secretory products used for different functions in reproduction. This occurrence has been observed in various other species of crustaceans and insects, particularly when the formation of a complex spermatophore or shell is involved in accessory gland functions (Adiyodi and Anikumar, 1988).

The SDS-PAGE used to separate the proteins of the accessory gland secretion provided inconclusive results. The accessory gland is situated in a region of the penis that is immediately adjacent to the vas deferens and is interspersed with muscle tissue. Because of its location and the difficulty of dissecting such a small structure, the vas deferens and muscle samples were used as control samples. Any protein bands present in the accessory gland sample that were also present in the muscle or vas deferens samples were considered to be caused by the presence of muscle fibers or vas deferens within the accessory gland sample. The results of the SDS-PAGE runs indicate that the bands present in the accessory gland samples were also present in the muscle and vas deferens samples. We believe that the proteins from the muscle that surrounds the vas deferens and connects to the accessory gland were the only proteins being detected by the SDS-PAGE in the vas deferens and accessory gland samples. Since the muscle used for the samples was taken from the clasper, a modified appendage located near the anterior end of the organism, contamination of the muscle samples by vas deferens or accessory gland tissue would be highly unlikely. Had the bands present in the accessory gland or vas deferens samples been from either of these two tissues, it would not be expected that the same bands would be present in the clasper muscle samples. This leads to the conclusion that the bands seen in all three samples were from the muscle which surrounds the vas deferens and accessory gland. The data obtained from this portion of the procedure did not provide information on the molecular weight of proteins in the accessory gland; therefore, our methods must be modified and the assays repeated.

This study provided a more detailed description of the accessory gland and the cells that make up the gland cell unit. It did not, however, provide any further information on the function of the accessory gland secretions. The inability to separate and isolate the accessory gland secretions using SDS-PAGE electrophoresis techniques prevented the identification of secretory proteins by molecular weight, and the subsequent labeling of these proteins and tracing into the female after copulation.

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Journal of the Pennsylvania Academy of Science 70(1):46-52, 1996

BRANCHIAL NA, K-ATPASE ACTIVITY AND OSMOTIC AND CHLORIDE ION REGULATION IN THE THAI CRAB, PSEUDOSESARMA MOESCHI[1]

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ABSTRACT

The Thai red crab, *Pseudosesarma moeschi,* is a strong osmoregulator over a wide range of external salinities: it hyperosmoregulates in 5%, 10% and 25% sea water (SW), it is nearly isoosmotic in 50% and 75% SW and it hyporegulates in 100% SW (1000 mOsm). Hemolymph chloride ion concentrations show the same trends as hemolymph osmotic pressure, with the hemolymph nearly isoionic in 50% SW (≅ 230 mM Cl⁻). The role of the branchial sodium pump (Na, K-ATPase) in osmotic and ionic regulation was investigated by assaying enzyme specific activity (ESA) in homogenized gills from crabs acclimated 14 d in the six sea water media. Branchial ESA is highest in the posterior gills (numbers 5-7) and in these gills ESA is minimal in crabs acclimated in 50% and 75% SW, media in which the crab is nearly isoosmotic with the medium. ESA of the posterior gills increased in both the dilute (25%, 10% and 5% SW) and concentrated (100% SW) media, in which the crab pumps ions either into or out of its hemolymph, respectively. ESA of the anterior gills (numbers 1-4) is minimal and shows few changes with acclimation medium, underscoring the importance of the posterior gills in osmotic and ionic regulation. The time course of decreased branchial Na, K-ATPase ESA in crabs released from osmotic stress by transfer from 5% SW to 50% SW is consistent with both rapid (0-24 h) decreases in the activity of existing enzyme and longer term (5-7 d) inactivation or destruction of the enzyme in the gills. [J PA Acad Sci 70(1):46-52, 1996]

[1]Received for publication consideration 28 December 1995; accepted 2 March 1996.

INTRODUCTION

Mantel and Farmer (1983), Towle (1984), Lucu (1990) and Taylor and Taylor (1992) have reviewed the many studies demonstrating high levels of Na, K-ATPase enzyme specific activity (ESA) in the gills of osmoregulating crabs. Crab branchial Na, K-ATPase is found in the basolateral cell membranes of epithelial cells (Towle and Kays, 1986) and there are patches of specialized "ionocytes", particularly in the posterior gills (Copeland, 1964; Barra et al., 1983). The branchial ionocyte patches have high Na, K-ATPase activity (Neufeld et al., 1980) and they enlarge in crabs acclimated in low-salinity media (Copeland and Fitzjarrel, 1968; Neufeld et al., 1980; Aldridge and Cameron, 1982). These branchial ionocyte patches are generally assumed to be responsible for osmoregulatory ion transport in crabs, although this has been proved only in brine shrimp, *Artemia salina* (Croghan, 1958) and an intertidal isopod, *Idotea woesnesinskii* (Holliday, 1988).

Burnett and Towle (1990) have reviewed studies of ion transport in the excised, perfused gills of *Carcinus maenas* and *Eriocheir sinensis.* They note the many difficulties inherent in this preparation, particularly the need to prevent gill tissue swelling by including glutathione and several other organic solutes in the perfusion medium to maintain a constant gill perfusion pressure. In addition, they note that most studies with perfused gills have used the same medium on both sides of the gill or unphysiological perfusion media, conditions which make it difficult to relate the data so obtained to normal crabs hyperregulating in dilute media. These authors also presented data showing that the perfused gills of *Callinectes sapidus* acclimated in 5% SW could transport ^{22}Na into the hemolymph against a large, outwardly-directed sodium gradient across the

gill and that ^{22}Na transport was inhibited by internal ouabain, external amiloride and by internal cyanide and iodoacetate. Their results are consistent with the currently accepted model for gill sodium transport: an apical sodium/proton exchanger (Shetlar and Towle, 1989) and a basolateral Na, K-ATPase. Schwarz and Graszynski (1989) and their collaborators have developed a split-lamella preparation of the posterior gills of *E. sinensis.* Onken et al. (1995) reviewed the results obtained to date with this preparation and note that they are consistent with coupled sodium and chloride uptake in the gills of seawater-acclimated crabs and with independent mechanisms for sodium and chloride absorption in the gills of freshwater-acclimated crabs.

Recent work suggests that the crab branchial sodium pump may be controlled by a dopamine/cyclic AMP system (Lohrmann and Kamemoto, 1987; Sommer and Mantel, 1988, 1991) and, possibly, by gill tissue concentrations of polyamines (Lovett and Watts, 1995). In addition, Riestenpatt et al. (1994) have found that cyclic AMP stimulates sodium uptake in split crab gill lamellae by increasing apical sodium channel number and, thus, apical sodium conductance.

The crab, *Pseudosesarma moechi* (Serene and Soh, 1970), occurs in the Gulf of Thailand, where it lives in burrows in Nippa forests adjoining mangrove areas in the lower reaches of estuaries, with water salinities averaging 20-28‰ (Naiyanetr, personal communication). To the best of our knowledge, the osmoregulatory abilities of *P. moeschi* have not been investigated. Because this crab lives in intertidal areas subject to large daily and seasonal changes in the osmotic pressure of its environment, it would be expected that it osmoregulates well. *P. moeschi* is now readily available in the North American pet trade and the present study was undertaken to determine: 1) the osmotic and ionic regulatory abilities of *P. moeschi*; 2) whether, like many euryhaline crabs, *P. moeschi* shows increased branchial Na, K-ATPase ESA when acclimated in media in which it hyperosmoregulates; 3) what effect hypoosmoregulation has on branchial Na, K-ATPase ESA, and 4) to determine the time course of changes in hemolymph osmotic pressure and branchial Na, K-ATPase ESA in crabs subjected to release of an osmoregulatory challenge (transfer from 5% to 50% SW). A preliminary account of some of the findings in this paper has been published as an abstract (McLaughlin and Holliday, 1994).

MATERIALS AND METHODS

Animals

Pseudosesarma moeschi imported by air freight from wholesale suppliers in Thailand are now commonly available as "Thai red crabs" in the North American pet trade. Most of the animals sold under this name are *P. moeschi,* but, in our experience, up to 20% of the crabs so offered for sale are other species. Adult male *P. moeschi* of 1-2 cm carapace width were purchased from The Pet Stop, Phillipsburg, New Jersey. In the laboratory crabs were maintained in 100 l aquaria on 3 cm of coarse sand for two weeks at room temperature (20-24°C) before use in experiments. The aquaria were slightly tilted so that the crabs could enter or leave 2 l of 10% artificial sea water (10% SW, 100 mOsm, "Instant Ocean", Aquarium Systems, Inc., Mentor, Ohio) at the lower end of the aquarium. Twice each week crabs were fed crushed dog food kibbles ("Gravy Train", General Foods, Inc., White Plains, New York) *ad lib.* and tank water was changed the next day. Only active, apparently healthy crabs which appeared to be in intermolt condition were used in this study. Mortality under maintenance conditions was 1% per week or less.

Crabs were acclimated completely submerged in 8 l of experimental sea water media (5%, 10%, 25%, 50%, 75% and 100% SW, where 100% SW = 1000 mOsm) for 14 d, except for the time course experiment, in which they were first acclimated 21d in 5% SW and then submerged for 0-14 d in 50% SW. In these experiments crabs were fed flake fish food ("TetraMin", Tetrawerke, Melle, Germany) *ad lib.* every 3-4 d. Mortality of experimental animals was less than 5% per week; most of the dead crabs appeared to have been killed and partially eaten by other crabs during molting.

Osmolality Measurement and Body Fluid Sampling

Sea water and hemolymph osmolalities were measured with a Wescor Model 5100C vapor pressure osmometer. Hemolymph was sampled by syringe through the arthrodial membrane at the base of a walking leg and quickly expelled onto a piece of plastic film. Osmotic pressure was measured immediately on an 8.00 µl aliquot of hemolymph placed on a filter paper disc in the osmometer; the total time that elapsed between expelling the sample onto the plastic film and placing the aliquot in the osmometer was approximately 5 seconds.

Chloride Ion Concentration Measurements

Sea water and hemolymph chloride ion concentrations were measured with a Buchler Instruments Model 442 coulometric titrator. Hemolymph samples were obtained as noted above for osmometry and 10.0 µl aliquots were immediately dissolved in 4.00 ml of "nitric/acetic acid reagent" (0.63 g nitric acid, 10.5 g acetic acid, 0.58 mg NaCl and 0.9 g polyvinyl alcohol, all per 100 ml solution) and titrated using the "low" range of the instrument.

Branchial Na, K-ATPase Assay

Crabs were killed by quickly removing the carapace (that is, the entire dorsum of the cephalothorax), taking care not to contaminate the gills with hepatopancreas tissue or gastric juice. The seven pairs of gills were excised, rinsed in homogenizing medium (HM; 0.250M sucrose, 6.00mM ethylene diamine tetraacetic acid) at 0-4°C and blotted dry on filter paper. Homogenates were prepared on ice using 20 strokes in a motor-driven, ground glass homogenizer (Kontes "Duall" #21 @ 200 RPM). Gills 1 and 2 are small and all four of these gills from each crab were combined for assay and homogenized in 0.500 ml HM. Gills 3-7 were assayed individually (only one of each pair) and homogenized in 0.500 ml HM. Protein concentrations in the crude homogenates varied between 0.1 and 0.5 mg x ml^{-1} and were measured colorimetrically at 595 nm using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, California) with bovine serum albumin as a standard.

The enzyme assay used to measure Na, K-ATPase enzyme specific activity (ESA) was identical to that of Holliday (1985). Briefly, phosphate liberated from ATP by each homogenate was measured in two media. One medium had optimum concentrations of all ions and cofactors (100 mM Na+, 30.0 mM K^+, 5.00 mM ATP, 10.0 mM Mg^{2+}, 20.0 mM imidazole, pH 7.20) and the activities of all ATPases present in the crude homogenate were assayed in this medium. The second medium lacked potassium and contained 130 mM Na^+, 5.00 mM ATP, 10.0 mM Mg^{2+} and 20.0 mM imidazole at pH 7.20; 1.00 mM ouabain was added to this medium to prevent stimulation of the Na, K-ATPase by K^+ present in the crude homogenates. Thus, the second medium assayed the activities of all ATPases present except the Na, K-ATPase. After incubation at 30°C for 15.0 min, the reaction was stopped with 1.50 ml of Bonting's reagent (560 mM H_2SO_4, 8.10 mM ammonium molybdate and 176 mM $FeSO_4$) and color was allowed to develop for 20 min. Phosphate concentration was measured colorimetrically at 700 nm as the reduced phosphomolybdate complex. Enzyme specific activity was calculated as the difference between phosphate (P_i) liberated by each homogenate in the two media and is expressed as µmol P_i x mg^{-1} homogenate protein x h^{-1}. All chemicals used for enzyme assays were reagent grade or better.

Statistical Analyses

Data are presented in the figures as mean values ± 1 standard error of the mean (SEM). Two-way analysis of variation with repeated measures (ANOVA, "PC-SAS", 1991 version) was used to determine the significance of effects of sea water concentration and gill number on branchial Na, K-ATPase ESA in crabs acclimated for 14d in the six SW media. Duncan's multiple range test ("PC-SAS", 1991 version) was used to compare mean Na, K-ATPase ESA's of individual gills from crabs acclimated in the various media. A probability (P) value ≤ 0.05 is considered to be significant. In the figures, error bars on symbols indicate ± 1 SEM; standard error values smaller than the symbols for the mean values are not shown.

RESULTS

Osmoregulatory Performance

The osmoregulatory performance of *Pseudosesarma moeschi* acclimated 14 d in various sea water media is shown in Figure 1. The crab's hemolymph was nearly isoosmotic in 50% and 75% SW, increasingly hyperosmotic to the medium in 25%, 10% and 5% SW, and hypoosmotic in 100% SW. Average hemolymph/external medium osmotic pressure gradients were 496 ± (16 (n=7) mOsm in 5% SW; 439 ± 9 (n=6) mOsm in 10% SW; 247 ± 14 (n=7) mOsm in 25% SW; 41 ± 15 (n=8) mOsm in 50% SW; -34 ± 18 (n=8) mOsm in 75% SW and -170 ± 13 (n=9) mOsm in 100% SW.

Chloride Ion Regulation

As shown in Figure 2, hemolymph chloride ion concentration closely follows the pattern seen above with hemolymph osmolality. The chloride ion gradient across the body wall is nearly zero in 50% SW (-15 mM ± 5 mM Cl^-) and increases progressively in the dilute media (102 ± 4 mM Cl^- in 25% SW; 162 ± mM Cl- in 10% SW; and 179 ± 5 mM Cl^- in 5% SW). In 75% and 100% SW,

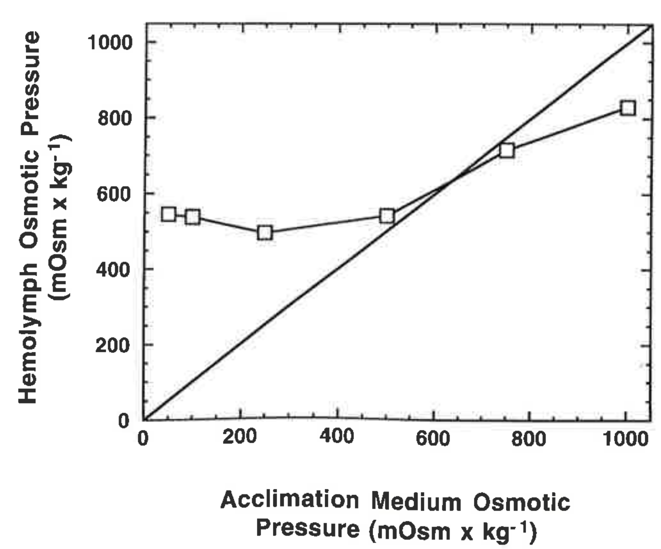

FIGURE 1. Osmotic performance of *Pseudosesarma moeschi* acclimated 14 d in 5%, 10%, 25%, 50%, 75% or 100% sea water (100% sea water = 1000 mOsm). Each symbol represents the mean value of measurements from 6-9 crabs; standard errors of the means are smaller than the symbols used. The solid line represents equal osmotic pressures in the hemolymph and in the external medium ("isoosmotic line").

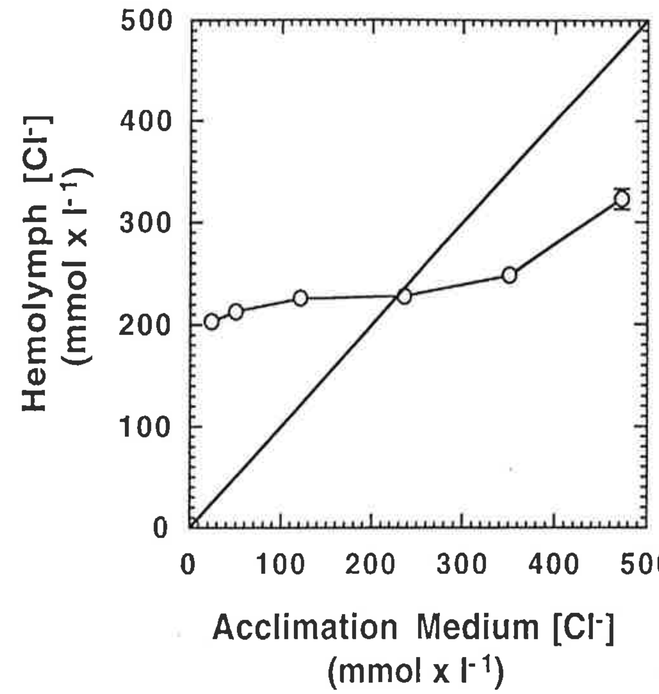

FIGURE 2. Regulation of chloride ion concentration in the hemolymph of *Pseudosesarma moeschi* acclimated 14 d in 5%, 10%, 25%, 50%, 75% or 100% sea water. Each symbol represents the mean value from 8-13 crabs; error bars indicate ± 1 SEM. The solid line represents equal ion concentrations in the hemolymph and in the external medium ("isoionic line").

P. moeschi increasingly hyporegulates chloride ion, with transepithelial gradients of -112 ± 8 mM and -165 ± 11 mM, respectively.

Branchial Na, K-ATPase ESA

Acclimation for two weeks in the six sea water media affected the branchial Na, K-ATPase of *P. moeschi* as shown in in Figure 3. In general, ESA was lowest in gills 1+2, 3 and 4 (hereafter referred to as the anterior gills) in all media, and the posterior gills (5, 6 and 7) had higher ESA's. Salinity of the acclimation medium had little effect on the ESA's of the anterior gills, but ESA in the posterior gills increased in crabs acclimated in 25%, 10% and 5% SW and in 100% SW compared to the values from crabs acclimated in 50% and 75% SW. Thus, both hyperosmotic and hypoosmotic regulation are associated with increases in branchial ESA in the posterior gills of *P. moeschi*.

Two-way ANOVA indicates that the ESA's for each gill pair differed significantly by gill number ($F_{5,729}$ = 42.29, P < 0.01) and that acclimation medium significantly affects gill ESA ($F_{5,226}$ = 13.09, P < 0.01). There is also a significant interaction between acclimation medium and gill number (F25,36 = 2.10, P = 0.024), indicating that the ESA's of some gills, the posterior ones, are affected more by salinity than others. Duncan's multiple range test indicates that in crabs acclimated in 5%, 25%

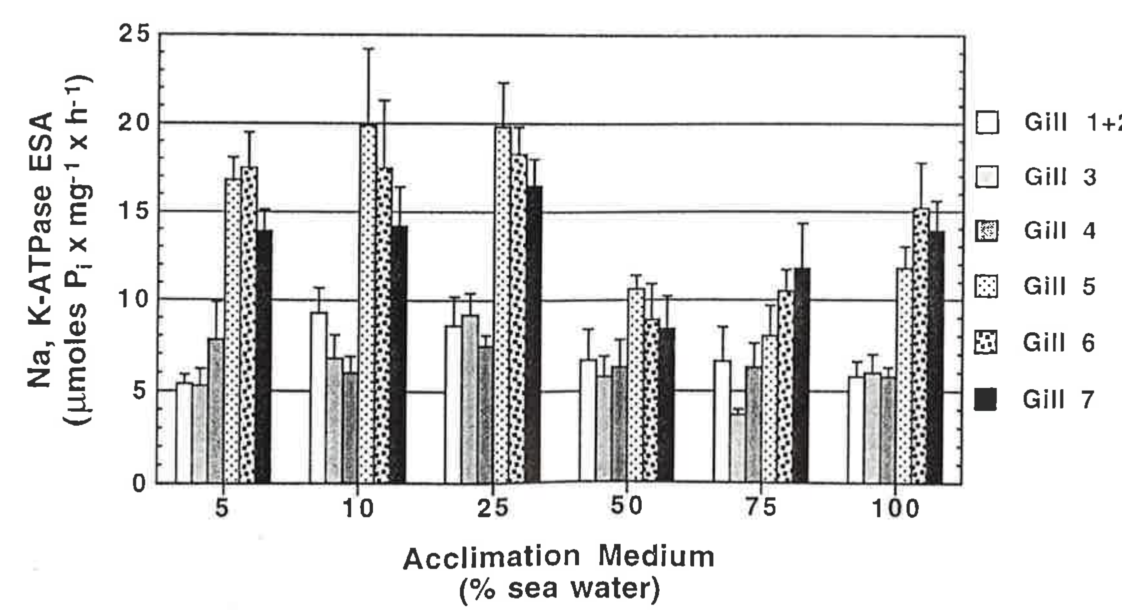

FIGURE 3. Na, K-ATPase enzyme specific activity (ESA) in homogenates of *Pseudosesarma moeschi* gills. Crabs were acclimated two weeks submerged in 5%, 10%, 25%, 50%, 75% or 100% sea water. Each symbol represents the mean value of homogenates from 6-9 crabs; error bars indicate ± 1 SEM. ANOVA indicates that: a) Na, K-ATPase ESA is significantly affected by external salinity in all gills ($F_{5,729}$ = 42.29, P < 0.01); b) ESA varies significantly by gill ($F_{5,226}$ = 13.09, P < 0.01); and c) that gill number and external salinity interact significantly ($F_{23,36}$ = 2.10, P = 0.024). Duncan's multiple range test showed that: a) in 5%, 25% and 100% SW, the ESA's of gills 5-7 are significantly larger than those in gills 1-4; b) in 10% SW the ESA's of gills 5 and 6 are significantly larger than those of gills 1-4 and that gill 7 ESA's are not; c) in 50% SW the ESA's of all gills except number 5 are not significantly different from each other and that gill 5 ESA is significantly larger than those of the other gills; and d) in 75% SW none of the gills have ESA's significantly different from each other.

and 100% SW, the posterior gills form a group that is significantly different from the anterior gills, which form a second group with significantly lower ESA's. In 10% SW, only gills 5 and 6 formed a group with significantly higher ESA's than the anterior gills and gill 7. In crabs acclimated in 50% SW all gills except number 5 formed a group which was significantly different from gill 5, which had a higher ESA than the other gills. Finally, in 75% SW, the gills formed a single group with no significant differences between the ESA's of any of the gills. Thus, the posterior gills seem to be most important in osmoregulation in *P. moeschi* in both dilute and concentrated media. Further, branchial ESA is minimal in 50% and 75% SW, media in which the crab performs minimal osmoregulatory work.

Time Course of Decreases in Branchial Na, K-ATPase in Isoosmotic Media

To determine the time course of decreases in branchial Na, K-ATPase ESA when the need for osmoregulation is removed, 5% SW-acclimated crabs were abruptly transferred to 50% SW and Na, K-ATPase ESA of the sixth gill and hemolymph osmotic pressures were measured for 14 d (Figure 4). Hemolymph osmotic pressure was not significantly affected by this treatment, indicating that the crabs were able to rapidly decrease the strong inward ion transport necessary for hyperosmoregulation in 5% SW. However, branchial Na, K-ATPase ESA measured *in vitro* did not decrease significantly from control values until between four and seven days after transfer. This time course is consistent with both rapid decreases in *in vivo* Na, K-ATPase activity in branchial ion transport cells in the short term and a longer term decrease in the amount of enzyme present in the cells (see Discussion).

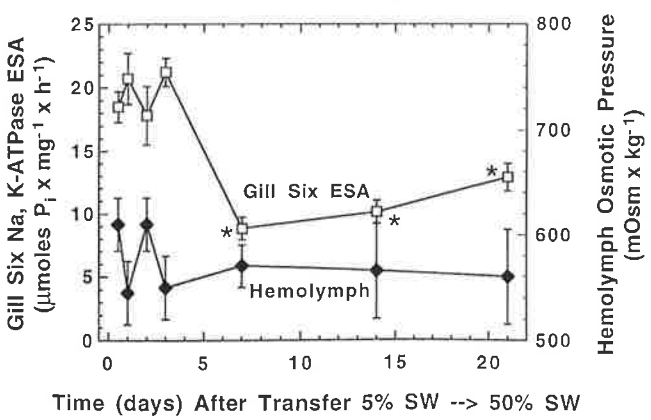

FIGURE 4. Time course of changes in Na, K-ATPase enzyme specific activity of gill 6 and hemolymph osmotic pressure in *Pseudosesarma moeschi* after transfer of crabs from 5% sea water to 50% sea water. Each symbol represents the mean value of homogenates or hemolymph samples from 5-6 crabs; error bars indicate ± 1 SEM. Asterisks indicate significant differences (Student's unpaired t test) between mean ESA's at time zero and at the time indicated. Hemolymph osmolality did not change significantly during the experiment.

DISCUSSION

The osmotic performance of *P. moeschi* acclimated submerged in sea water media in the present study is most similar to that reported for *Eriocheir sinensis* (DeLeersnyder, 1967), *Sesarma meineri* (Gross et al., 1966) and *Sesarma reticulatum* (Foskett, 1977). This pattern of hyperosmoregulation in dilute media and hyporegulation in 100% SW with an "isoosmotic" point of about 600-700 mOsm is unusual in crabs. Several euryhaline fiddler crabs show a similar pattern when they are allowed to enter or leave the acclimation media at will (Baldwin and Kirschner, 1976 a, b; D'Orazio and Holliday, 1985; Holliday, 1985; Rablais and Cameron, 1985). However, the isoosmotic point in *P. moeschi* is much lower than that of the fiddler crabs used in the studies cited immediately above (about 900 mOsm). The pattern of chloride ion regulation in *P. moeschi* is also unusual in crabs and it most closely resembles that reported for another Asian crab, *Barythelphusa guerini* (Venkatachari and Vasantha, 1981).

The data from our osmotic and ionic performance experiments show that both inwardly and outwardly directed ion transport are under physiological control in *P. moeschi* and they justify a search for changes in branchial ion transport enzyme activity (*e.g.*, the Na, K-ATPase) which might power these processes. As expected, branchial Na, K-ATPase ESA increased in crabs acclimated in increasingly dilute sea water media. Further, the posterior gills of *P. moeschi* (numbers 5, 6 and 7) have higher branchial ESA's, underscoring the importance of the crab's posterior gills in osmoregulatory ion transport. This phenomenon has been reported in several other crabs (reviewed by Towle, 1984; Taylor and Taylor, 1992). An unexpected finding in the present study was increased ESA in the posterior gills of crabs acclimated in 100% SW, a medium in which they, unlike most other crabs tested, hypoosmoregulate. We know of only one other crab, *Uca tangeri* (Drews, 1983) which shows this pattern of changes in branchial ESA, a pattern which is similar to that shown by some teleosts (*e.g., Cyprinodon salinus,* Stuenkel and Hillyard, 1980) and which implicates the sodium pump in both inward and outward ion transport by the gills of these animals.

Although the posterior gills of crabs are clearly important in ion transport, the anterior gills of many crabs, but not *P. moeschi,* also show significant changes in Na, K-ATPase ESA when the animals are subjected to low salinity stress (*e.g.*, Péqueux and Gilles, 1977; Neufeld et al., 1980; Holliday, 1985; Corotto and Holliday, 1996, in press) and it seems likely that these gills may also play a significant role in ion transport, particularly in semiterrestrial crabs such as *Eriocheir, Hemigrapsus* and *Uca.*

When osmoregulatory stress was removed by transfer of crabs acclimated in 5% SW to an nearly isoosmotic medium of 50% SW, branchial Na, K-ATPase ESA measured *in vitro* showed no significant decrease until 4-7 after transfer (Figure 4). Thus, it is clear that long term reductions in salt uptake from the medium are associated with decreased activity of branchial Na, K-ATPase ESA, probably by enzyme degradation in the basolateral membrane of the gill ionocytes or by regression of the ionocyte patches. The opposite processes, branchial ionocyte proliferation (Copeland and Fitzjarrel, 1968; Aldridge and Cameron, 1982), and increased Na, K-ATPase activity within the growing branchial ionocyte patches (Neufeld et al., 1980) have been reported in blue crabs, *Callinectes sapidus*, acclimating to dilute media. However, because hemolymph osmotic pressure was essentially unchanged after transfer of crabs from 5% SW to 50% SW in the present study, it is clear that rapid, *in vivo* reduction of inward ion transport by exiting sodium pumps in the gills of *P. moeschi* also occurs. It may be that these short term changes occur, as in cultured mammalian cells, by stimulation of Na, K-ATPase activity by changes in intracellular Na^+ concentration (reviewed by Pressley, 1988) and/or by addition of K^+ "leak" channels in the basal-lateral cell membranes (reviewed by Schultz and Hudson, 1986). Robinson (1994) has recently demonstrated rapid modulation of unidirectional ^{22}Na fluxes across the gills of *C. sapidus* subjected to acute changes in external salinity.

With regard to control of changes in branchial ion transport, Lohrmann and Kamemoto (1987) and Sommer and Mantel (1988) have reported, respectively, that in the crabs, *C. sapidus* and *Carcinus maenas,* branchial ^{22}Na influx is rapidly changed by injected dopamine, pericardial organ extracts and dibutryl cyclic AMP. Sommer and Mantel (1988) also reported increased branchial Na, K-ATPase ESA with injected cAMP and, in a second study, showed that branchial cAMP concentrations were increased by low-salinity stress (Sommer and Mantel, 1991). Recent work also suggests that crab branchial Na, K-ATPase ESA may be controlled by gill tissue concentrations of polyamines (Lovett and Watts, 1995). Schwarz and Graszynski (1989) have developed a split-lamella, Ussing-type gill preparation which is proving to be useful in the *in vitro* study of the mechanisms and physiological control of ion transport in crab gills (reviewed by Onken et al., 1995). Using this preparation, Riestenpatt et al. (1994) have found that cyclic AMP stimulates sodium uptake in gill lamellae from freshwater-acclimated *E. sinensis* by increasing apical sodium channel number and apical sodium conductance. Thus, both the apical and basolateral transport of sodium seem to be under physiological control.

ACKNOWLEDGMENTS

We thank Dr. Raymond B. Manning, National Museum of Natural History of the Smithsonian Institution, USA, and Dr. Peter K.L. Ng, Department of Zoology of the National University of Singapore, for identifying *Pseudosesarma moeschi*. We also thank Dr. Phailbul Niayanetr of The Faculty of Science, Chulalongkorn University, Thailand, for providing information on the distribution and habitat of *P. moeschi.*

This study was funded by Lafayette College.

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