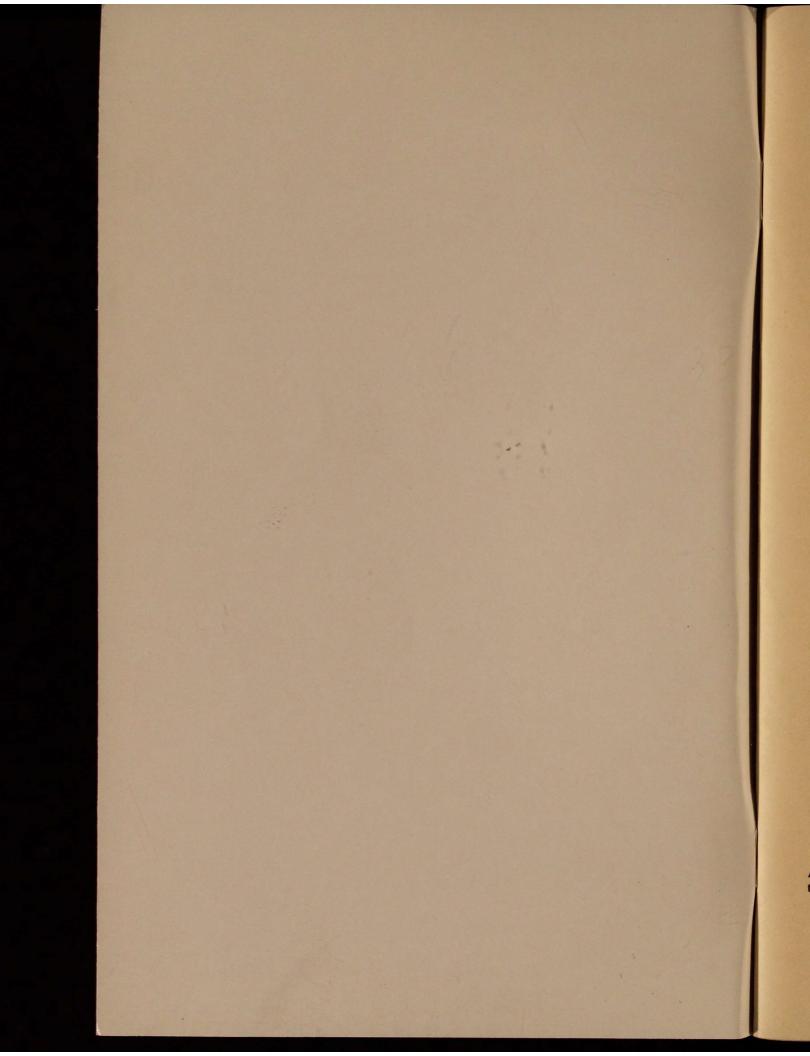
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Papers of the Sixty-Ninth Annual Session







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Papers of the 1994 Annual Meeting

NEW RECORDS FOR THE LAND SNAIL TRIODOPSIS PLATYSAYOIDES, A WEST VIRGINIA ENDEMIC

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ABSTRACT

Triodopsis platysayoides was described by Brooks in 1933 from a specimen collected at Coopers Rock. Monongalia County. When this species was listed as federally threatened in 1978, it was still known only from the vicinity of Coopers Rock. Surveys by WV Division of Natural Resources and U.S. Fish and Wildlife Service employees increased the number of localities for this species to 7 before the publication of the T. platysayoides recovery plan in 1983. All seven sites were associated with sandstone outcrops and boulders within the Cheat River Gorge near Coopers Rock. In subsequent years, 34 sites south of Interstate Highway 68 (I-68) were visited by WVDNR personnel and student assistants from West Virginia University, Morgantown. These surveys identified 11 additional locations for this snail. These 18 sites are located in a 3.4 km X 5.4 km region of Monongalia and Preston counties south of I-68. One additional site was discovered approximately 10.3 km southeast of the nearest known location. This site is associated with a cave in the limestone stratum below the sandstone. All known sites for this species are found in the Cheat River Gorge or in the steep valleys of its tributaries; no site is more than 1.88 km from the Cheat River. Sites range from 396 m to 622 m in elevation. Thirty-eight sites north of I-68 were surveyed in 1991; no T. platysayoides were found.

The flat-spired three-toothed land snail was described as <u>Polygyra platysayoides</u>, based on a specimen collected at Coopers Rock Overlook, Monongalia County, WV, by Brooks in 1933. Although the taxonomic status of the species was questioned, Pilsbry (1940) considered it to be a distinct species, but transferred it to the genus <u>Triodopsis</u>. Vagvolgyi (1968) considered <u>T. platysayoides</u> to be a subspecies of <u>T. complanata</u>, but Solem (1974,1976) suggested that <u>T. platysayoides</u> was a good species. Emberton (1988) recognized <u>T. platysayoides</u> as a valid species on

the basis of the morphology of uneverted penial tubes and electrophoresis utilizing 12 enzyme systems.

In 1974, the species was still known only from the type locality, and Solem (1974) stated that <u>T. platysayoides</u> was one of six land snails in eastern North America he considered "immediately endangered." In 1978 the U.S. Fish and Wildlife Service (USFWS) added the species to the list of federally threatened animals (Fed. Reg. 1978).

Surveys by WV Division of Natural Resources (WVDNR) and USFWS biologists following the listing of the species located additional populations of <u>T. platysayoides</u> in the vicinity of Coopers Rock (WVDNR, unpub. data). In 1981 the known range was extended approximately 1.4 km upstream (east); the first population on the opposite (south) side of the gorge was discovered in 1982. When the recovery plan for the species (USFWS 1983) was published, seven locations for the species were known. All sites were in the vicinity of Coopers Rock and associated with cliffs and boulders of the Upper Connoquenessing Sandstone (Pottsville Series) (Hare 1957). Limited surveys in 1983 through 1987 failed to located new populations.

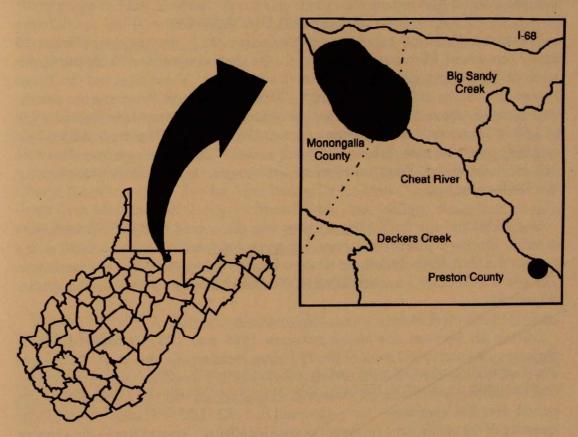


Figure 1. Known range of the land snail <u>Triodopsis platysayoides</u> in Monongalia and Preston counties, West Virginia.

METHODS

Using a helicopter, WVDNR biologists observed the Cheat River gorge area near Coopers Rock in "leaf off" condition 1983. Potential habitat for <u>T</u>.

platysayoides (cliff and boulder areas) was identified and marked on 7.5' topographic maps. WVDNR biologists and students from West Virginia University, Morgantown, visited known localities and 34 areas of potential habitat for this snail in 1988 and 1989; all sites were located south of Interstate Highway 68 (I-68). Survey methods included looking for snails on the surface of rocks and leaf litter, examination of leaf litter for snails hidden within the litter, and examination of crevices and small caves using flashlights. The distinctive shell morphology of this species made it possible to identify specimens in the field. Thirty-eight additional sites located in the Cheat Gorge north of I-68 were searched in 1991 by Thomas Pauley and graduate students from Marshall University, Huntington, WV.

All T. platysayoides located were identified as adult (shell possessing an aperatural lip) or immature (shell not possessing an aperatural lip). Data from a captive population indicate that these snails can develop the lip during their hatch year, but most do not develop it until their second year (Stihler, unpub. data). The greatest diameter of the shells of most snails was measured using vernier or dial calipers. During the early surveys, the greatest diameter of adult snails was measured including the aperatural lip; during later surveys, greatest diameter was recorded both including and excluding (just posterior of) the lip.

RESULTS AND DISCUSSION

During the 1988-1991 surveys mentioned above, 91 T. platysayoides (79 live, 12 dead) were found. Eleven new localities for the species were identified; live specimens of T. platysayoides were found at 10 of the sites. These sites and the 7 sites known previously, are located within 1.88 km of the Cheat River and are associated with the Upper Connoquenessing Sandstone. The 18 sites are located south of I-68 in Monongalia and Preston counties (Figure 1) and can be bounded by a rectangle approximately 3.4 km X 5.4 km in size. The sites range from 396 m to 622 m in elevation. Empty shells found during the surveys were placed in the WVDNR collection.

One additional site for <u>T. platysayoides</u> was discovered by WVDNR biologists in 1986. During a winter bat survey, <u>T. platysayoides</u> was found associated with a cave in the Greenbrier limestone stratum located below the Pottsville sandstone. The site is located 10.3 km southeast of the nearest known locality for the species (Figure 1).

During all surveys conducted between 1983 and 1993, a total of 223 <u>T. platysayoides</u> was found at 18 of the 19 known locations for this snail. At one site this snail is known only from one empty shell, but live specimens were found at the other 17 sites. At the only site where <u>T. platysayoides</u> was not found during this period, five live specimens were observed in 1982 (USFWS, unpub. data). The majority of the snails were found at the type locality at Coopers Rock; the number of specimens found at the other sites ranged from 1 to 13 individuals. Size range and mean diameter are given in Table 1. Because of the small sample size at all sites except the type locality, statistical comparisons were not made.

Table 1. Range, mean, and standard deviation of greatest diameter measured on specimens of the lands snail <u>Triodopsis platysayoides</u> from Monongalia and Preston

counties, West Virginia, 1983-1993. All measurements in millimeters.

and the same of	MEAN	STD.	N	RANGE
Adult (diamterer including lip)	22.2	1.20	71	19.4 - 25.1
Adult (diameter without lip)	20.9	1.00	66	19.0 - 23.2
Immature	14.2	4.69	103	5.0 - 22.8

Although recent survey efforts have increased the known range of <u>T. platysayoides</u> the species is still known from only 19 populations in a restricted area of the Cheat River gorge in West Virginia. It is likely that future studies will locate additional populations, but it is also likely that such efforts will confirm that this species occupies a restricted geographic range. Because of this, classification as "federally threatened" is still warranted.

ACKNOWLEDGMENTS

Funding for these studies was provided by the U.S. Fish and Wildlife Service through Section 6 of the Endangered Species Act and the West Virginia Division of Natural Resources' Nongame Wildlife Fund. The author wishes to thank all persons who assisted in conducting surveys for this snail including Kenneth Knight, Cheryl Hamilton, Bryce Findle, John Young, Dr. Thomas Pauley and graduate students from Marshall University, Andrew Moser, Jack Wallace, Scott Butterworth, and Donna Mitchell.

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Freshwater Bivalves in Elk River, West Virginia with Emphasis on

Federally Endangered Species

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ABSTRACT

During 1991 and 1992 an extensive survey was conducted of the unionid bivalve population in the Elk River. The purpose was to determine the status of three federally endangered species, *Pleurobema clava* (Lamarck, 1819), *Epioblasma torulosa rangiana* (Lea, 1839), and *Lampsilis abrupta* (Say, 1831). Twenty-one stations were surveyed in the Elk River below Sutton Lake, and three stations above. Methods used were water scopes, snorkeling, scuba, and bank/midden searches. The Elk River maintains a very diverse and abundant bivalve population throughout most of its length below Sutton Dam (24 species). A limited population exists above Sutton Lake (6 species). Live individuals of *P. clava* were found at six of the 21 stations surveyed below the dam, and reproduction was evident. No specimens of *E. t. rangiana* were collected. Recently however, two live individuals of *E. t. rangiana* were located at one site in the lower river. Limited numbers of *L. abrupta* were found inhabiting the lower reaches of the river although no evidence of reproduction has been seen.

INTRODUCTION

From August 1991 through May 1992 an extensive survey was conducted of the unionid bivalve population of the Elk River, West Virginia. The primary purpose of the survey was to determine the status of three federally endangered species, *Pleurobema clava* (Lamark, 1819), *Epioblasma torulosa rangiana* (Lea, 1858)(both listed in 1993), and *Lampsilis abrupta* (Say, 1831). Taylor and Hughart (1981) made the only other recorded extensive survey of the Elk River's bivalve fauna in 1978 and 1979. At that time Taylor reported on numerous collections that had been deposited at various museums. Most of this historic work was conducted over 70 years ago by Dr. A. E. Ortman of the Carnegie Institute, Pittsburgh, PA.

The Elk River, or "Tiskelwah" (river of the fat elk) as the native Americans called it, originates in Pocahontas County, West Virginia. It flows approximately 291 km to its confluence with the Kanawha River in Charleston, Kanawha County, West Virginia. The majority of the mainstem flows through Webster, Braxton, Clay, and Kanawha counties. The river falls from an elevation of about 1219 m in Pocahontas County to 172 m at its mouth. The principle rock types within the basin are sandstone and shale with numerous beds of coal and limestone. The four largest tributaries on the Elk River are Big Sandy Creek, Buffalo Creek, Birch River, and Holly River.

In 1961 Sutton Dam (Braxton County) was completed by the U.S. Army Corp of Engineers on the Elk River, approximately 160 km above its mouth. The reservoir was constructed to provide flood control, pollution abatement, fish and wildlife habitat and general recreation. Sutton dam had a coldwater release prior to 1980 at which time a multilevel release was completed (Jim McCormik pers. comm., 2 Feb 94, USACOE, Sutton Lake) returning the Elk River nearer to its more natural pre-impoundment temperature regime.

Methods

Site selection was initially based on the historic sites as listed by Taylor and Hughart (1981). After these sites were surveyed, additional sites were chosen based on accessibility and habitat (shoal areas). The lower reaches of the river below Clendenin were not surveyed due to the availability of time. A qualitative survey of the unionids was done using waterscopes, snorkeling, scuba, and midden searches. Water scopes and snorkeling were the primary methods used. Once live individuals were identified and enumerated they were returned to the substrate. Taxonomy used was based on Turgeon, et al. (1988).

Results

Twenty-one stations were surveyed in the Elk River below Sutton Lake and three stations were surveyed above (Table 1, Figure 1). A total of 25 species were collected, with 24 being found below Sutton Lake and 6 above (Table 2). Two species [Villosa fabalis (Lea, 1831) and Epioblasma triquetra (Rafinesque, 1820)] not previously reported were represented only by weathered empty shells collected from muskrat middens. They probably were present during previous studies but were not collected due to their small size.

The largest number of individuals were collected at Station 2 (1552) and Station 3 (1469). The highest species abundance (20) was found at Station 1.

Pleurobema clava was collected alive at six stations and empty shells were found at three additional stations. At the most downstream station where P. clava was found (Station 5) only empty shells were collected. At Station 3 several fresh dead shells were collected from a muskrat midden, including several small shells indicating reproduction. The uppermost site where P. clava was collected (Station 17), resulted in only four live individuals. The abundance of P. clava was probably under represented due to our collection techniques and the burying nature of this species.

The status of the other two federally endangered species is more questionable. E. t. rangiana was not collected during this survey and only one individual of L. abrupta was collected at Station 1.

Habitat degradation was very evident throughout the Elk River. For example, at Station 6, only three live bivalves were collected in the riffle/island complex immediately below the confluence with Buffalo Creek. Buffalo Creek carries a heavy load of sand and the Elk River in this area is heavily embedded (rocks cemented together with the deposited sand). Immediately above Buffalo Creek a diverse bivalve fauna was collected (13 species) including the most downstream collection site for live *P. clava*.

Discussion

In 1982 and 1983 a water quality survey was conducted on the Elk River Basin

(WVDNR 1985). At that time water quality of the river basin was generally characterized as excellent. Most of the water quality standards violations were due to high fecal coliform bacteria from domestic sewage and agricultural runoff. During the 1991-92 unionid survey, several open sewers were still evident along the river. Coker, et al. (1921) found healthy, even exaggerated growth of mussels in water influenced by sewage. However, Grantham (1969, cited in Fuller 1974) suggested that with too great enrichment, water bodies develop increased bacterial oxygen demand, increased carbon dioxide, and lowered pH. Many sources of organic pollution also include toxic substances such as heavy metals which can also be detrimental to bivalve populations. The 1982-83 water quality survey also found violations of water quality standards for dissolved oxygen, phenols, and several heavy metals such as iron, manganese, and lead. Most notable were 19 violations for the pH standard (WVDNR 1985). All violations were below a pH level of 6.0.

Timbering, oil and gas extraction, and coal mining have existed throughout the Elk River drainage basin for years. It is the author's belief that discharges from coal mining activities have long been detrimental to the bivalve population in the river. Buffalo Creek, which is one of the four largest tributaries to the Elk River, was noted as having a pH of 5.1 at its mouth in August of 1983 (WVDNR 1985). It is this reduced pH along with the high sediment load in Buffalo Creek which is probably the cause for the reduced bivalve fauna below the confluence. Many of the bivalves observed during the survey, primarily downstream of site 11 (mouth of O'brion Creek), appeared to be extremely "eroded" at any point where the periostricum had been removed. Simpson (1899, cited in Fuller 1974) stated that acid could eat holes through the shell to the animal and a few live bivalves located during this survey were found with holes eaten completely through the nacre.

Fuller (1977) reported that siltation associated with poor agricultural practices and deforestation of much of North America was probably the most significant factor impacting mussel communities. This was evident at the mouth of Strange Creek (Station 20). The substrate of the river below the confluence of Strange Creek was good for bivalves but highly embedded with silt and sand. Although Strange Creek watershed has been timbered extensively over the past decade, the heavy sand load evident in the river immediately below Strange Creek was probably caused by timbering in the early 1900's or in part due to natural erosion (Doug Wood, pers. comm., WVDEP, MacArthur, WV). Excluding the embeddedness, the available habitat for bivalves was much greater below Strange Creek than above, yet densities of bivalves were greater above than below. Other areas of high embeddedness were evident throughout the Elk River.

Another impact that was quite evident on the Elk River was a pipeline crossing at Station 19. While 11 species of unionids were observed above the pipeline crossing, not a single unionid was found within the suitable riffle habitat below the crossing. Such impacts are becoming more of a concern as the number of requests

for permits to place pipelines across the Elk River increases.

Taylor and Hughart's (1981) 1978-79 bivalve survey of the Elk River took place after the river had been under approximately 20 years of abnormally low temperatures due to the coldwater release on Sutton Dam. While some unionids were able to survive this period, reproduction may have been slowed or ceased completely depending on species. USFWS (1993) suggests that abnormally low water temperatures, such as found below dams, may prevent reproduction from ever taking place. Arey (1921, cited in Fuller 1974) suggests low temperatures dull glochidial

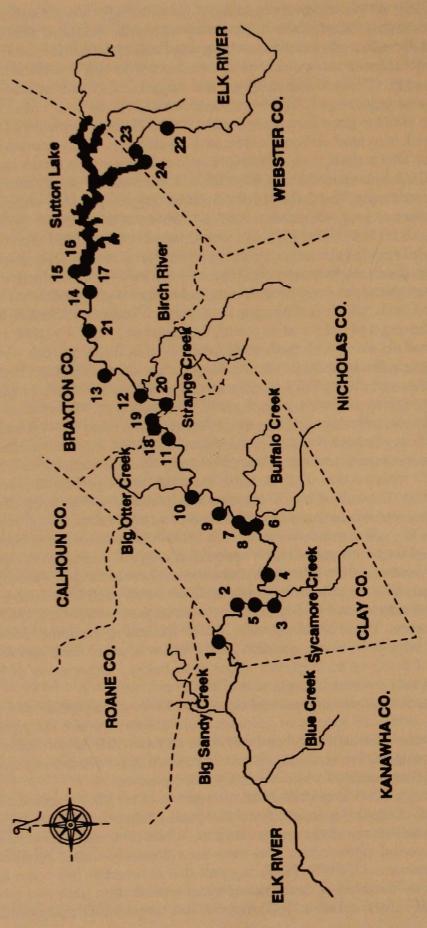


Figure 1. Location of freshwater unionid survey stations on the Elk River, West Virginia, 1991-1992.

Table	1. Station August 1	locations for	r the Elk River unionid survey conducted from May 1992.		
Stat	Latitude	Longitude	Station Location		
1	38°30'20''	81°14'55''	Kanawha Co. approx. 1/2 mi blw King Shoals Ck at a large island		
2	38028.25	81611.50	Clay Co. mth Twistabout Ck upstream to abv		
3	38°25'55''	81°12'00''	Clay Co. large island blw Sycamore Ck		
4	38°27'05''	81009.05	Clay Co. island area abv bridge, jct CRS & 18, Elkhurst		
5	38º27 '15''	81611.35	Clay Co. approx. lmi upstream of Laurel Ck at riffle just blw Jack Bend		
6	38°27'40''	81004.52	Clay Co. island area at Clay, mth Buffalo Ck, jct CR16 & 11		
7	38º29'00''	8103.30	Clay Co. island 1.9mi abv Clay Junction, CR16/4		
8	38°28'10''	81°04'45''	Clay Co. island 1/2mi N of Clay on CR16		
9	38°29'45''	80004'20''	Clay Co. mth Spread Ck, CR 16/4, 4.1mi abv Clay Junction		
10	38032'00''	81001'55''	Clay Co. island abv bridge, mth Big Otter Ck at Ivydale		
11	38°33'55''	80°57'15''	Clay Co. mth O'brion Ck, jct CR44 & 4		
12	38°35'25''	80°53'20''	Braxton Co. blw mth Birch River CR4 Glendon		
13	38°38'10''	80°51'45''	Braxton Co. abv jct CR4 & 21 at Frametown		
14	38039'25''	80044'35''	Braxton Co. blw 179 bridge, Gassaway exit		
15	38°40'00''	80°43.05	Braxton Co. CR4, behind Sutton GoMart		
16	38°39'40''	80°41'45''	Braxton Co. riffle area below Sutton Dam		
17	38039'45''	80042'20''	Braxton Co. approx. 0.75mi blw Sutton Dam		
18	38°35'10''	80°56'10''	Braxton Co. mth Duck Ck, jet CR4 & 11		
19	38°35'05''	80°55'40''	Braxton Co. mth Laurel Fk at pipeline crossing, near jct CR4 & 13/2		
20	38°33'55''	80°54'00''	Braxton Co. mth Strange Ck jct CR4 & 40		
21	38039'25''	80048'15''	Braxton Co. mth Sugar Ck behind church, CR4		
22	38035'55''	80029.20	Webster Co. blw 1st bridge abv Braxton Co. line, CR7		
23	38°37'00''	80°31'55''	Webster Co. blw Bear Rn, CR7 near Co. line		
24	38°37'05''	80°33'50''	Braxton Co. riffle abv Bakers Run Camping area, CR17/10		

Station numbers are in order of sampling time.

Species composition of unionid bivalves collected from the Elk River from August 1991 through May 1992 (Stations listed in ascending stream order). Table 2.

IISICO III ASCEIIC	chung sucam order).	35	3	5	130																			
Station	1 2	1100	5 3	3 4	9 1	00	7	6	10	=	18	19	20	THE RESERVE	12	13	21	14	15	17	16	77	23	22
Alasmidonta marginata			343														100					0	a	•
Anodonta grandis																								
Strophitus undulatus																			D					
Lasmigona c. costata			17		*	1		*				*					*		*					
Quadrula pustulosa				-	•						*	-							*					
Amblema plicata																								
Fusconaia subrotunda						*					*		The same of											
F. flava		-	-			*	*									0								
Cyclonaias nuberculata																								
Pleurobema clava		-	IC	0									0											
Elliptio crassidens					•				*			•												
E. dilitata						*	*					•												
Prychobranchus fasciolaris						*				*		•												
Actinonaias ligamentina *						*	*												*		D			
Obovaria subrotunda	:		-		0					0	D	0						Q						
Potamilus alatus																								
Ligumia recta	:	-						*	*		0	•	0											
Villosa fabalis		0	0																					
V. iris	•																							
Lampsilis siliquoidea		-	-							*														
L. abrupta																								
L. cardium	:				*																			
L. fasciola	:	0	-		*																	0		Q
L. ovata					*																			
Epioblasma triquetra [DD	-	D	-																				
D = empty shells only					7																			1

response to opportunities for infection. Historic records indicated four species of unionids immediately below Sutton Dam [Amblema plicata (Say, 1817), Fusconaia subrotunda (Lea, 1831), P. clava, Obovaria subrotunda (Rafinesque, 1820)] (Taylor and Hughart 1981). Taylor and Hughart (1981) did not find any live unionids at their site located 60 yards below Sutton Dam in 1978-79. The present survey found 22 live individuals of three species of unionids [A. plicata, Ligumia recta (Lamarck, 1819), Lampsilis cardium (Rafinesque, 1820)] immediately below Sutton Dam; one relic shell of Actinonaias ligamentina (Lamarck, 1819) was also found. No recent reproduction was evident below the dam. Approximately 1200 m below Sutton Dam, 12 species of live unionids were found. Except O. subrotunda, all species that were historically found below the dam were collected at this site. This was the uppermost site for the collection of P. clava as well as a new record for the river with the collection of Anodonta grandis (Say, 1829).

The recovery plans for the federally endangered clubshell (P. clava) and northern riffleshell (E. t. rangiana) suggest that P. clava was once widespread and abundant throughout the Ohio River drainage. It appears that existing populations throughout the drainage are at the edge of the original range (USFWS 1993). Historical records, some 50-100 years old, reported in Taylor and Hughart (1981) show P. clava in at least 11 sites throughout the Elk River below Sutton Dam. By 1978 and 1979 Taylor and Hughart (1981) found P. clava only at one of the 15 sites they surveyed. In this present study, P. clava was found alive at five historic sites with relics and/or fresh dead shells found at an additional two historic sites.

The northern riffleshell has never been known to be widespread throughout West Virginia (Files, WVDNR) although the federal recovery plan (USFWS 1993) suggests that this species ranged similar to that of P. clava and further north. Historical records reported by Taylor and Hughart (1981) indicate E. t. rangiana at only two sites on the Elk River and failed to collect any during their 1978-79 survey. During the 1991-92 survey E. t. rangiana was not found at any site. The only recent record of E. t. rangiana in the Elk River is by Ecological Specialists, INC who found two live individuals approximately nine years of age while doing a survey for a proposed pipeline crossing (ESI 1993).

The recovery plan for the federally endangered Pink Mucket Pearly Mussel (L. abrupta) suggests that it was once widespread throughout the Ohio River drainage but was never found in abundance (USFWS 1985). Historical records reported by Taylor and Hughart (1981) showed L. abrupta at only one site below King Shoals. In 1978 and 1979, Taylor and Hughart did not find any individuals of L. abrupta in the Elk River. In 1993 L. abrupta was found in the lower reaches of the river near Clendenin (files WVDNR, Elkins, WV) and since L. abrupta is primarily a large river species it is only expected to be found in this lower portion of the river which

was not extensively surveyed in 1991-92.

Historical records indicated 21 species of unionids in the Elk River. Of the 21, Pleurobema coccineum (Conrad, 1834), T. truncata (Rafinesque, 1820), and E. t. rangiana were not collected during this survey. However, in addition V. fabalis and E. triquetra, A. grandis, Potamilus alatus (Say, 1817), and Lampsilis siliquoidea (Barnes, 1823) were collected yet were not historically reported prior to Taylor and Hughart's (1981) work when they did collect L. siliquoidea in the summers of 1978 and 1979. Stein (1990) also collected P. alatus, and L. siliquoidea above Queen Shoals.

There appears to be a few unionid species on the brink of or already extirpated

from the Elk River. One that appears to be extirpated is T. truncata. This species has not been collected alive or dead in any surveys conducted probably in the last 25 years. E. triquetra has been represented by weathered shells as has V. fabalis. P. coccineum was probably on the outer limits of its range when collected in the lower reaches of the river. As this survey did not extend into the lower river reaches, any inferences made about this species would be misleading. Elliptio crassidens (Lamarck, 1819) was found at 12 of the 21 sites surveyed below Sutton Dam. E. crassidens was most abundant at Station 3 (114) and Station 21 (43). While decent populations of E. crassidens were found at several sites, no reproduction (small shells) was evident. The fish host known for E. crassidens is the skipjack herring [(Alosa chrysochloris (Rafinesque, 1820)]. While this fish is generally not known from the Elk River it is fairly common in the Ohio mainstem and has shown no indication of declining populations. It is generally a big river fish and if found in the Elk River, would be mainly in the lower reaches (Dan Cincotta, pers. comm. 24 Feb, 1994, WVDNR, Elkins, WV). It therefore appears that E. crassidens may have another fish host or that the skipjack herring did indeed range much farther than is presently believed.

Conclusions

The Elk River still maintains a very diverse and abundant bivalve population throughout its entire length below Sutton dam and a limited bivalve-population above Sutton Lake. Over the last several years, improvements have been made in the water quality of the river. It is this author's belief that returning the river to its normal temperature regime by modification of Sutton Dam played a large role in minimizing the loss and allowing for recovery of the unionid population. P. clava appears to be surviving and reproducing although any increased pollution and/or habitat degradation may be very detrimental to this species' existence. This survey did not find any individuals of E. t. rangiana although ESI consultants found two individuals inhabiting the lower reaches of the river. This species is severely threatened with extirpation from the Elk River. This survey and recent surveys conducted by other individuals found L. abrupta inhabiting the lower reaches of the river although no evidence of reproduction has been found. It appears this species is at the limit of its historical range within the Elk River. Though the water quality of the Elk River is presently considered good, any increase in pollutants and more importantly in habitat destruction via increased sedimentation, dredging, filling, pipeline construction, etc., could place the bivalve fauna once again in jeopardy.

Acknowledgements

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Acute respiratory responses of larval fathead minnows (<u>Pimephales promelas</u>) and swordtails (<u>Xiphophorus helleri</u>) to low pH.

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We investigated the respiratory responses of larval fathead minnows and sword-tails to low pH. Fish were placed in a buffered pH solution (pH 5 to 8) of reconstituted soft water at 25°C. After 2 hours initial exposure, their rate of oxygen consumption was measured for 2 hours in a Gilson Differential Respirometer. On a dry weight basis, the mean respiratory rate of fathead minnows on day-of-hatch was twice that of swordtails on day-of-birth. There was no significant difference in the mean respiratory rate (per mg dry weight) between 1-day-old and 30 day-old swordtails. Low pH negatively affected respiration. The magnitude of the respiratory response varied between the two species and with age in swordtails.

Introduction

The physiological effects of acid stress have received much attention in recent years (Fromm 1980; Peterson et al. 1982; Wood and McDonald 1982; Wood 1989). Several physiological and morphological effects relating to respiration have been noted in larval fishes exposed to acid waters, including abnormal gill development (Peterson et al. 1982), disruption of ionic uptake by chloride cells located in the gills (Wood et al. 1990), and acidosis (Ultsch et al. 1980). Research on ventilation (Neville 1979) and oxygen consumption rates (Ultsch 1978; Ultsch et al. 1980) in adult fish demonstrate respiratory responses to acid waters. The embryolarval and early juvenile life stages of fishes are generally the most sensitive to environmental toxicants (McKimm 1977; Van Leeuwen et al. 1985). Sensitivity to low pH stress also increases during and immediately after hatching (Daye and Garside 1979; Peterson et al. 1980).

The purpose of this study was to evaluate a methodology to measure the respiratory response of larval fishes to pH. Fathead minnows (<u>Pimephales promelas</u>) and swordtails (<u>Xiphophorus helleri</u>) were studied because these two species are conveniently propagated in laboratory culture and their reproductive strategies differ (Lagler et al. 1962). The fathead minnow is oviparous and lays eggs in a nest that the male guards. The swordtail is ovoviviparous; embryonic development takes

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place within the body cavity of the female and the young are born alive. While both species demonstrate forms of parental care, the two strategies result in distinct differences in weight and levels of development at hatch/birth. The objectives were: (1) to evaluate the effectiveness and suitability of buffering test solutions to stabilize pH, (2) to evaluate the effectiveness of measuring larval fish respiration via manometric techniques, and (3) to measure the respiratory response of larval fishes to pH stress.

Methods

Fathead minnows and swordtails were cultured in the laboratory at the West Virginia Cooperative Fish and Wildlife Research Unit, Morgantown, West Virginia. Test organisms included 1-day-old fathead minnows and 1-day-old and 30-day-old swordtails.

Larval fish were randomly placed in 50-ml beakers filled with pH-buffered, reconstituted soft water at 25°C. The pH of the test solutions was buffered with 0.05 M 2-(N-morpholino)ethansulfonic acid (MES) or 3-(N-Morpholino)propanesulfonic acid (MOPS). The initial pHs of MES buffered test solutions were pH 5.0, 5.5, 6.0, 6.5, or 7.0; those of MOPS buffered were pH 6.0, 6.5, 7.0, 7.5, and 8.0. Fish and test solution were transfered to manometer flasks for acclimation for 2 hours prior to measurement of respiration. Following acclimation, respiration was measured for 2 hours in a Gilson Differential Respirometer³ by standard manometric techniques (Umbreit et al. 1972). Fish remained in the manometer flasks for 24 hours and any mortality was noted. Fish were sacrificed and dried at 50°C for 12 hours. The dry weights of individual fish were measured to the nearest 1 ug on a Cahn C-30 microbalance. The pH of the test solutions was measured prior to exposure, immediately following respiratory measurements, and after 24 hours of exposure.

Statistical computations were performed using the Statistical Analysis System (SAS 1988). The effects of species, age, pH, and buffer were assessed by analysis of covariance. The relationship between the first and second hour of exposure was evaluated by the Cochran T-Test with significance at p 0.05.

Results and Discussion

A 0.05 M concentration of MES and MOPS satisfactorily maintained the pH of test solutions through 24 hours of exposure (Table 1).

Table 1. Mean pH (± s.d.) of test solutions during the larval fish exposure.

Initial	4 Hours	24 Hours
5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.1
5.5 ± 0.0	5.5 ± 0.0	5.5 ± 0.0
6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0
6.5 ± 0.0	and the latest terminal to the latest terminal t	6.5 ± 0.0
7.0 ± 0.0		7.0 ± 0.1
7.5 ± 0.0		7.5 ± 0.0
8.0 ± 0.0	8.0 ± 0.1	8.0 ± 0.0
	5.0 ± 0.0 5.5 ± 0.0 6.0 ± 0.0 6.5 ± 0.0 7.0 ± 0.0 7.5 ± 0.0	5.0 ± 0.0 5.0 ± 0.0 5.5 ± 0.0 5.5 ± 0.0 6.0 ± 0.0 6.0 ± 0.0 6.5 ± 0.0 6.5 ± 0.0 7.0 ± 0.0 7.0 ± 0.0 7.5 ± 0.0 7.5 ± 0.0

The maximum deviation from nominal test pH was 0.1 pH units. MES and

³⁾ Mention of trade names or commercial products does not constitute endorsement or recommendation by the U.S. Government.

MOPS are zwitterionic aliphatic amines with the following properties: pKs of 6.1 and 7.2 at 25°C, respectively, water soluble, low membrane permeability, and low interference with biological processes (Good et al. 1966; Good and Izawa 1972; Ferguson et al. 1980).

Acute mortality was observed during the measurement of respiration of fathead minnows at pH 5.0, 5.5, and 6.0. Mortality was 100% after 24 hours exposure at the above pH range. Survivorship was 100% after 24 hours exposure to pH 6.5 and 7.0. McCormick et al. (1989) showed that recruitment of larval fathead minnows declined at pH 6.0 and approached zero at pH 5.5, in part due to reduced survival within the first few days of hatch. The mean respiratory rate of 1-day-old fathead minnows at pH 6.5 and 7.0 was 20.3±6.5 and 19.1±3.1 mg O2/mg/hr (mean ± sd; Figure 1), respectively.

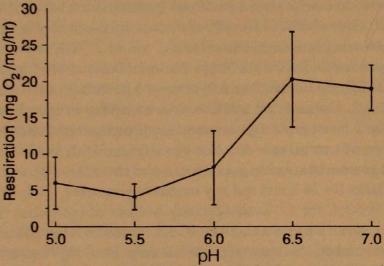


Figure 1. Respiration rate of 1-day-old fathead minnows exposed to test solutions of pH 5.0 to 7.0, buffered with 0.05M MES. During the measurement of respiration rate, mortality occurred in each treatment pH 6.0 and below.

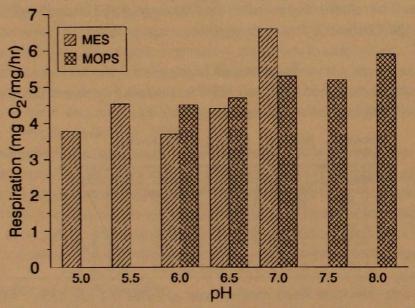


Figure 2. Respiration rate of 1-day-old swordtails exposed to test solutions of pH 5.0 to 8.0, buffered with 0.05M MES or MOPS.

There was no significant difference in the rate of respiration between pH 6.5 and 7.0, nor was there a difference between hours one and two. Respiration declined significantly at pH 6.0 and below. However, interpretation of the effect of pH on respiratory rate is complicated by the obvious implications of mortality during the measurement.

The survivorship of 1-day-old and 30-day-old swordtails was 100 % through 24 hours exposure to all test solutions. Oxygen consumption of 1-day-old swordtails declined 36%, from 5.9 to 3.8 mg 02/mg/hr as pH declined from 8.0 to 5.0 (Figure 2). There were no significant differences in the rates of oxygen consumption of 30-day-old swordtails over the tested pH range (Figure 3).

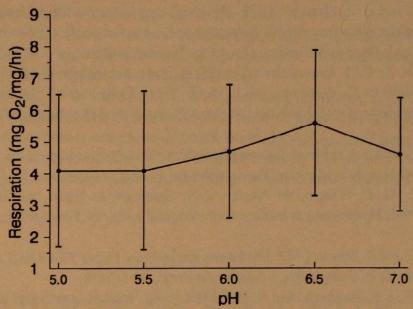


Figure 3. Respiration rate of 30-day-old swordtails exposed to test solutions of pH 5.0 to 7.0, buffered with 0.05M MES.

The respiratory rate of 30-day old swordtails was approximately twice that of 1-day-old swordtails. However, there was no significant difference when respiratory rate was calculated on a dry weight basis. Duration of exposure may affect 30-day-old swordtails, since the respiratory rate declined significantly between hour one and hour two when measured on a per fish basis. However, on a dry weight basis, oxygen consumption did not differ between hour one and hour two (p = 0.07).

In conclusion, the zwitterion buffers (MES and MOPS) adequately maintained pH during the course of exposure. Biological buffers may provide an alternative to continuous-flow diluters as a means of controlling pH under static conditions. Measurement of respiration of larval fishes was precise enough to detect differences in respiratory rates of the two species, as well as differences due to pH of the test solutions. On a dry weight basis, the mean respiratory rate of fathead minnows on day-of-hatch was twice that of swordtails on day-of-birth. As pH declined, respiration of 1-day-old fathead minnows and swordtails declined significantly. Acute mortality was encountered with fathead minnows as pH declined to

6.0 and below. Respiration of 1-day-old swordtails was significantly less at lower pH levels.

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Spatial and short-term temporal water quality variations in a medium-sized stream affected by human-induced factors

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Abstract

Deckers Creek, a tributary of the Monongahela River, cuts through the Chestnut Ridge anticline, exposing coal, limestone, and other sedimentary rocks of Mississippian-through Pennsylvanian-age. Water samples were collected every two weeks for a six-month period at 13 (10 main stream and 3 tributary) stations, and every three days near the mouth of the main stream. Chemical analyses showed high levels of iron, acidity, and sulfate derived from water draining coal mines and other areas of coal-related activity. Acidity is partially neutralized, and iron and sulfate decrease where the streambed is in limestone. Chloride increases downstream, probably due to a combination of sewage discharge and road salting. Higher flows cause a rise in pH and a decrease in concentration of most of the dissolved constituents due to dilution. Total iron concentration does not correlate significantly with discharge.

Introduction

Deckers Creek is a tributary of the Monongahela River in Monongalia and Preston counties, West Virginia (Figure 1). Drainage area is about 100 km2; average discharge is about 2900 liters per second (L/s) and ranges from less than 10 to more than 160,000 L/s (U. S. Geological Survey, 1970). There is considerable mineral extraction in the basin; coal mines are prevalent in the region, and limestone is produced in one area along the stream. A highway and a railroad parallel much of the stream, and several towns have been established along this route. As a consequence of these factors, mine drainage, sewage, and road runoff affect the chemical character of the stream water.

Deckers Creek basin is within the Allegheny Plateau and straddles the Chestnut Ridge anticline. It heads at the crest of the anticline, flows east down the dip slope to the axis of the Ligonier syncline, then turns to the west and cuts perpendicularly through the anticline before flowing to the Monongahela River. The surface rocks in the basin are sedimentary, ranging in age from limestones of the Middle Mississippian Greenbrier Group through shales, sandstones and coals of the Pennsylvanian Conemaugh Group. Several coals and the Greenbrier limestone are important in affecting the water quality of Deckers Creek and its tributaries. Coals of the Allegheny Group, especially the Upper Freeport, have been mined extensively. When coals and associated rocks weather, acid mine drainage (AMD) may be produced. The Upper Freeport coal has a relatively large potential for producing AMD (Renton and others, 1973). In crossing the Chestnut Ridge anticline, Deckers Creek

encounters the Allegheny Group coals twice, once before and once after the Greenbrier limestones.

Previous studies. Previous studies of the chemical character of the waters in the stream have been few, and have not attempted to define systematic spatial and temporal variations.

The U. S. Geological Survey maintained a monitoring station near the mouth of Deckers Creek from April 1914 to September 1915 and February 1946 to September 1969 (U. S. Geological Survey, 1970, p. 70). A number of investigations have been done in connection with modifications to the channel of Deckers Creek and its tributaries by the U. S. Soil Conservation Service and the U. S. Army Corps of Engineers in the early 1960's. These studies were concerned primarily with physical hydrology of the basin, especially of the lower part.

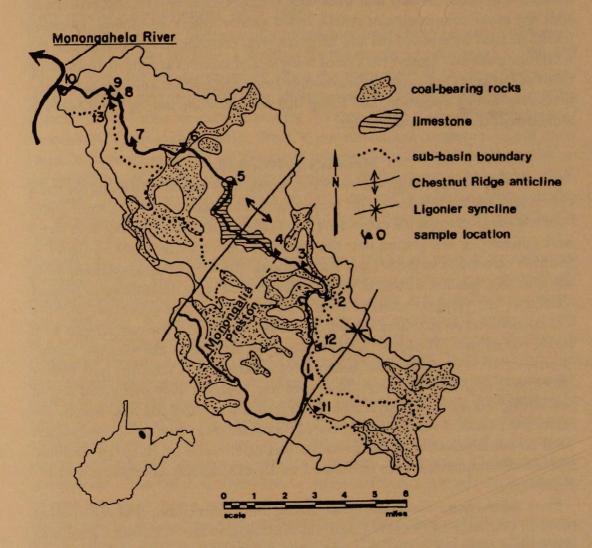


Figure 1. Map of Deckers Creek basin showing sample collection sites. Numbers without t prefix indicate stations on the main stem, t indicates a tributary station (t1: Kanes Creek, t2: Dillan Creek, t3: Aaron Creek). Axis of the Chestnut Ridge anticline, Ligonier syncline, and approximate areas of coal and limestone are also shown. Geology is from Hennen and Reger (1913, 1914).

As part of the comprehensive river basin plan (West Virginia Department of Natural Resources, 1982), point-source effluent discharges were identified and samples for chemical analysis were taken in most tributaries to the Monongahela River; twenty-one of these are from Deckers Creek or its tributaries. Specific conductance, dissolved oxygen, and temperature are reported for all samples, and fecal coliform for about half. The components of acid mine drainage û sulfate, iron, and manganese û are reported for a few of the samples. This report notes that "in the Monongahela River basin, the two most significant [non-point pollution sources] are runoff from abandoned mining activities, including seepage from coal refuse piles and inadequate residential sewage and septic systems" (p. 3).

Teti (1975) sampled 29 stations (10 on tributaries) on eight occasions in order to determine effects of various industries along the stream. He found no significant difference between Sunday and mid-week samples, coal mining and processing adds the usual components of AMD, and a nearby limestone mining operation results in local increase of pH and hardness with corresponding decreases in the AMD components. Smith (1978) took three sets of samples at eight stations along a part of Deckers Creek and a seven-sample storm sequence at one station near the limestone mining operation, and found that the limestone mining contributes to reduction of the AMD components.

Materials and Methods

Water samples were collected every two weeks at 13 stations (10 along the main stream and three at tributaries) for the period 4 November 1979 to 21 April 1980. Four additional samples per two-week period (one approximately every three days) were collected at one station near the mouth of Deckers Creek (#9 on figure 1, hereafter referred to as the Marilla Park station). All were grab samples and were not filtered. Specific conductance and temperature were determined at time of collection, and pH was measured within two hours of sample collection. Acidity was determined on the same day as collection by titrating the raw sample with 0.02N NaOH to a pH of 8.3. Analysis for chloride was by the mercuric nitrate titrametric method. After these analyses, each sample was acidified with hydrochloric acid to pH 2.0 or lower. The remaining analyses were performed within two weeks. Sulfate was analyzed by the turbimetric method, and total iron by the FerroZine∑ method, using Hach-prepared chemicals on a Hach DR-1 colorimeter. Total hardness (divalent metal hardness) was determined by NaEDTA titration. Sodium determinations were performed on an EEL atomic absorption spectrophotometer.

Flow measurements were not made during this investigation. The relationship between discharge of Deckers Creek and the adjacent basin, Cobun Creek, was determined by linear regression using data for a four-year period (water years 1966-1969) when U. S. Geological Survey gaging data were available for both sites (U. S. Geological Survey, 1967, 1968, 1969, 1970). More than 90% of the variation in discharge at Deckers Creek is predictable by the Cobun Creek data. The equation for the relationship:

 $DD = 1.75 + 7.58D_{c} - 0.0264D^{2} + 0.0000466D^{3},$

where DC is the discharge of Cobun Creek, and DD is the calculated discharge of Deckers Creek, was used to calculate the probable discharge of Deckers Creek for the period of investigation based on the U. S. Geological Survey (1981) data for Cobun Creek. These synthetic hydrograph data were used with the Marilla Park station chemical data to calculate the flux of material transported past this station.

Results

Meteorological conditions. Conditions for the six months of sampling were not especially unusual. The temperature at Morgantown Airport (normal mean for the November-April period is 4.0+C) was about 1.3+C warmer and precipitation at Morgantown Dam and Lock (normal November-April total precipitation is 470 mm) was about 1.5 mm per month greater than the average (National Oceanic and Atmospheric Administration, 1979, 1980).

General Relationships. Water quality parameters varied both spatially and temporally. Table 1 shows the simple statistics for the measured parameters for the 130 samples from the regular sampling along the main stem of Deckers Creek, and table 2 lists the simple statistics for all samples from the Marilla Park sampling station. Table 1 also includes values for the spatial and temporal coefficients of variation (CV) for each variable. The CV is a convenient measure to use in comparisons between variables for groups of samples because it is not dependent on the measurement scale as the standard deviation is (Snedecor and Cochran, 1967, p. 62-64). The values given in table 1 are the means of CV calculated for each date for all samples taken for the spatial CV, and for each station for all dates for the temporal CV.

Pearson's correlation coefficients (r) were computed for all pairs of the measured variables (table 3). Pearson's product-moment coefficient of correlation, often simply called the correlation coefficient in statistics textbooks, is the parametric correlation coefficient. It was developed by Karl Pearson by extension of work of others in a series of papers published at the turn of the century [see especially Pearson (1896) and the discussion by Wyatt and Bridges (1967, p. 200)]. With many samples, even a small correlation can be statistically significant. For that reason, a smaller alpha error than the usual 0.05 was used to indicate significant relationships in tables 3-5. Even at the 1% significance level (a=0.01), most of the correlation coefficients shown in table 3 are significant. Only temperature and distance upstream (indicated by variable *position*) are not well correlated with the chemical constituents of the water. Correlations between variables at a single station tend to be even greater as shown in tables 4 and 5.

Spatial effects. As can be seen from the values for the spatial coefficient of variation in table 1, there is considerable variation along the main stem of Deckers Creek. The effects of areas of coal mining and limestone, and the additive or dilutional effects of tributaries are readily evident in the plots of pH, sulfate, total iron, and chloride (figure 2).

Temporal effects. Considerable variation is shown at each site during the study. The Marrilla Park sampling station shows the variation best because of more-frequent sampling. Representative plots of temporal variation in pH, sulfate concen-

tration, total iron flux, and chloride flux are shown in figure 3; the calculated discharge data are also included in each plot.

Acidity and pH. When sulfide minerals (usually one of the iron disulfides) react with air and water, oxidation of the metal and sulfur components produces hydrogen ions, which results in acidity and a decrease of pH. Figure 2a clearly shows the effect of discharge from the coal-bearing terrain in the four upstream locations, which show progressive reductions in pH. Once the stream reaches the limestone terrain (the next two stations downstream), pH rises. The next station downstream once again shows a decrease in pH, reflecting additional discharge from coal mines in this area. The last downstream stations indicate partial neutralization by tributary stream water coming from areas unaffected by AMD. Acidity and pH are inversely correlated. In general, pH changes proportionally less than acidity in the downstream direction, indicating that perhaps the buffering becomes greater downstream. On a temporal basis, pH decreases with increasing flow (figure 3a); most likely this results from dilution. This is reflected in the high correlation between discharge and pH for the Marilla Park station (r=0.767).

Sulfate. Another major contribution from AMD is sulfate. The mean sulfate concentration (figure 2b) does not change much spatially in the portion of Deckers Creek sampled in this study. Most of the change is due to dilution from tributaries that bring in water of much lower solute concentration, such as the tributary between stations 8 and 9. There is also a decrease of solutes between stations 4 and 6 due to a combination of dilution and precipitation of gypsum because of the influx of dissolved calcium in the Greenbrier limestone belt. The effect of dilution over time, because of increased discharge, is readily apparent in figure 3b.

Iron. The analysis is for total recoverable iron. It is likely that ferric iron is the prevalent species, because of the good aeration of the Deckers Creek water, and much may be particulate rather than dissolved (based on visual observation of the raw water samples). The iron concentration is generally inversely proportional to pH (compare figures 2a and 2c). However, the correlation between pH and total iron is much lower than the correlation between pH and acidity (table 3) because iron can precipitate out of the water because of oxidation, without a rise of pH. Figure 2c shows the increases in total iron concentration contributed from tributaries draining the mining areas just before stations 2 and 7. However, although pH decreases slightly after station 2 from additional AMD contributions or acid produced by oxidation of ferrous iron, iron concentration falls almost immediately downstream. In this area, oxidation and subsequent precipitation of the iron compounds is progressing faster than iron is added to the stream.

As with the other chemical constituents in the water, there is an inverse relationship between total iron concentration and discharge at the Marilla Park station (table 4), which is due to the dilution effect of the higher discharge. However, unlike the other constituents, the flux of iron does not correlate with discharge. Whereas the fluxes of the other constituents have a high positive correlation with discharge (table 5), total iron flux has a small non-significant negative correlation with discharge. Examination of a temporal plot (figure 3c) shows that iron flux actually decreases at some times of high flow. The cause of this relationship is unclear, but it may be due to the interrelationship of elevated pH at the higher flows and the chemical behavior of dissolved iron; as discharge increases, pH and aeration of the water increases, and iron is less mobile in an alkaline and oxidizing environment.

Hardness. Only the total divalent metal hardness was determined, so the relationship between the various components of hardness is unknown, but the most important component probably is calcium. The sources for hardness are primarily dissolution of the Greenbrier limestone in the basin and limestone or lime treatment of acid discharges from operating coal mines. Both of these sources are likely to contribute mostly calcium. The only removal is likely to be if calcium and sulfate concentrations, in combination, are high enough to exceed the solubility of gypsum; this did not occur for any of the samples taken during this study, nor for any reported by either Teti (1975) or Smith (1978), on the basis of calculations using the reported analytical values. However, some calculated values were so close to saturation that the difference could be accounted for by analytical error typical for samples such as these. There are pebbles in the stream coated with a mixture of gypsum and clay (coatings were analyzed by x-ray diffraction) just downstream of the limestone outcrop, so the solubility of gypsum must have been exceeded at some times.

Chloride and sodium. West Virginia Route 7 follows the portion of Deckers Creek sampled in this study. This is a major traffic route, and is generally heavily salted during winter snow storms. As the snow melts, meltwater generally runs directly into Deckers Creek. Additional sources may be sewage from the communities in the valley. An additional source of sodium is from AMD treatment with soda ash, which may be the cause of the peak in sodium concentration unrelated to chloride concentration during the autumn months. The correlation of chloride and sodium concentrations is low (tables 3 and 4), and is probably related to soda ash treatment of AMD.

Discussion

The source of material carried by stream waters is often problematic. In Deckers Creek, most sources are related to local industry or land use. Mining and processing of coal are prevalent, and there are limestone extraction operations in the basin. There is also sewage, treated and untreated, from towns and individual houses, and nearby roads are salted in winter. The major constituents in stream water come from one or more of these sources.

Coal mining and processing. As noted above, there is a high correlation between specific conductance and several of the chemical constituents. It is well known that weathering of coal and coal-mining wastes will produce AMD, which contains sulfate, iron and other metals, and acidity, and which is generally low in pH. The high correlation between these constituents and the specific conductance suggests that the majority of dissolved matter is from AMD. An indirect effect of mining is the high divalent metal concentration, due largely to solution of calcium and to a lesser extent magnesium from lime and ground limestone that are used to treat acid water on mine sites, and from dissolution of limestone by acidic water.

An additional contribution to dissolved matter is sodium from soda ash used to raise the pH of mine discharge. Correlation between pH and acidity and the other AMD products is fairly low. This is due largely to the effect of neutralization of acidity which occurs within the limestone belt (figure 2a), while the other products are less affected, for example sulfate (figure 2b).

Limestone extraction. The belt of limestone in the midst of the coal mining terrain serves to counteract some of the effects of the drainage from the coal terrain. The quarrying and mining operations have disturbed the area and thereby add to the natural effect of limestone terrains by making the rock more accessible to weathering. Smith (1978) assigned effects of the limestone terrain mostly to increase in exposure and surface area of limestone by the extraction operations. There is also a significant contribution of dissolved limestone in the tributaries flowing from the limestone outcrop area, as well as from the groundwater passing through bedrock into Deckers Creek. The relative effect of these various contributions requires further investigation through mass balance studies in that area.

Highway salting. Although salting of highways is common during the winter months, and the stream is directly adjacent to the highway for much of its length, there is relatively little effect on water quality. Chloride flux (figure 3d) increases when salting for snow occurs after the end of November, but in comparison to the other chemical parameters, chloride concentration shows only a small variability at the Marilla Park station (see CV in table 2). Application of salt to the highway has little long-term effect. Chloride movement is probably retarded by the soil between the highway and the stream, and does not arrive at the stream until rain or snowmelt carry it along. There is also a constant source such as sewage discharge. Because of the relatively small variation in chloride concentration, the main influence on the chloride flux is discharge. The small increase in mean chloride concentration in the downstream direction (figure 2d) is largely caused by a higher density of highways in the downstream end of the basin.

Conclusions

Deckers Creek showed a large variability in chemical character during the period of this study, both temporally and spatially. The stream shows effects of various land uses within its drainage basin, but the most important process controlling the chemical composition of the stream is coal mining. However, although large quantities of acid mine drainage reach the stream, natural neutralization due to a belt of limestone, and several other, lesser contributions of alkalinity serve to ameliorate the effects of the acid in the lower reaches of the stream (at least during the period of this study). In general, the concentration of most of the chemical components of the water relate inversely to discharge, and flux correlates positively, except for total iron.

Table 1. Simple statistics for 130 bi-weekly samples from the main stem of Deckers Creek.

Variable	Mean	Standard deviation	Minimum	Maximum	Spatial Coefficient of	Temporal f variation	Units
Temperature	4.7	3.7	-1.0	14.0	12%	79%	degrees C
Specific conductance	279	84	160	570	9%	28%	microsiemens/ cm
pH	5.14	0.78	3.91	6.74	13%	8%	standard units
Chloride	11.8	2.5	5.0	20.4	14%	16%	mg/L
Total iron	2.34	2.00	0.35	10.45	38%	66%	mg/L
Acidity	6.5	5.5	0.8	31.2	58%	71%	mg/L as CaCO ₃
Sulfate	71	30	25	215	. 16%	38%	mg/L
Hardness	104	32	60	276	9%	28%	mg/L as CaCO ₃
Sodium	7.5	6.3	2.8	46.3	28%	68%	mg/L

Table 2. Simple statistics for 64 samples taken at the Marilla Park station.

Variable	Mean	Standard Deviation	Minimum	Maximum	Coefficient of variation	Units			
Temperature	4.1	4.4	-1.0	21.0	107%	degrees C			
Specific Conductance	291	73	170	490	25%	microsiemens/ cm			
pH	6.04	0.63	4.72	7.62	10%	standard units			
Concentration:									
Total iron	2.96	2.28	0.20	9.46	77%	mg/L			
Acidity	4.7	3.3	0.6	11.6	70%	mg/L as CaCO ₃			
Chloride	15.1	2.9	9.8	24.8	19%	mg/L			
Sodium	8.9	4.0	3.9	27.3	45%	mg/L			
Sulfate	73	31	15	150	42%	mg/L			
Hardness	112	31	70	212	27%	mg/L as CaCO ₃			
	The same of the same		Flux:						
Flow	4300	4130	765	24000	99%	L/sec			
Total iron	7.3	3.2	1.2	19.8	43%	g/sec			
Acidity	12.4	5.9	4.0	47.0	50%	g/sec as CaCO ₃			
Chloride	59.9	50.0	13.5	325.7	87%	g/sec			
Sodium	31.4	24.2	8.8	174.8	81%	g/sec			
Sulfate	232	104	113	662	46%	g/sec			
Hardness	418	381	148	2874	96%	g/sec as CaCO ₃			

Table 3. Correlation coefficients (Pearson's r) for the chemical parameters and relative location for the 130 bi-weekly samples from the main stem of Deckers Creek. Statistical significance: $\alpha \le 0.001$ shown as bold italic, $0.001 < \alpha \le 0.01$ shown as bold normal, remainder are less significant ($\alpha > 0.01$).

	T				No.				
SpC	-0.101	SpC							
рН	-0.016	-0.407	рН						
CI	-0.387	0.319	0.297	CI	1				
Total iron	-0.358	0.686	-0.248	0.511	Total iron	1			
Acidity	-0.168	0.747	-0.682	0.037	0.516	Acidity			
Sulfate	-0.171	0.880	-0.378	0.219	0.644	0.554	Sulfate		
Hardness	-0.183	0.916	-0.274	0.334	0.673	0.508	0.907	Hardness	
Sodium	0.077	0.628	-0.229	0.276	0.325	0.689	0.340		Sodium
Position	-0.029	0.065	-0.669	-0.560	-0.207	0.342	0.154	0.068	-0.129

Table 4. Correlation coefficients for the chemical parameters and synthetic discharge for 64 samples from the Marilla Park sampling station on Deckers Creek. Statistical significance: $\alpha \le 0.001$ shown as bold italic, $0.001 < \alpha \le 0.01$ shown as bold normal, remainder are less significant ($\alpha > 0.01$).

	THE RESERVE AND ADDRESS OF								
SpC	-0.318	SpC	*						
рН	0.287	-0.850	рН						
CI	-0.562	0.580	-0.450	CI	The same of				
Total iron	-0.542	0.802	-0.780	0.619	Total iron				
Acidity	-0.389	0.881	-0.858	0.550	0.852	Acidity			
Sulfate	-0.284	0.948	-0.834	0.499	0.792	0.854	Sulfate		
Hardness	-0.356	0.942	-0.788	0.552		0.816	0.898	Hardness	
Sodium	-0.092	0.667	-0.472	0.431	0.448	0.610	0.640	0.471	Codin
Discharge	0.290	-0.615	0.767	-0.439	-0.554	-0.575	-0.652	-0.494	-0.400

Table 5. Correlation coefficients (Pearson's r) for chemical flux and synthetic discharge for 64 samples from the Marilla Park sampling station on Deckers Creek. Statistical significance: $\alpha \le 0.001$ shown as bold traile, $0.001 < \alpha \le 0.01$ shown as bold normal, remainder are less significant ($\alpha > 0.01$).

	Discharge					
Total Iron	-0.058	Total iron				
Acidity	0.424	0.117	Acidity			
Chloride	0.969	0.026	0.328	Chloride		
Sodium	0.872	-0.015	0.318	0.910	Sodium	
Sulfate	0.793	-0.033	0.461	0.751	0.677	Sulfate
Hardness	0.952	-0.048	0.310	0.962	0.923	0.658

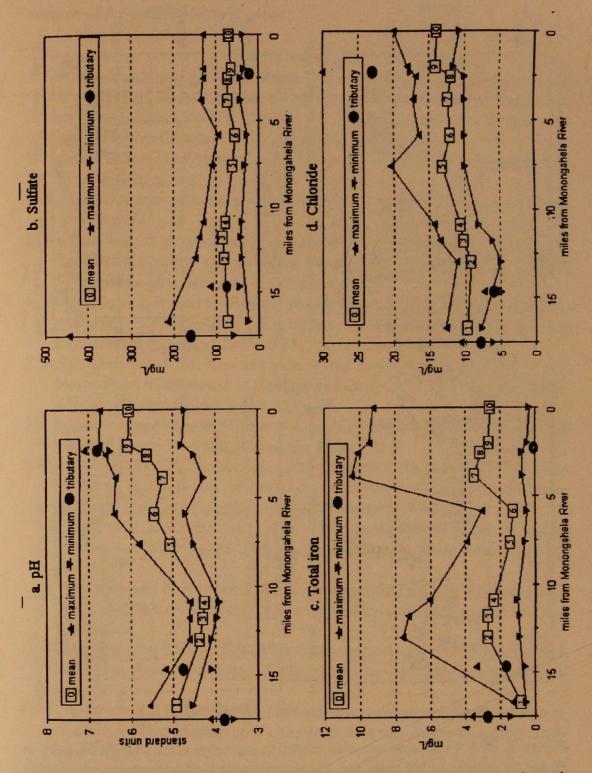


Figure 2. Plots of water quality results showing average, minimum, and maximum values at sampling stations along Deckers Creek and three tributaries; a) pH, b) sulfate concentration, c) total iron concentration, and d) chloride concentration. Numbers in the station-mean boxes correspond to station numbers on the main stem of Deckers Creek.

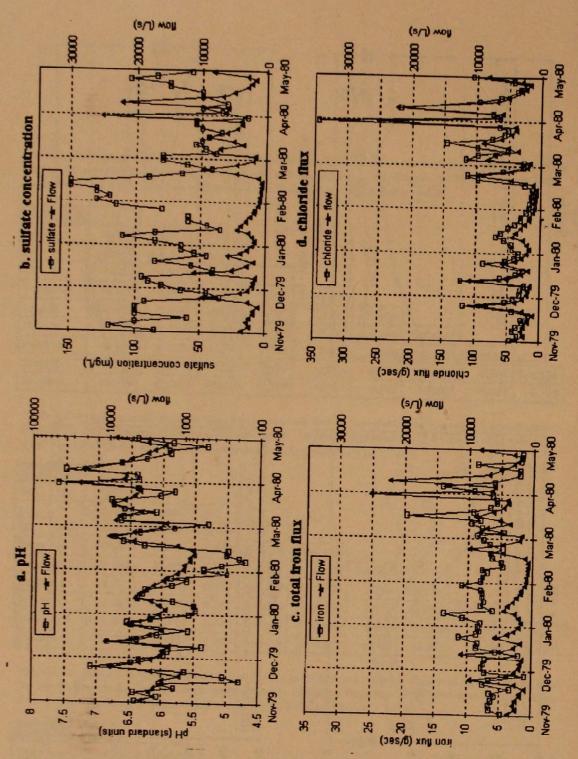


Figure 3. Plot of temporal variation related to discharge at the Marilla Park (#9 in figure 1) station; a) pH (note use of logarithmic scale for discharge), b) sulfate concentration, c) total iron flux, and d) chloride flux. Positions shown on the horizontal axis are for the first day of the month.

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Leaf Species Selection by the Shredding Stoneflies *Peltoperla arcuata* and *Tallaperla maria* (Plecoptera: Peltoperlidae)

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ABSTRACT

Experiments were conducted to examine food selection by two West Virginia species of peltoperlids. Nymphs of *Peltoperla arcuata* and *Tallaperla maria* were collected at the Fernow Experimental Forest, Tucker County, West Virginia. They were placed in aerated containers and given a choice of 12 leaf species on which to feed: American beech, black birch, black cherry, dogwood, red oak, red maple, rhododendron, sugar maple, sassafras, sycamore, hickory, and tulip popular. Nymphs were exposed to different leaf combinations to determine preferential consumption. After two weeks, the nymphs and remaining leaf material were weighed. Dogwood, tulip popular, sassafras, maple, and cherry were the most preferred leaves while red oak, sycamore, and rhododendron were least preferred. Dogwood was the most preferred leaf species for *P. arcuata*, while tulip popular was the most preferred leaf species for *P. arcuata*, while tulip popular was the most preferred leaf species for *P. maria*. Overall, however, leaf species preferences were similar for *P. arcuata* and *P. maria*. We conclude that different selections of leaf species probably do not account for observed distributional differences between the stonefly species at the Fernow Experimental Forest.

INTRODUCTION

Many aquatic invertebrates depend on leaf detritus for energy in forested headwater streams. Nelson and Scott (1962) found that 66% of the energy of primary consumers on a Piedmont stream rock-outcrop was derived from allochthonous organic material consisting largely of autumn-shed leaves. In forested headwater streams, where leaf litter inputs are high, leaf shredding insects are one of the dominant functional feeding groups in the benthos (Vannote et al., 1980). In Appalachian streams, shredders such as the stoneflies *P. arcuata* Needham and *T. maria* Needham and Smith are important in breaking down leaf detritus into smaller particles which can be used as energy sources for other stream organisms. Thus, shredders play a prominent role in the processing of coarse detritus, facilitating

energy flow in streams through direct conversion of detrital energy into shredder biomass and by production of fine particulate organic matter (Perry et al., 1987). The two peltoperlid species selected for this experiment are morphologically very similar with the exception that P. arcuata nymphs usually have paired dark spots on the meso-and meta-nota. These spots are absent on T. maria nymphs. Both species were in the same genus until 1981, when Stark and Stewart (1981) split the T. maria complex from Peltoperla. Similarities in morphology and feeding habits may suggest that these two genera prefer similar habitats. Such is not the case in the Fernow Experimental Forest, Tucker County, West Virginia. Peltoperla arcuata nymphs are most abundant in the headwaters (1st and 2nd order), whereas T. maria nymphs are most abundant farther downstream (3rd and 4th order, Kevin Yokum, personal observation). Egglishaw (1964) has shown that the distribution of aquatic macroinvertebrates is closely associated with plant detritus that accumulates in streams. Macroinvertebrate species often exhibit selective or preferential feeding when offered a variety of leaf types (Wallace et al., 1970). The objective of this study was to examine whether leaf preference may be responsible for distributional differences between P. arcuata and T. maria nymphs on the Fernow Experimental Forest.

METHODS

Approximately 100 P. arcuata and 100 T. maria nymphs (range, 0.12-2.73mm head width, X=1.2mm) were collected with dip nets at the Fernow Experimental Forest in November, 1993. They were brought back to the Marshall University Aquatic Biology Laboratory where they were kept in closed styrofoam containers within an environmental chamber at 15°C. The containers were supplied with aerated stream water and a random mix of leaf material until the experiment was ready to begin. Freshly fallen leaves were collected at the Fernow Experimental Forest. Three leaf groups, each consisting of four species, were used in this experiment: Group 1, red oak, Quercus rubra L., American beech, Fagus grandifolia Ehrh., tulip popular, Liriodendron tulipifera L. and sugar maple, Acer saccharum Marsh; Group 2, sycamore, Platanus occidentalis L., black cherry, Prunus serotina Ehrh., sassafras, Sassafras albidum (Nutt.) Kees, and rhododendron, Rhododendron maximum L; and Group 3, black birch, Betula lenta L., red maple, Acer rubrum L., dogwood, Cornus florida L., and hickory, Carya ovata (Mill.) K. The leaves were washed and preleached for 10 days in stream water. The preleached leaves were removed, dried at 60°C for 24 hours, and transferred to desiccators until a constant dry weight was maintained. Each species of leaf was then divided into 500 mg portions using a Sartorius balance. Five hundred mg portions of four leaf species were placed in plastic trays (7 x 15 x 29 cm) containing 1.5 liters of stream water from the Fernow site. Twelve leaf species were used in the experiment. Three rows of six trays each were placed in an environmental chamber where the temperature was maintained at 15°C and the light regime was adjusted to natural day-night conditions. In each row of six trays, two contained P. arcuata, two contained T. maria, one contained a mixture of both species, and one contained only leaves. Leaves were placed in the trays for 24 hours prior to the addition of ten

nymphs to each tray. The trays were examined daily to remove dead nymphs and exuviae. These were dried, ashed in a muffle furnace and added to final weights at the termination of the experiment. Mortality rates did not exceed 5% during the two week period.

After two weeks the nymphs and leaves were removed from the trays, dried in a drying oven for 24 hours at 100°C and then placed in desiccators until a constant dry weight was reached. Nymphs were ashed in a muffle furnance at 500°C for an ash-free dry weight. The average amount of leaf material eaten (dry weight in mg) per mg nymphs (ash free dry weight) was calculated for each leaf species. The water from each tray was filtered using Whatman #5 paper and a buchner apparatus to remove frass. The tannin and lignin content of the water was determined using a Hach Kit Model TA-3 tannin-lignin test kit. The test was not specific for tannin-lignin because other reducing materials present in the water may give similar results. Therefore, the results are expressed as tannic acid substances (mg/L).

A second experiment was performed using the five most preferred leaf species of each peltoperlid species. This was done to determine the most preferred leaf species for each peltoperlid species and to insure that no leaf species was slighted due to group composition. Two trays for each species were set up with twenty nymphs per tray; each tray contained a bundle of the five preferred leaf species. In a fifth tray, 10 of each nymph species were combined with or without leaves; the sixth tray contained leaves but lacked nymphs.

Data were analyzed using M rankings (Norman and David, 1969) which detect the likelihood of individual leaf species preferred or rejected by both *P. arcuata* and *T. maria*. High M rankings reflect strong probability that both stoneflies prefer a given leaf species, while low rankings indicate that both stoneflies rejected a certain leaf species.

RESULTS AND DISCUSSION

When nymphs of both *P. arcuata* and *T. maria* in the first experiment were placed in aerated containers, they congregated on certain leaf bundles. Nymphs fed on cuticle, epidermis, and mesophyll of the leaves while xylem and phloem of the leaves were avoided. This feeding pattern created a skeletonized appearance to the leaves. Wallace et al. (1970) and Ward and Woods (1986) both reported similar appearance of leaves in their experiments with nymphs of the family Peltoperlidae.

Tulip popular and sugar maple were the most preferred leaves for both *P. arcuata* and *T. maria* in Group 1 (Table 1) (Figs. 1,2). Tulip popular was the most preferred for *P. arcuata*, while sugar maple was the most preferred by *T. maria*. Preference for both was significantly higher than other species at the 0.06 confidence level. Red oak was the least preferred by both species at the 0.10 significance level.

Leaves in Group 2 included sycamore, cherry, sassafras, and rhododendron. Cherry was the most preferred leaf species by *T. maria*, while sassafras was the most preferred leaf species for *P. arcuata* (Table 1) (Figs. 1,2). Both species ranked significantly high at the 0.06 confidence level. Sycamore was the least preferred by *P. arcuata* while rhododendron was the least preferred by *T. maria*. Rhododendron scored significantly low at the 0.10 confidence level.

Group 3 consisted of birch, red maple, dogwood and hickory. Dogwood was the most preferred for *P. arcuata*, while *T. maria* preferred red maple the most (Table 1) (Figs. 1,2).

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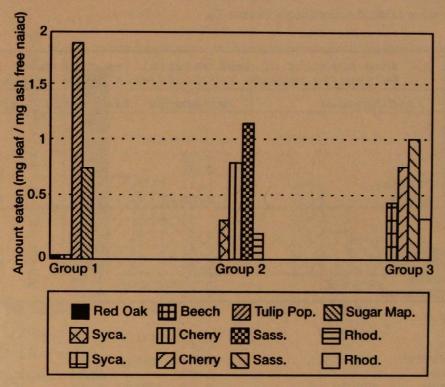


Figure 1. Leaf consumption by *Peltoperla arcuata* nymphs in experiment 1. Legend reads horizontally with 1st row corresponding to group 1, etc.

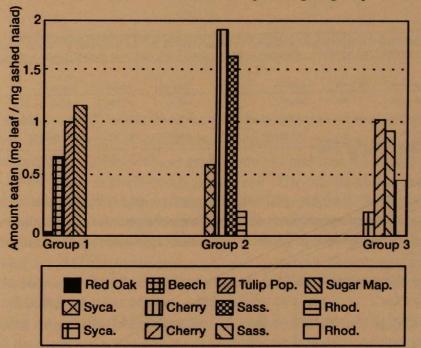


Figure 2. Leaf consumption by *Tallaperla maria* nymphs experiment 1. Legend reads horizontally with 1st row corresponding to group 1, etc.

Tallaperla maria avoided birch, while P. arcuata avoided hickory. Red maple and dogwood ranked significantly high at the 0.06 confidence level.

M rankings were calculated using the data from the entire experiment and leaf species were ranked accordingly (Table 2).

Table 1. Mean quantity of leaf material (mg dry) eaten by Peltoperla arcuata and Tallaperla maria nymphs.									
Leaf Species	Peltoperla	Tallaperla	Mixture						
Group 1	Marine Marine								
Red Oak	0.02	0.04	0.01						
Beech	0.01	0.67	0.52						
Tulip Popular	1.95	1.02	2.46						
Sugar Maple	0.80	1.11	0.91						
Group 2	3/1								
Sycamore	0.27	0.66	0.79						
Cherry	0.72	1.94	1.75						
Sassafras	1.15	1.59	1.42						
Rhododendron	0.24	0.20	0.40						
Group 3	TAN PERSONAL PROPERTY.		Mary and						
Birch	0.45	0.17	0.48						
Red Maple	0.82	0.93	0.65						
Dogwood	1.00	0.84	0.70						
Hickory	0.35	0.48	0.55						
Totals	7.78	9.65	10.64						

	consumed	i/mg nymph). Maximum	ranking s	um = 16; mi	nimum ran	nd T. maria (mo king sum = 4.	
Leaf Group	Species	H rank	Species	M rank	Species	H rank	Species	# rank
Group 1	Red Oak	5**	Beech	8	Tulip	14*	Sugar Haple	13
Group 2	Sycamore	6	Cherry	14*	Sassafras	14*	Rhododendron	5**
Group 3	Birch	6	Red Haple	14*	Dogwood	12	Hickory	

On a scale of 16-4, cherry, red maple, sassafras, and tulip popular all scored 14, which shows a strong preference at the 0.06 confidence level. Rhododendron and red oak scored very low (5) indicating a nonpreference at the 0.10 confidence level.

Part two of the experiment (Fig. 3) showed that dogwood was the most preferred leaf species for *P. arcuata*, and tulip popular was the most preferred by *T. maria*. The two species shared four of the top five preferred leaf species: tulip popular, red maple, sugar maple, and sassafras.

Analysis of filtered water showed that the experimental containers had a higher tannic acid content than did corresponding control containers. In Part One, the water from experimental containers averaged 3.82 mg/L, while control containers

averaged 2.83 mg/L. In Part Two, experimental containers averaged 5.97 mg/L versus 4.20 mg/L for corresponding controls. No tannic acid was detected in the original stream water used in the experiment.

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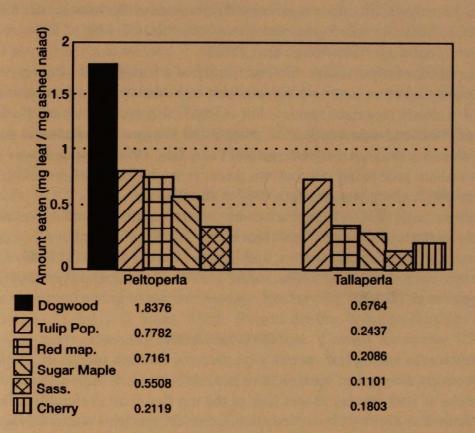


Figure 3. Leaf consumption by *Peltoperla arcuata* and *Tallaperla maria* nymphs in experiment 2.

Both *P. arcuata* and *T. maria* nymphs used in this experiment preferred certain leaf species. Both species of stoneflies preferred tulip popular, sugar maple, red maple, and sassafras. Differences in preference showed that *P. arcuata* preferred dogwood, while *T. maria* preferred black cherry. The results of our experiments generally agree with other studies. Ruggles (1990) found that nymphs of *P. tarteri* preferred tulip popular and sugar maple, while red oak, sycamore, and beech were avoided. Wallace et al. (1970) observed that *T. maria* preferred tulip popular and dogwood and avoided rhododendron, oak, and sycamore. Tulip popular was preferred by the shredding caddisfly *Hydatophylax variabilas*, while birch was avoided (Iron et al., 1988).

Differences in preference in leaves in our experiment were related to variation in inferred food quality among leaf species. Food quality of leaf species relates directly to chemical components of the leaves, processing time, and bacteria or fungi conditioning of leaves (Cummings et al., 1989). The preferred leaf species

in this experiment had high concentrations of nitrogen and soluble carbohydrates and were lower in concentrations of polyphenols, tannins and lignins (Irons et al., 1988). Condensed tannins may decrease the availability of cell wall and membrane-bound protein to these organisms. Differences in breakdown rates have been linked to differences in lignin content and thus accessibility of leaf material as microbial substrate (Sinsabaugh et al., 1981).

Processing rate influences the food quality of a leaf species. Leaves with fast processing times are more readily available to be utilized as a food source compared to slowly processed species. For example, dogwood leaves are quickly processed, while red oak is processed much more slowly and therefore is often unavailable as a food source (Peterson and Cummins, 1974). Maple leaves have an intermediate processing rate but are prone to microbial attack which gives the leaves a high nitrogen content (Gollidy et al., 1983).

Peltoperla arcuata and T. maria avoided sycamore, red oak, and American beech leaves in our experiment. These leaves are slowly processed and contain high amounts of polyphenols, tannins, and lignins (Ward and Woods, 1986). Similar findings were reported by Wallace et al. (1970), Kaushik and Hynes (1971) and Ruggles et al. (1990).

CONCLUSION

Preferences among leaf species were detected for both species of peltoperlids. Preferences among leaf species were similar between *T. maria* and *P. arcuata*. Nymphs of both species shared four of the top five most preferred leaf species, and both also avoided the six least preferred leaf species. Inferred food quality (tannin and lignin concentrations, processing time, and bacterial and fungal conditioning) was related to preference. Due to the overall similarities in leaf selection between *P. arcuata* and *T. maria*, we consider it unlikely that food preference is responsible for the distributional differences between the two peltoperlids at the Fernow Experimental Forest.

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The Effect of Lunar Cycling on Collision Injuries in Raptors

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Abstract

Many studies have examined the effect of lunar cycling and light intensity on the activity of small mammals; however, few studies have examined the behavioral effects of light intensity on raptorial birds. Raptor injury reports from four raptor rehabilitation centers were used in this study. The data sets were divided into diurnal (hawks, falcons and eagles) and nocturnal (owls) raptors. The lunar month was divided into four sections of equal length with each section having its midpoint on a different moon phase. ANOVA was used for analysis. New and full moon phases were found to have significantly higher occurrence of collision injuries (P>0.003) than the quarters. This was true for both diurnal and nocturnal raptors. Because diurnal raptors did not respond differently from nocturnal raptors, the cause of this difference is believed to be differences in small mammal behavior that occur with change in moon phase.

Introduction

Numerous studies have documented that moon phase has an impact on small mammal activity. Lockard and Owings (1974) showed that activity increased three-fold during moonless nights when compared to full moon in bannertail (Dipodomys spectabilis) and Fresno (D. nitratoides) kangaroo rats. Captive-bred Chihuahua deer-mice (Peromyscus maniculatus blandus) were much more active at light levels similar to those of moonless nights when exposed to a caged owl (not able to prey on them) (Blair 1943). Old field mice (P polionotus) in outdoor enclosures were inhibited in activity by 70 % during full moon phase when compared to moonless nights (Wolfe and Summerlin 1989). Ord's kangaroo rats (D. ordii)(Kaufmann and Kaufmann 1981) and P. floridanus and P. gossvpinus (Layne 1971) use different micro-habitats during various moon phases. Denser cover is used when conditions of greater moonlight exist; however, during a new moon, small mammals may occupy more open cover. No research was found suggesting diurnal changes in small mammal behavior during different moon phases.

Clarke (1983) suggests that while the hunting efficiency of short-eared owls (Asio flammeus) increases with increase in moonlight, deer-mouse (P. maniculatus) activity decreases which results in a relatively similar cost-benefit ratio during the lunar month.

Less moon light for navigation around obstacles during the new moon and increased use of dense cover by small mammals and decreased small mammal activity levels during the full moon may result in increased number of collision injuries for owls during these moon phases. Also, increased hunting in open cover during the new moon may draw the owls into more dangerous areas (roads, housing developments) where collision injuries are more likely to occur and injured owls are more likely to be spotted. Diurnal raptors, such as hawks, are expected to be unaffected by moon phase.

Methods

Requests for a listing of raptor injury reports were made to all registered raptor rehabilitation centers in the United States. Raptor injury reports for the years 1985 through 1990 (only years with complete records) from the four responding raptor rehabilitation centers (Wildlife Arc, Milwaukee, Wisconsin; Curative Workshop Rehabilitation Center, Greenbay, Wisconsin; Willowbrook Forest Preserve Wildlife Haven, Glen Ellyn, Illinois; West Virginia Raptor Rehabilitation Center, Morgantown, West Virginia) were used. From these data, only collision injuries were used. Each set of data from the individual centers was divided into two subsets: noctumal raptors (owls) and diurnal raptors (hawks, eagles and falcons). Each phase of the lunar month was allocated 7.4 days with its mid-point being the peak of that phase (first quarter, full, second quarter and new moon). Since the exact time of the injury could not be determined, the injury was designated to have occurred at the mid-point of the day it was reported (12:00 noon). While many other methods of designation of moon phase have been used (Mills 1986), the indirect nature of the observation (raptor injury reports) made this method the only viable option. ANOVA with orthogonal comparisons was used for analysis (Table 1.).

Table 1. ANOVA for the lunar influence on collision injuries in raptors.

Source	Degrees of freedom	Error term	F-value	P>F
Site	3	Residual	42.4	.0001
Moon Phase (with orthogonal comparisons)	3	Residual	3.1	.03
New and Full vs. the Quarters	1		9.0	.003
New vs. Full	1	920	0.06	NS
First Quarter vs. Second Quarter	1		0.26	NS
Class	1	Residual	11.4	.0009
Site X Phase	9	Residual	1.1	NS
Site X Class	3	Residual	2.8	.04
Phase X Class	3	Residual	0.06	NS
Year	5	Residual	9.2	.0001
Site X Phase X Class	9	Residual	0.5	NS
Residual	155			

Note: Class refers to the classification as diurnal or nocturnal raptor.

N=192

Results

A total of 192 collision injuries were reported (Table 1). A significantly higher number of injuries (means of 10.4 and 10.9 injuries/year/site, respectively) occurred during the new and full moon phase than during the first and second quarter (means of 8.5 and 8.8 injuries/year/site, respectively) (P<0.003). Site and year were also significant but are considered to be blocking factors (because differences between sites and years can be attributed to a great many causes). Class (diurnal or nocturnal) and site by class were significant (P<0.001 and P<0.04, respectively). Phase by class was not significant (p<0.98).

Discussion

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The limited moonlight available during the new moon may result in more collisions because the birds may have difficulty navigating around stationary objects. Although owls are able to capture mice with little or no visible light, more small mammals escape capture due to loss of visual contact during the new moon (Clark 1983), suggesting that more hunting is needed for the same amount of food (more hours of hunting may result in more injuries). Additionally, small mammals may concentrate around roads and other open areas (Layne 1971) resulting in more injuries and more recoveries of injured birds. During the full moon, small mammals may not venture far from cover or inhabit dense microhabitats (Kaufmann and Kaufmann 1981), which results in predator pursuits through dense cover (possibly leading to more injuries). The first and second quarters are possibly an intermediate stage that results in less behavioral extremes and more light for maneuvering (Clark 1983), resulting in fewer injuries.

Hawks experience the same peaks of injury occurrence as do owls during the new and full moon although of different magnitudes. Although nocturnal and diurnal raptors utilize many different prey species, several prey species are utilized by both. Use of different micro-habitat by small mammals could result in more exposure of hawks to human disturbances (cars and housing developments). Once again, the first and second quarters may be less prone to behavioral extremes and as a result, fewer injuries occur.

The difference in injuries may be due solely to behavior differences in the prey; however, raptors may exhibit different behavioral pattems in response to direct lunar cue. Raptors have not been documented to respond directly to lunar cues, only indirectly in response to changes in small mammal behavior (Clarke 1983). The nature of raptor response to lunar cues is beyond the scope of this research; however, since a difference in occurrence of collision injuries does exist, research on the nature of the lunar cue would be justified.

For verification of the nature of lunar cues, experiments need to be conducted using altered light environments in controlled enclosures during different periods in the lunar cycle. Differences in micro-habitat use by the raptors during different periods in the lunar cycle would also require study. Insight into behavioral cues of predators would be gained by understanding the effects of lunar cycling in raptors.

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