

Volume 58, Numbers 2, 3, 4

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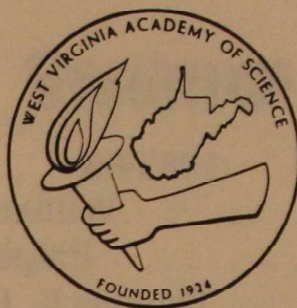
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1986**

Vol. 58—Nos. 2, 3, 4

**PAPERS OF
THE SIXTY-FIRST ANNUAL SESSION**

Marshall University

Huntington, West Virginia

April 4-5, 1986

Printed by
McClain Printing Company
Parsons, West Virginia
April, 1988

Cover Design
Meredith Pearce
West Virginia University
Office of Publications
Morgantown

West Virginia Academy of Science, Inc.

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Biology Section Botany

Aquatic Macrophytes in Doddridge and Harrison Counties, West Virginia

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Abstract

Aquatic plant communities from Doddridge and Harrison Counties were studied during the fall of 1982. Frequency, relative frequency, and sociability were calculated for the various taxa found. The taxa most encountered were *Chara foliolosa*, *Najas flexilis*, *N. minor*, *Potamogeton diversifolius*, *P. foliosus*, and *P. pusillus*. Four taxa were found that are listed as rare and endangered for West Virginia: *Elodea nuttallii*, *Myriophyllum exalbescens*, *Brasenia schreberi*, and *Nymphaea odorata*.

There is little information in the literature concerning the distribution of the aquatic taxa of West Virginia. A generalized discussion on aquatic vegetation in West Virginia was undertaken by Core (1966) and the general ranges of most of the vascular aquatic macrophytes may be found in Strausbaugh and Core (1978). In the present study a total of 46 localities were sampled for aquatic macrophytes in two counties in north-central West Virginia. Of these localities, 19 are in Doddridge County and 27 are in Harrison County. These counties are in the Mixed Mesophytic Forest Region in the Low Hills Belt of the Cumberland and Allegheny Plateaus (Braun 1950). Harrison County is immediately underlain by rock of the Pennsylvanian System which contains the Dunkard, Monongahela, and Conemaugh Groups. The younger Dunkard Group is restricted to the western one-fourth of Harrison County where it overlies the older Monongahela and Conemaugh Groups. The Dunkard Group covers all of Doddridge County except for areas on tributaries that have been eroded to expose the underlying Monongahela Group (Cardwell et al. 1968).

Materials and Methods

The localities sampled include three city reservoirs, one large strip mine pond, and the remainder were small warm-water ponds. Sampling was accomplished by walking the shore, wading in the water, and using a reptile rake. All sites were studied in the late summer of 1982. Voucher specimens were deposited in the West Virginia University Herbarium (WVU). Nomenclature follows Strausbaugh and Core (1978) and Wood (1967).

Each locality was treated as though it were a single plot. From the data obtained, the frequency and the relative frequency were calculated for each species using the following formulas (Vogel and Ebinger, 1979):

$$\text{Frequency (\%)} = \frac{\text{Total habitats of occurrence of a species}}{\text{Total habitats examined}} \times 100$$

$$\text{Relative Frequency} = \frac{\text{Total habitats of occurrence of a species}}{\text{Total habitats of occurrence of all species}} \times 100$$

Sociability of the aquatic macrophytes was determined by the number of times each taxon occurred as the only species present, or as associated with one, two, or three or more taxa (Table 1).

Results and Discussion

A total of 21 species of aquatic macrophytes were sampled. These species are listed in Table 1 along with the county in which they are found, their frequency, relative frequency, and sociability. Taxa having frequencies greater than 7 are considered to be common. These include *Chara foliolosa*, *Najas flexilis*, *N. minor*, *Potamogeton diversifolius*, *P. foliosus*, and *P. pusillus*. Of these, *Najas flexilis* was not found in Doddridge County and *Potamogeton pusillus* was found in only one pond in Doddridge County. *Potamogeton diversifolius* and *P. foliosus* were reported by Strausbaugh and Core (1978) as being throughout the state, while *P. pusillus* and *Najas minor* were reported from 2 counties and *N. flexilis* from 3 counties. In addition to *Najas flexilis*, thirteen taxa were found in Harrison County but not in Doddridge County. These include *Lemna minor*, *Chara braunii*, *C. globularis*, *Elodea canadensis*, *E. nuttallii*, *Heteranthera dubia*, *Myriophyllum exalbescens*, *Potamogeton crispus*, *P. pectinatus*, *Brasenia schreberi*, *Nelumbo lutea*, *Nuphar advena*, and *Nymphaea odorata*. This discrepancy might be due to the lesser number of localities sampled (eight fewer in Doddridge County), the typically smaller sites, or the differences noted in geology. Two taxa, *Potamogeton epihydrus* var. *epihydrus* and *Najas guadalupensis*, were found in Doddridge County but not found in Harrison County. Strausbaugh and Core (1978) list the former taxon as common throughout most of the state. The latter taxon, however, is not included in the *Flora of West Virginia*. McNeill (1965) found *Najas guadalupensis* in Mercer County and this survey adds a second county for this species.

A large number of ponds surveyed contained no aquatic macrophytes. In Harrison County 11% of the ponds were sterile, while in Doddridge

County 37% were sterile. These figures combine to make a total of 21% which lacked aquatic macrophytes. Ponds in heavily wooded areas were sterile, as were many ponds with very riled waters caused by wading livestock. The ponds with the livestock also appeared to have high concentrations of livestock sewage. This sewage may have contributed to the formation of algal blooms which were observed in these ponds. The increased turbidity of the ponds might well provide an effective deterrent to higher plant growth. Similarly, the ponds in wooded situations are subjected to both lower light intensities, due to shading, and greatly decreased oxygen levels, due to the organic matter continually leaching from the leaves and the subsequent respiration of decomposer organisms (Smith 1980).

Most taxa have a high degree of sociability, usually occurring with one or more species. A few consistent associations were found (Table 1). *Potamogeton diversifolius* was commonly associated with *Chara foliolosa* (6 of the 9 locations) and with *P. pusillus* (5 of the 9 locations), while *Najas flexilis* was commonly associated with *N. minor* (5 of the 7 locations). In contrast, at half of the locations in which *Potamogeton foliosus* was found it was the only species present and in nearly another third of its localities it was associated with only one other taxon.

Four aquatic macrophytes, *Elodea nuttallii*, *Myriophyllum exalbescens*, *Brasenia schreberi*, and *Nymphaea odorata* are included in the listing of Rare and Endangered Vascular Plant Species in West Virginia (Clarkson et al. 1981). *Elodea nuttallii* is classified as local and had been previously vouchered from the same station. *Myriophyllum exalbescens* is classified as local and vulnerable (Clarkson et al. 1981) and had been previously vouchered from Harrison County. *Myriophyllum exalbescens* was found in abundance during this study from the same station and from two additional stations in Harrison County. *Brasenia schreberi* and *Nymphaea odorata* are classified as vulnerable and neither had previously been found in Harrison County where *Brasenia schreberi* was found at a single station and *N. odorata* from two stations.

One aquatic macrophyte, *Najas guadalupensis*, found during this study is not included in Strausbaugh and Core (1978). The taxon was found at two stations in Doddridge County and should be included in the next revision of the West Virginia Flora. Although *N. guadalupensis* was previously reported by McNeill (1965), no voucher specimens were located.

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Table 1. Sociability, frequency, and relative frequency of submersed aquatic macrophytes in Doddridge and Harrison counties during the late summer of 1982

	Only species	Associated with one other species	Associated with two other species	Associated with three or more other species	Dodd. Co.			Harr. Co.			Total		
					# Ponds	Freq. %	Rel. Freq.	# Ponds	Freq. %	Rel. Freq.	# Ponds	Freq. %	Rel. Freq.
FREE-FLOATING SPECIES													
<i>Lemna minor</i> L.	*	2	—	2	—	—	—	4	15	5.7	4	9	4.4
SUBMERSED SPECIES													
<i>Chara braunii</i> Gmel.	*	—	1	1	—	—	—	2	7	2.9	2	4	2.2
<i>C. foliolosa</i> Muhl.	+	3	1	5	3	16	15	6	22	8.5	9	20	10.0
<i>C. globularis</i> Thuill.	*	2	—	1	—	—	—	3	11	4.3	3	7	3.3
<i>Elodea canadensis</i> Michx.	*	—	—	1	—	—	—	1	4	1.4	1	2	1.1
<i>E. nuttallii</i> (Planch.) St. John	*	—	—	1	—	—	—	1	4	1.4	1	2	1.1
<i>Heteranthera dubia</i> (Jacq.) MacM.	*	—	—	1	—	—	—	1	4	1.4	1	2	1.1
<i>Myriophyllum exalbensens</i> Fern.	*	—	—	1	—	—	—	3	11	4.3	3	7	3.3
<i>Najas flexilis</i> (Willd.) Rostk. & Schmidt.	*	1	—	6	—	—	—	9	33	12.9	9	20	10.0
<i>N. guadalupensis</i> (Spreng.) Magnus	+	—	2	1	2	11	10	—	—	—	2	4	2.2
<i>N. minor</i> All.	+	—	—	5	2	11	10	5	19	7.1	7	15	7.8
<i>Potamogeton crispus</i> L.	*	—	—	1	—	—	—	1	4	1.4	1	2	1.1
<i>P. diversifolius</i> Raf.	+	5	2	4	3	16	15	9	33	12.9	12	26	13.3
<i>P. epiphydrus</i> Raf. var. <i>epiphydrus</i>	+	1	—	1	3	16	15	—	—	—	3	7	3.3
<i>P. foliosus</i> Raf.	+	5	2	1	6	32	30	10	37	14.3	16	35	17.7
<i>P. pectinatus</i> L.	*	—	—	1	—	—	—	1	4	1.4	1	2	1.1
<i>P. pusillus</i> L.	+	2	1	5	1	5	5	8	30	11.4	9	20	10.0
EMERGENT SPECIES													
<i>Brasenia schreberi</i> Gmel.	*	—	—	—	—	—	—	1	4	1.4	1	2	1.1
<i>Nelumbo lutea</i> (Willd.) Pers.	*	—	—	1	—	—	—	1	4	1.4	1	2	1.1
<i>Nuphar advena</i> (Ait.) Ait. f.	*	1	1	—	—	—	—	2	7	2.9	2	4	2.2
<i>Nymphaea odorata</i> Ait.	*	—	—	2	—	—	—	2	7	2.9	2	4	2.2

+New county record Doddridge County
 *New county record Harrison County

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NEW COUNTY RECORD HARRISON COUNTY

Updates on the Vascular Flora of West Virginia. II.

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Abstract

During the past year, seven species of vascular plants have been identified or verified at the West Virginia University Herbarium (WVA) as new records for the state of West Virginia (based on Strausbaugh and Core, 1978). Unless otherwise noted, nomenclature and distribution information follows Fernald, 1950.

The new species here reported are:

Puccinellia distans (L.) Parl. (Poaceae). Ohio County, *Allison Cusick*, 23,559, June 14, 1984. This plant is naturalized from Europe.

Carex echinata Murr. (Cyperaceae). Preston County, *Allison Cusick*, 23,510, June 11, 1984. A more northern species, this is probably a southern range extension.

Rorippa sessiliflora (Nutt.) A. S. Hitchc. (Brassicaceae). Wayne County, *Allison Cusick*, 24,214, May 10, 1985. This species is in our range and to be expected.

Thermopsis villosa (Walt.) Fern. & Schub. (Fabaceae). Wyoming County, *Dana Evans*, s.n., 1973. Plant is to our south and to be expected. (Radford, et al, 1968).

Vicia cracca L. (Fabaceae). Logan County, *Anna L. Scott*, s.n., May 21, 1973; Putnam County, *Margaret E. Dennison* s.n., June 30, 1973. This species is naturalized from Europe.

Ludwigia peploides (H.B.K.) Raven ssp. *glabrescens* (Kuntze) Raven (*Jussiaea repens* var. *glabrescens* Kuntze) (Onagraceae). Wood County, *Allison W. Cusick*, 24,960 & *Marilyn Ortt*, October 23, 1985. Plant occurs in our area and is to be expected in West Virginia.

Liatris squarrulosa Michx. (Asteraceae). Morgan County, *Allison W. Cusick*, 23,862, September 5, 1984. This is a southern species, but is expected in our area. (Cronquist, 1980).

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RNA Synthesis in Mesophyll Protoplasts of a Sunflower Hybrid and Its Parents with and without the Addition of Exogenous Phytohormones

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Abstract

Total RNA synthesis in protoplasts derived from two parental lines of sunflower (RHA 297 and 290x89) and their hybrid progeny were compared in their response to the phytohormones, indoleacetic acid (IAA), naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP). Significant differences were found in responses which were dependent upon genetic background. Inbreds and hybrids displayed lower levels of synthesis in response to 10^{-6} M IAA when compared to controls; the greatest reduction occurred in 290A x 89B. BAP and NAA at 10^{-6} M produced a significant increase in RNA synthesis in the hybrid over that of its parents.

Introduction

Heterosis occurs when hybrids exceed the mean performance of their parents or of the superior parent in some defined specific trait, or a general one such as organic matter accumulation. It is also associated with various metabolic processes such as ascorbic acid turnover (1), oxidative phosphorylation (15), and altered template and RNA polymerase activity (4). The sunflower hybrid H9097 is superior to its parents (290A x 89B and RHA290) in oil production; however, it does not manifest heterosis in the production of leaf and stem tissue under the conditions of the W. Va. Univ. Exp. Sta. Plant Sciences Farm in Morgantown, W.

Va. It was intermediate to both parents in leaf and stem yield. Since it did not manifest vegetative heterosis, it has been utilized to investigate whether RNA synthesis follows the vegetative growth pattern or manifests enhanced synthesis as the result of heterozygosity. The studies were conducted with and without phytohormone stimulation. Phytohormones increase the levels of RNA in various plant tissues (14). Twenty-four hours after the application of auxin, an increase in membrane bound ribosomes has been observed (23) as well as poly A + RNA (18,29). Vigor has been positively correlated with increased poly A + RNA (12). Indoleacetic acid (IAA) acts on the chromatin of the nucleolus (21) and on a nucleoplasmic receptor protein to form a complex which triggers the synthesis of various RNA species (16). The phytohormone receptor has been found in the chromatin (20).

Hormones also alter the permeability of protoplasts to nucleic acid precursors (28). Zelcer et al. postulated that the absence of either naphthaleneacetic acid (NAA) or 6-benzylaminopurine (BAP) prevents cell division in protoplasts and cause a leveling off of uridine incorporation. A 24 hr preconditioning of potato leaf tissue in a solution containing 1 and 2 mg/l, respectively of BAP and NAA, produced higher and more consistent survival percentages of protoplasts during the first 2 days of regeneration (22). Inclusion of 1 mg NAA/l in a culture medium sustained cell division in the formation of callus from protoplasts of cultured grape pericarp (25). A positive correlation has been established between the amount of BAP present in the culture media and grapevine pericarp callus protoplast survival and division (24). Reduction of BAP concentration (1 mg/l is optimum) from 1 to 0.2 mg/l lowered protoplast survival and division.

Materials and Methods

Seeds of sunflower (*Helianthus annuus* L.) hybrid H9097 and of its male sterile parent 290A x 89B and restorer parent RHA297 (Sheyenne Foundation Seeds, Inc.) were planted in 20 cm pots of moistened autoclaved soil fertilized with 20:20:20 (N-P-K). Additional fertilizer solutions were applied at weekly intervals. Plants were maintained at 30°C, with a 12 hr photoperiod of 10,000-Lux, white fluorescent and incandescent light. They were irrigated with tap distilled water and kept at 70% relative humidity. The fully expanded middle leaf of plants in the three-leaf stage was used for the production of protoplasts since young leaves are more active in transcription.

The Bhojwani technique (2) was modified by the addition of two percent Ficoll for the isolation of protoplasts. Leaves were surface sterilized for 20 min in 0.5 percent sodium hypochloride and 0.1 percent sodium dodecylsulfate (SDS), rinsed thoroughly in autoclaved double distilled water, and cut into 3-5 mm² pieces in a petri plate.

A digestive enzyme solution consisting of osmoticum/OS:0.6 M sorbitol, 5 mM MgCl₂ and 1 mM Ca(H₂PO₄)₂•H₂O monobasic, pH 5.8 to which digestive enzymes had been added [2% Cellulysin (Calbiochem), 1% pectinase in glycerol (Sigma) and 0.5% Driselase (Calbiochem)] was

poured over the 1-3 mm² pieces of sunflower leaf tissue in a petri plate, sealed with parafilm and incubated in darkness at 25°C for 16 hrs.

Protoplasts were released by gently pressing leaf tissues and filtering through four layers of cheese cloth and one layer of miracloth. The cells were pelleted by centrifugation at 100 xg (swinging bucket) for 8 mins, washed twice in OS, suspended in a known volume of OS plus 2% Ficoll (Sigma) and counted with a hemocytometer (13). Live cells were distinguished from dead ones by their spherical shape and/or exclusion of Evans blue dye (0.1%). Chloramphenicol (10 ug/ml) was included in the incubation media.

For each experiment 10⁵ cells were suspended in a volume of 0.495 mls of OS containing 10 ug/ml chloramphenicol with and without the appropriate hormones, IAA, NAA and BAP. Cells were incubated in a closed water bath at 37°C with a Grow-lux light. After 1 hr of preincubation 5 uCi ³H-uridine (37 mCi/mmol) were added to each tube; incubation was resumed for 5 more hrs. Incorporation was terminated in two sets of triplicate tubes by adding 50 ul of 20% (w/v) SDS and vortexing each tube for 15 sec to lyse the protoplasts. Two mls of ice cold 10% trichloroacetic acid (TCA) were added to each tube, and the tubes were incubated on ice for 30 min. The TCA precipitated material was poured onto a GF/A filter under suction and washed three times with 6 mls of 5% TCA. The filters, after thorough drying, were placed into scintillation vials with 10 mls of toluene POPOP-PPO scintillation cocktail and counted twice, for ten min each, in a Beckman LS-1000 C scintillation counter.

Results

Differences in viability between the protoplasts of the three strains as measured by Evans blue (0.1%) exclusion were unremarkable (Fig. 1).

The incorporation of ³H-uridine into total RNA by the mesophyll protoplasts of the hybrid and its two parents (290A x 89B and RHA298) did not differ significantly for the first two hrs. Differences in incorporation began to appear by three hrs of incubation and decided differences were evident by 5 hrs (Fig. 2).

The basis for testing heterosis is the comparison between the incorporation of labelled precursor into cells of hybrid H9097 and the average of incorporation of its inbred parents (19). Since this is an *a priori* approach, that is, the contrast was planned before the experiment, a one degree of freedom comparison is applied to test the following:

$$\text{hypothesis: } u_{H9097} - \frac{u_{290A \times 89B} + u_{RHA297}}{2} = 0$$

Incorporation of ³H-uridine by sunflower protoplasts during a 5 hr incubation period, with and without hormone is given in Table 1. The analysis of variance (ANOVA) for incorporation for the control IAA, NAA and BAP are given in Tables 2, 3 and 4 respectively. A significant difference at the 1% level exists between H9097 and its parent, 290A x 89B, in incorporation of ³H-uridine without hormone. A similar difference exists between RHA297 and 290A x 89B. The difference between

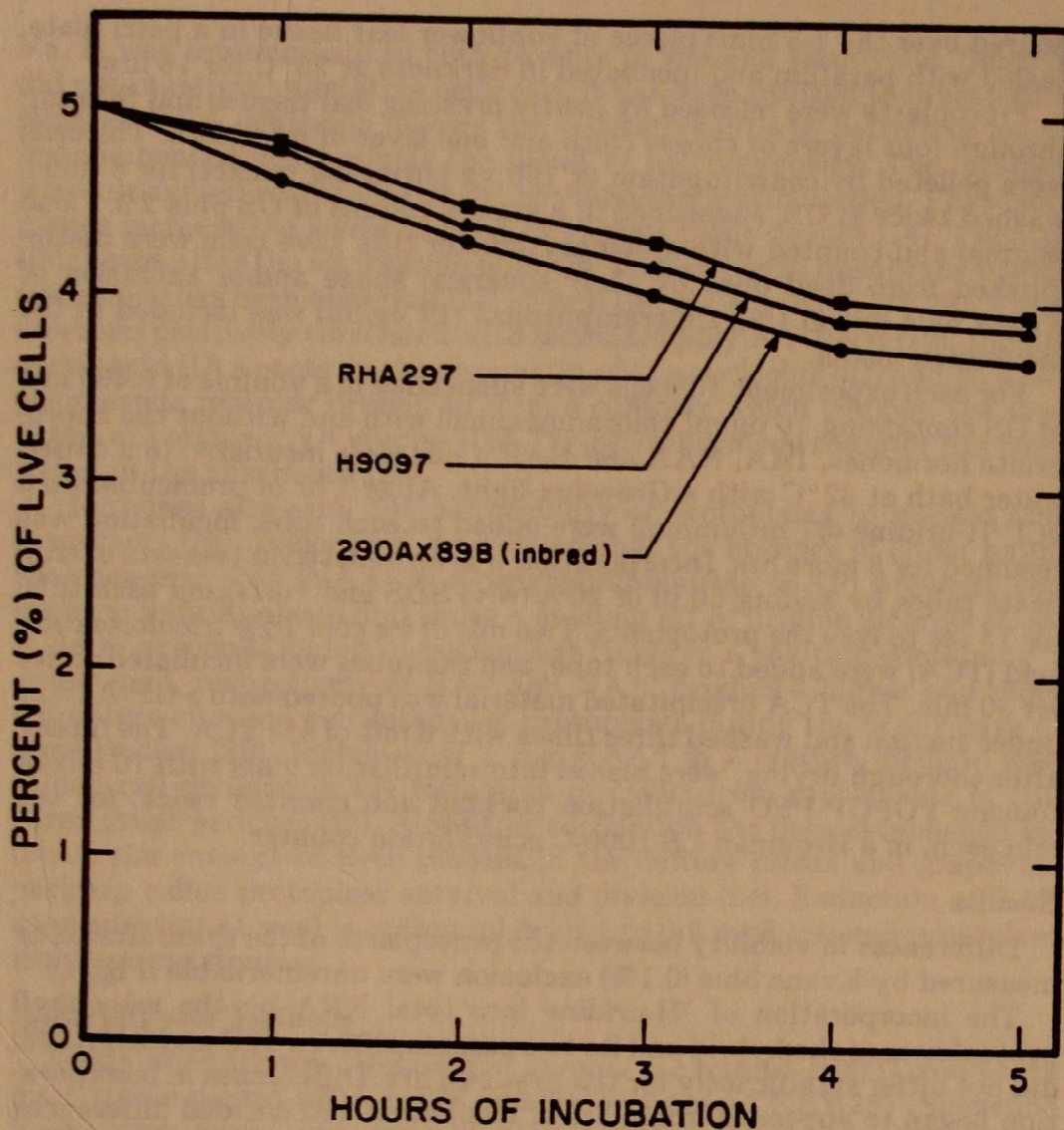


FIGURE 1. Viability curves of RHA297, 290Ax89B (inbred) and H9097 (hybrid). 2.5×10^5 protoplasts were incubated in osmoticum for 5 hrs at 30°C . Percent live cells at hourly interval was determined by exclusion 0.1% Evans blue dye.

the treatment and control is also significant. IAA generally depressed incorporation but, percentage wise less so in the hybrid H9097 than its parents (Table 2). Incorporation is 95.1% of the control with hormone in H9097, 92.7% in RHA297 and 76.8% in 290A x 89B.

With NAA treated protoplasts significant differences were found between strains (Table 3). The interaction of strain x hormone was not significant, but the difference between the hybrid and its parents was. The hybrid was superior in incorporation to its vegetatively more vigorous RHA297 parent when stimulated by NAA (Table 1). However, no significant difference was found between NAA treatment and control. The hybrid H9097 manifested a heterotic response by incorporating significantly more ^3H -uridine than the mean incorporation of its inbred parents.

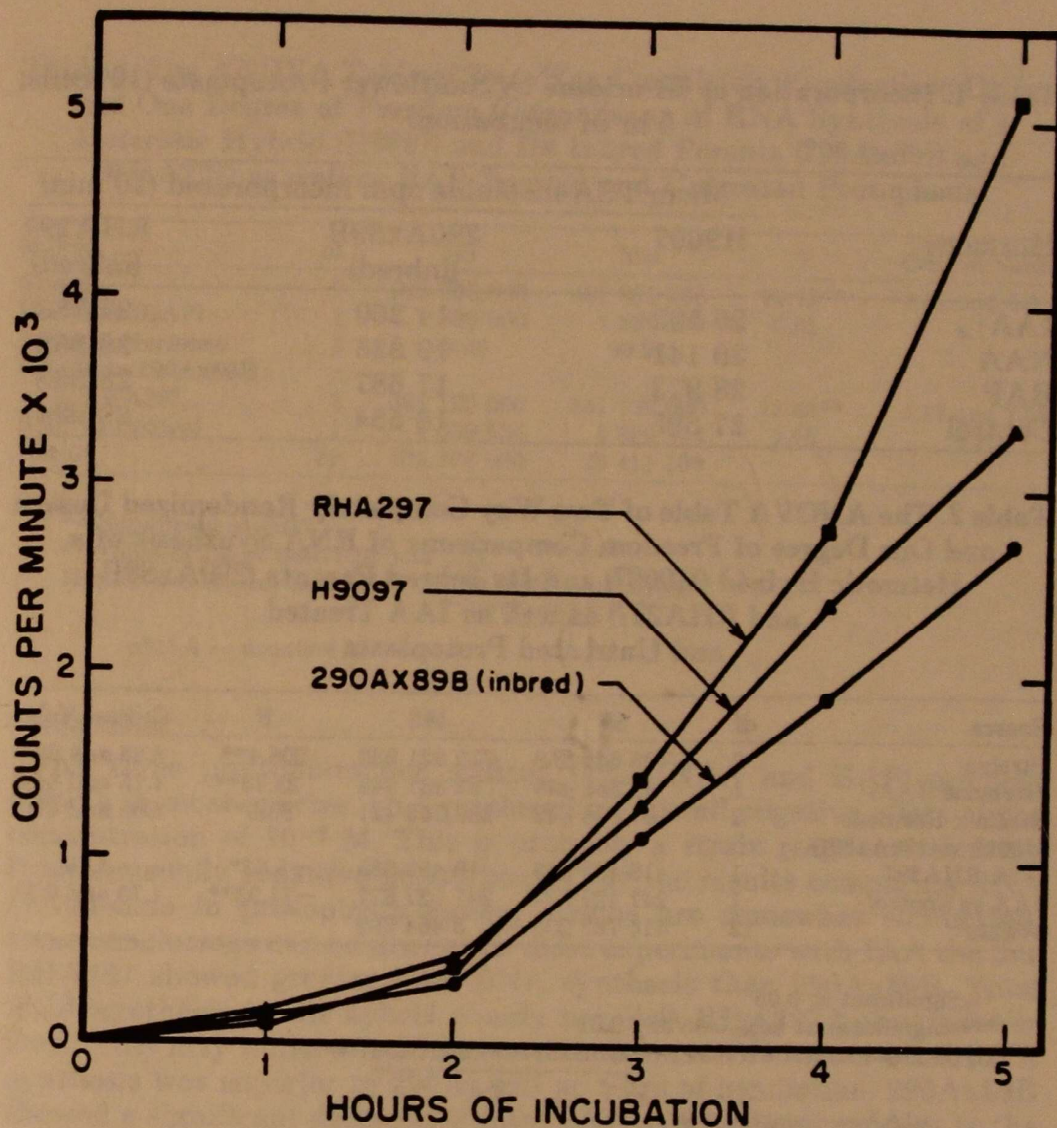


FIGURE 2. Incorporation of ^3H -uridine into TCA-insoluble material of sunflower protoplasts of H9097, 290Ax89B and RHA297. A 5-hour study of incorporation of tritiated uridine into 1×10^5 protoplasts ($10\mu\text{Ci}/\text{ml}$ ^3H -uridine) was carried out. The TCA insoluble material was filtered onto a glass fiber filter. The incorporated labelled precursor was measured in a toluene based scintillation cocktail (POPOP-PPO). Each point represents a mean of 3 experiments which were triplicated.

The ANOVA for the BAP treatment (Table 4) showed that the hybrid had a significantly superior rate of incorporation to the mean performance of its parents. However, the difference in incorporation between hybrid H9097 and RHA297, (the more vigorous of the two parents), is only 465 cpm; the hybrid and RHA297 are therefore equal. Also, both parental lines manifested a slight but nonsignificant decline in incorporation over the controls.

Table 1. Incorporation of ³H-uridine by Sunflower Protoplasts (10⁵ cells; 5 hr of incubation)

Hormone	Mean TCA-insoluble cpm incorporated (10 min)		
	H9097	290Ax89B (inbred)	RHA297 (inbred)
IAA	26 533	14 250	27 783
NAA	30 141	19 835	28 362
BAP	28 973	17 687	28 508
Control	27 895	18 554	29 984

Table 2. The ANOVA Table of Two-Way Completely Randomized Design and One Degree of Freedom Comparisons of RNA Synthesis of a Heterotic Hybrid (H9097) and Its Inbred Parents (290Ax89B and RHA297) as well as IAA Treated and Untreated Protoplasts

Source	df	SS	MS	F	Critical Value
Strains	2	1 403 643 859	715 321 930	206.4**	3.88 and 6.93
Hormone (IAA)	1	82 381 448	82 381 448	23.73**	4.75 and 9.33
Strain x Hormone	2	567 506 842	283 753 421	85.5**	3.88 and 6.93
H9097 vs 290Ax89B & RHA297	1	19 483 385	19 483 385	-5.62*	4.75
IAA vs Control	1	247 127 677	247 127 677	-71.33**	4.75 and 9.33
Within	12	415 783 233	3 464 609		

*=Significant at 0.05

**=Significant at both 0.05 and 0.01

Hypotheses tested: $u_{H9097} - u_{290Ax89B} + u_{RHA297} = 0$

2

$u_{IAA} - u_{control} = 0$

Table 3. The ANOVA Table of Two-Way Completely Randomized Design and One Degree of Freedom Comparisons of RNA Synthesis of a Heterotic Hybrid (H9097) and Its Inbred Parents (290Ax89B) and RHA297) as well as NAA Treated and Untreated Protoplasts.

Source	df	SS	MS	F	Critical Value
Strains	2	784 333 000	392 166 250	27.05**	3.32 and 5.39
Hormone (NAA)	1	322 200 000	322 200 000	22.22**	4.17 and 7.56
Strain x Hormone	2	23 445 000	11 722 500	0.81	3.32 and 5.39
H9097 vs 290AX89B & RHA297	1	373 910 000	373 910 000	25.79**	4.17 and 7.56
NAA vs Control	1	10 877 552	10 877 552	0.75	11
Within	30	435 000 000	14 500 000		

* = Significant at 0.05

** = Significant at both 0.05 and 0.01

Hypotheses tested: $u_{H9097} - u_{290Ax89B} + u_{RHA297} = 0$

$u_{NNA} - u_{control} = 0$

Table 4. The ANOVA Table of Two-Way Completely Randomized Design and One Degree of Freedom Comparisons of RNA Synthesis of a Heterotic Hybrid (H9097) and Its Inbred Parents (290Ax89B and RHA297) as well as BAP Treated and Untreated Protoplasts

Source	df	SS	MS	F	Critical Value
Strains	2	923 833 000	461 916 500	19.73**	3.32 and 5.39
Hormone (BAP)	1	1 889 000	1 889 000	0.81	4.17
Strain x Hormone	2	99.96	99.96		
H9097 vs 290Ax89B & RHA297	1	361 120 000	361 120 000	15.43**	4.17 and 7.56
BAP vs Control	1	4 809 534	4 809 534	2.05	4.17
Within	30	702 333 000	23 411 100		

*=Significant at 0.05

**=Significant at both 0.05 and 0.01

Hypotheses tested: $u_{H9097} - \frac{u_{290Ax89B} + u_{RHA 297}}{2} = 0$

$u_{NAA} - u_{control} = 0$

Discussion

All three phytohormones utilized (IAA, NAA and BAP) promote growth in most species. IAA produced an overall negative effect at the concentration of 10^{-6} M. This is probably a strain response resulting from unusually sensitive genotypes. While the results comparing RNA metabolism in protoplasts among strains are somewhat conflicting, some conclusions can be drawn. In most experiments with IAA the line RHA297 showed greater total RNA synthesis than 290Ax89B. Total RNA synthesis in the hybrid closely parallels RHA297 but at a lower level. This may indicate some form of dominance. However total RNA synthesis was superior to 290Ax89B at 5 hrs of incubation. 290Ax89B showed a significant decrease in total RNA synthesis in response to the addition of 10^{-6} M IAA. RHA297 and the hybrid H9097 showed no significant response to the phytohormone (Tables 1 and 2). Further work is required to study auxin-kinetic or auxin-cytokinin balances in sunflower in connection with transcription. Murayama et al (17) reported occasional decreases in plant growth due to auxin. Auxin alone has an adverse effect on some cells (5,7). Skoog has suggested that a balance between auxin and kinitin is critical in determining the type of growth obtained in tobacco (26,27). Although IAA has been found to enhance vacuolation in tomato root protoplasts (eventually resulting in bursting) this effect was not observed with sunflower leaf protoplasts. Possibly the concentration of 10^{-5} M IAA did not alter membranes sufficiently to produce a significant increase in water uptake (6).

It is unlikely that pH influenced these results since the physiological range for IAA activity is between 5.5 and 6.5 and the medium used had a pH of 5.8(6).

The genetic composition of the strain probably influences the effect of NAA (Table 3). Both the treated hybrid and the male sterile line (290A x

89B) display an increase in the incorporation of tritiated RNA precursor into the acid-insoluble material of the protoplasts. However, NAA has a negative effect on 290A x 89B. Differences in response to plant hormones among petunia strains, and lines of barley aleurones, have been linked to genetic differences among genotypes. In NAA and 2,4-D supplemented media, the ability of the protoplasts (derived from petunia) to divide is genetically determined. All the petunia hybrids tested displayed superior response to various hormone combinations over their inbred parental lines.

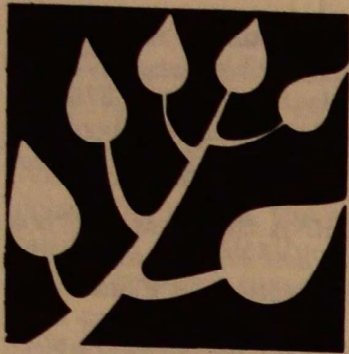
BAP stimulated RNA synthesis in the hybrid, while having an adverse effect on the inbred parents. The accessibility of labelled precursors to the DNA-dependent RNA polymerases might vary according to strains. The hybrid state may result in more hormone receptors, either on the cell membrane and/or chromatin, and produce superior hormone responsiveness. Alternatively, the hybrid state may have higher affinity for hormone at the same number of receptors. This would make concentrations, that were insufficient for producing stimulating effects in parental lines, adequate for the production of the heterotic response.

Although heterosis in stem and leaf production are not apparent under field conditions with H9097, statistically significant higher levels of RNA synthesis were stimulated by NAA and BAP. The heterozygous state may provide a superior system for transcription through regulation and may not be reflected as a component of vegetative growth. Instead the energy so conserved may have been partitioned into greater oil synthesis by the hybrid.

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Biology Section Zoology

Additional Notes on the Range and Distribution of the Cheat Mountain Salamander *Plethodon nettingi*

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Abstract

Field surveys conducted in the summers of 1982 to 1985 helped to further define the range and distribution of *Plethodon nettingi*. These studies resulted in 54 known disjunct populations. One of these populations is the most northern and lowest in elevation to be reported.

Introduction

Brooks (1948) described the range of *P. nettingi* as an area extending from the headwaters of Condon Run, Randolph County, south to Thorny Flat, Pocahontas County. In 1971, Highton found that the range extended north and east to the high elevations of the Allegheny Front. In 1981, Pauley described the range as an area extending east from McGowan Mountain through Mozark Mountain to Dolly Sods, south through Roaring Plains to Spruce Knob, southwest to Thorny Flat and north through Barton Knob to McGowan Mountain. Pauley also reported that of 181 sites surveyed, *P. nettingi* occurred in just 27 and that the elevation range was from 4,500 ft (1372 m) to 3,450 ft (1052 m).

Methods and Materials

During the summers of 1982 to 1985, an additional 183 sites were surveyed for *P. nettingi*. All sites were above 3,400 ft (1036 m) and were surveyed for at least one-man hour and within 48 hours of a rainfall.

Results and Discussion

A total of 5,928 salamanders was observed in the 364 sites (181 sites prior to 1982 and 183 sites surveyed between 1982 and 1985) and 682 (11.5%) of these were *P. nettingi*. The major species to occur other than *P. nettingi* were: *P. cinereus*, 2,486 (41.9%); *Desmognathus ochrophaeus*, 1,942 (32.7%); *P. wehrlei*, 451 (7.6%); and *P. glutinosus*, 273 (4.6%).

In the most recent sites surveyed (183) *P. nettingi* was found in 27 (14.7%). The total number of known *P. nettingi* sites is now 54 (14.8% of all sites surveyed). One of these recent sites is located at an elevation of 3,120 ft (951 m) southeast of Olson Fire Tower in Tucker County. This population is the most northern and the lowest in elevation known (Pauley, 1987). The previous most northern population was on Mozark Mountain, Tucker County; and the previous low elevation population was near Bear Heaven (3,451 ft or 1,052 m), Randolph County (Pauley, 1981).

The status of *P. nettingi* now appears to be as follows. There 54 disjunct populations in a range that extends from Canyon Rim Trail north of Blackwater River Canyon east through Mozark Mountain to Dolly Sods, south through Roaring Plains to Spruce Knob, southwest to Thorny Flat and north through Barton Knob and McGowan Mountain to Blackwater River Canyon. Two of the 54 populations have been destroyed and at least three more have been seriously disturbed by various types of activities such as coal mining, timbering, ski slope construction, etc.

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A New Record of the Diploid Species of Gray Treefrog, *Hyla chrysoscelis*, in West Virginia

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Abstract

The diploid species of gray treefrog, *Hyla chrysoscelis*, is reported for the first time to inhabit spruce bogs at higher elevations in West Virginia.

Introduction

Two morphologically indistinguishable species of gray treefrogs are allopatrically distributed in West Virginia. A diploid species ($2n=24$), *Hyla chrysoscelis*, is in the Unglaciated Allegheny Plateau and the Allegheny Mountains of the Appalachian Plateau Province. A tetraploid species ($4n=48$), *H. versicolor*, is primarily in the Ridge and Valley Province and in the portion of the Unglaciated Allegheny Plateau which is drained by the Greenbrier River. Sympatry of these two species occurs only along the New River in Summers and Monroe counties. Neither species has previously been reported from the higher elevations along the Allegheny Front (Little, 1983). Although the male reproductive calls of these species are distinct when heard in the field (the call of *H. chrysoscelis* is more rapidly pulsed), these species can be reliably identified only by comparing numbers of nucleoli, chromosomes, or pulses in male reproductive calls (Ralin and Selander, 1979).

On the night of July 8, 1986, a number of male gray treefrogs were calling in Olson Bog (elevation 978 m) on Backbone Mountain in Tucker County. We initially identified these as *H. chrysoscelis*, because the male reproductive calls sounded to be fast pulsed. This identification was confirmed by an analysis of chromosome numbers for two of these males (by John Wiley, East Carolina School of Medicine). These are the first gray treefrogs which have been reported from elevations above 900 m in West Virginia. Both species are absent from high elevations in the drainages of Dry Fork, Glady Fork and Red Creek, Randolph County, and from Canaan Valley, Tucker County.

Discussion

The natural history of *H. chrysoscelis* in Olson Bog is not consistent with that of conspecific populations in Texas and Wisconsin. Ralin (1968) reported that Texas males of *H. chrysoscelis* call primarily from vegetation and males of *H. versicolor* call primarily from the ground. Most male *H. chrysoscelis* which were heard in Olson Bog were calling on the ground from mats of sphagnum moss near the water. We have observed males of *H. chrysoscelis* calling on the ground near water at many other sites in the Allegheny Mountains and the Unglaciated Allegheny Plateau of West Virginia. In the western portion of its range (Texas to Wisconsin) *H. chrysoscelis* is most frequent found in association with grasslands and open areas (Blair, 1958). The *H. chrysoscelis* which we found in Olson Bog were in a heavily forested area. In West Virginia *H. versicolor* is the species of gray treefrog which occupies the more open areas of the Valley and Ridge Province.

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Herpetology Field Notes: Two Record Size Salamanders

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Abstract

Two salamander species, *Notophthalmus v. viridescens* and *Plethodon wehrlei*, collected during the summers of 1985 and 1986 established new records in length.

Discussion

Notophthalmus v. viridescens (Red-spotted Newt)

Conant (1975) reports the minimum total length for an adult *N. v. viridescens* to be 7.0 cm. A gravid female with a snout-vent length of 3.20 cm and a total length of 5.80 cm was collected on 13 June 1985 in a sphagnum bog 9.17 km north of the town of Fisher, Hardy County, West Virginia.

Plethodon wehrlei (Wehrle's salamander)

Conant (1975) lists the maximum total length of *P. wehrlei* to be 16.00 cm. A female with a snout-vent length of 7.48 cm and a total length of 16.80 cm was collected on 6 June 1986 in Blackwater Falls State Park, Tucker County, West Virginia.

Both specimens had the typical adult pattern, color, and other morphological features. They were deposited in the West Virginia Biological Survey at Marshall University (*N. v. viridescens* — WVBS 4675 and *P. wehrlei* — WVBS 4676).

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Pollutant-induced Changes in the Respiratory Response of the Green Crab, *Carcinus maenas* (L.) (Decapoda: portunidae) to Hypoxia

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Abstract

The green crab *Carcinus maenas* was exposed to eight common aquatic pollutants: PCB, PCP, DDT, 2-4-D, Hg, Cd, H₂S and NH₃ in experiments designed to examine alterations in emersion behavior and aerobic shutdown. Exposures to these chemical substances caused significant alterations in respiratory response. Exposure to PCP during hypoxia caused a significant decrease in the PO₂ level at which emersion behavior occurred. Exposure to H₂S during hypoxia caused a significant increase in the PO₂ level at which aerobic shutdown occurs.

Introduction

At high concentrations, most common aquatic pollutants have been shown to be lethal to most organisms studied (e.g., Eisler and Hennekey, 1977; Johnson and Gentile, 1979). However, to determine more accurately the overall impact of pollutants, a search for subtle physiological and/or behavioral changes that are induced in exposed organisms must also be studied. The respiratory mechanisms of many aquatic organisms seem to be particularly susceptible to pollutant-induced changes.

Heavy metals have been intensively studied, primarily with respect to their effects on the rate of oxygen consumption ($\dot{V}O_2$) (Reeve et al., 1977; Calabrese et al, 1975; and Moraitou-Apostolopoulou et al, 1979). Specific respiratory effects vary widely, from metal to metal, from species to species, and across concentrations for a given metal in a given species. Of particular interest is the interactive effect of heavy metal exposure and hypoxia (e.g., Alderdice, 1963). There appears to be a generalized, in-

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verse relationship between metal tolerance and environmental oxygen partial pressures (PO_2) (Voyer, 1975). Since, for many aquatic species (respiratory conformers), $\dot{V}O_2$ is proportional to PO_2 , the decreased toxicity at high PO_2 appears to be coupled with decreased absorption resulting from generally decreased metabolic rates (Clubb et al, 1975). However, since $\dot{V}O_2$ is itself affected by heavy metal exposure, the interactive effects are extremely complex and causal relationships with mortality and sublethal effects are difficult to determine.

Complex situations also exist for the other major classes of pollutants, for example: pesticides (Jackim et al, 1970; Anderson, 1971), polychlorinated phenols "naphthalene" (Laughlin and Neff, 1979; Stekoll et al, 1980), and reducing gases (Evans, 1967). Variation in environmental pH also has a significant effect on the respiratory physiology of aquatic organisms, both as a primary factor (i.e., changes in hydrogen ion concentration) and as it affects the valence states and dissociation constants of other substances (Dietrich et al, 1980).

Other factors of respiratory physiology such as extraction efficiencies, branchial ventilation rates, aerial respiration, and aerobic shutdown also have been studied in relation to pollutant exposure in aquatic organisms. Therefore, a significant but complex relationship appears to exist between various aspects of an organism's respiratory response and the interacting effects of hypoxia and pollutant exposure.

The green crab, *Carcinus maenas* (L.), demonstrates a particularly complex respiratory response to progressive environmental hypoxia. This response is a "mixed strategy" for survival involving two distinct mechanisms, one behavioral, i.e., emersion (Taylor et al, 1973; Becker and Valinski, 1980), and one physiological, i.e., aerobic shutdown, (Becker and Bayne, 1976; Burke, 1979). When exposed to progressive hypoxia, *Carcinus* first emerges the anterior margin of its carapace and ventilates its branchial chambers with air; this occurs at about a PO_2 of 65 torr, the critical pressure (P_c) for *Carcinus* (i.e., the point above which the animal regulates $\dot{V}O_2$ and below which it conforms to environmental PO_2). If the animal is prevented from emerging, it will return to aquatic ventilation until the environmental PO_2 decreases to about 24 torr, at which point the animal initiates aerobic shutdown. Aerobic shutdown involves the complete cessation of ventilatory activity and a shift from aerobic to anaerobic metabolic pathways.

The objective of this study was to investigate the potential effects of a group of common aquatic pollutants (viz., PCB, PCP, DDT, 2-4-D, Hg, Cd, H_2S , and NH_3) on this "mixed strategy" response to hypoxia by *Carcinus*.

Materials and Methods

Green crabs, *Carcinus maenas* (L.) were obtained from the Marine Biological Laboratory, Woods Hole, MA and maintained in a 400 liter sea water table with re-circulating synthetic sea water (Instant Ocean R) at 25 0/00 Salinity (S) and 22°C. Animals were held for at least two weeks until latent tidal rhythmicities were completely dampened. The animals were fed a mixed diet of ground beef heart and fish during hold-

ing, and were starved for 14 days prior to the experiment (Ahsanullah and Newell, 1971; Wallace 1973).

The apparatus used in the experiments was a modification of that used by Bayne (1971). A one liter respirometer chamber was partially filled with 600 ml of synthetic sea water, a crab was placed inside, and allowed to acclimate for one hour. The respirometer chamber was immersed in a light-proof water bath which was maintained at 22°C. Water was continuously circulated within the chamber by means of a submersible stirring unit to prevent localized variations in oxygen concentration. At the end of the acclimation period the stopcocks were closed, sealing off the chamber. All pollutant additions and/or pH adjustments were made at this time. Test substances were introduced by withdrawing a certain volume of water and then refilling the chamber with the same volume of test solution in order to obtain the desired concentration. This method permitted acclimation to the chamber under maintenance conditions and also allowed for pollutant addition without aerial exposure of the organisms. The exposure levels were: Aroclor 1254 (PCB) — 40 ppm, sodium pentachlorophenate (PCP) — 80 ppm, chlorophenothane (DDT) — 120 ppm, 2, 4 — dichlorophenoxyacetic acid (2-4-D) — 20 ppm, $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (H_2S) — 50 ppm, NH_3Cl (NH_3) — 120 ppm, HgCl_2 (Hg) — 40 ppm, and CdCl_2 (Cd) — 20 ppm. These pollutant concentrations are similar to those commonly measured in the field (Becker and Thatcher, 1973). All are soluble at these concentrations. The pH levels were adjusted to the indicated levels with HCl or NaOH, except when sulfide effects were being studied. In that case the pH levels were adjusted prior to the addition of $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$.

At 30 minute intervals, 50 ug/L samples of water were withdrawn from the chamber with a syringe and cannula, and the partial pressure of oxygen (PO_2) was measured using a Radiometer (type E-5046) oxygen electrode with a PHM 71 acid base analyzer. The animal was observed for the emersion response and the PO_2 was recorded at the time of emersion. When two successive PO_2 determinations indicated that there had been no decrease in PO_2 within the chamber for a period of one hour, aerobic shutdown was considered to have occurred, and the experiment was terminated.

There was no mortality during the experiment.

The tables present the summarized data and include the means and standard deviations and the number of organisms tested (N). The statistical analyses were done using the analyses of variance.

Results

Exposure to the test substances induced significant changes in the respiratory response to hypoxia of *Carcinus*. The average PO_2 at which *Carcinus* emersed with 59.9 ± 8.9 torr, while the average PO_2 at which aerobic shutdown was initiated was 25.2 ± 5.1 torr (Table 1). Exposure to PCP induced a significantly ($P < 0.01$) later emersion at a PO_2 of 44 torr; other PO_2 levels for emersion behavior ranged from 59 to 69 torr, which were not significantly different from the controls. Although only

PCP greatly altered emersion PO_2 , all test substances decreased the standard deviation of the PO_2 value as compared to the control (Table 1). Aerobic shutdown was significantly ($P < 0.01$) affected by exposure to H_2S (Table 1). *Carcinus* exposure to H_2S caused shutdown at a PO_2 of 38 torr, while all other shutdown values ranged from 21 to 25 torr. Exposure to H_2S also caused considerable increase in the variability (as measured by the standard deviation) of the PO_2 value. The differential aerobic shutdown response to sulfide was examined at various concentrations of Na_2S (Table 2). A threshold level was determined to be 50 $\mu g/L$ but there was no detectable effect of increased sulfide concentration on either emersion or shutdown.

To determine if the differential shutdown response was persistent (i.e., if *Carcinus* continued to shut down at elevated PO_2 levels after removal from exposure) the aerobic shutdown level was determined at one, six, and twelve hours after removal from the sulfide treatment (Table 3). Shutdown PO_2 was not significantly elevated after removal from sulfide rather it returned to control levels within the first hour (Table 4).

The effects of environmental pH on both the emersion and aerobic shutdown responses were also investigated (Table 4). No significant effects of pH were detectable for either emersion PO_2 or shutdown PO_2 . Because of the significance of pH in the complex physical chemistry of the sulfide ion (S^{2-}) in an aqueous solution, the interactive effects of sulfide and pH were examined (Table 5). Although there were increases in the PO_2 level at shutdown for the two pH extremes (6.5 and 8.5), the PO_2 levels were not significantly different (at $P = 0.05$) nor were the changes in the emersion PO_2 levels.

Discussion

The biological effects of pentachlorophenols and their sodium salts have been extensively studied (Rao, 1978; Buikema et al, 1979). Pentachlorophenols are powerful metabolic poisons which uncouple oxidative phosphorylation (Bevenue and Beckman, 1976). The biological effects of pentachlorophenols include ultrastructural changes (Doughtie and Rao, 1978), alterations in energy metabolism (Bostrom and Johansson, 1972), osmoregulatory stress (Thomas et al, 1981), changes in immune response (Leibovitz and Siegel, 1978), changes in hypoglycemia (Thomas et al, 1981), and changes in respiratory responses (Brett, 1964).

The decrease in PO_2 level at the point of emersion, indicating an increased respiratory regulatory ability, was most interesting. These crabs seemed to increase their respiratory ability with the addition of PCP, since they emersed at a lower PO_2 . Considering that most of the previous research reported increased metabolic costs associated with PCP exposure, regardless of the mechanism(s), studied, the lowering of P_C for *C. maenus* seems somewhat incongruous. Chlorinated phenols are a unique class in that the hydrogen of the phenol is acidic, the pK_a being influenced by the chlorines. It is the oxygen which interferes with electron transport. Thus, this compound behaves quite differently from, and is a poor analogy to, other chlorinated compounds whose toxicity is of a

Table 1. Effects of pollutants on the PO₂ levels (mean ± SD) at which emersion and aerobic shutdown occurred in *Carcinus maenas*, N = 10

Variable	Test Condition								Average for all assays	
	Control	PCB	PCP	DDT	24D	H ₂ S	NH ₃ ¹	H _g ¹		Cd ¹
Emersion PO ₂ (torr)	58.7 ± 18.0	61.0 ± 10.6	43.6 ± 5.1	60.1 ± 8.8	67.3 ± 6.4	68.6 ± 9.2	NA	NA	NA	59.9 ± 8.9
Shutdown PO ₂ (torr)	22.8 ± 4.2	24.0 ± 1.8	22.8 ± 3.9	24.6 ± 2.7	21.3 ± 2.3	38.4 ± 8.9	24.6 ± 4.0	25.4 ± 6.4	23.4 ± 7.8	25.2 ± 5.1

¹The effects of NH₃, Hg, and Cd on emersion response were not investigated for NH₃, Hg, or Cd.

Table 2. Effects of sulfide concentration on the PO₂ levels (mean ± SD) at which emersion and aerobic shutdown occurred in *Carcinus maenas*, N = 5

Variable	Sulfide concentration (mg/L)				
	0.005	0.050	0.500	5.000	50.000 ¹
Emersion PO ₂ (torr)	62.2±15.4	66.8±9.4	67.6±16.3	72.2± 9.1	68.0±11.2
Shutdown PO ₂ (torr)	24.8± 5.2	39.6±8.6	54.0±12.6	41.6±11.8	42.2± 4.9

¹This concentration treatment approximates the H₂S treatment in the pollutant assays.

Table 3. Effects of the persistence of H₂S-induced changes on the PO₂ levels (mean ± SD) at which emersion and aerobic shutdown occurred in *Carcinus maenas*, N = 5¹

Variable	Time after H ₂ S exposure (hrs.)			
	0	1	6	12
Emersion PO ₂ (torr)	68.0±11.2	60.4±12.0	68.4±11.4	67.4±7.5
Shutdown PO ₂ (torr)	42.8± 4.9	25.4± 8.2	27.0± 2.4	24.6±5.7

¹Treatment 0 hours differs from the H₂ treatment in the pollutant assay in that in the latter case the organism was still exposed to the H₂S.

physical nature. Thomas et al (1981) found that PCP's caused an increase in the plasma osmolality of the mullet (*Mugil cephalus*); however, in a study of chlorinated hydrocarbons in *Carcinus maenas*, there was a stimulation effect on Na-K ATPase activity in spite of Mg ATPase activity inhibition. Therefore, if the ATP required by the increased enzyme activity could be supplied by, some unknown, anaerobic metabolic pathways, the decreased osmoregulatory stress could indeed allow for continued regulation. However, many feel that osmoregulation involves relatively minor metabolic alterations.

Hydrogen sulfide is a strong metabolic poison (Evans, 1967), causing inactivation of specific enzymes (Cavallini et al, 1970). Numerous studies have been done on the effects of H₂S on aquatic invertebrates (e.g., Oseid and Smith, 1974). The increase of PO₂ level at which aerobic shutdown occurs in *Carcinus maenas* differs from the effects of sulfide exposure on the infaunal asteroid *Ctenodiscus crispatus* (Shick, 1976). *Ctenodiscus* showed a decrease in PO₂ level at aerobic shutdown levels ranging from 24 to 0.2 torr. This difference may be related to decreased permeability of the integument of *Ctenodiscus* rather than to a systemic response (Van Winkle and Mangum, 1975).

The physical chemistry of sulfide is rather complex. In these experi-

Table 4. Effects of pH on the PO₂ levels (mean ± SD) at which emersion and aerobic shutdown occurred in *Carcinus maenas*; data represent, N = 5

Variable	pH level				
	6.5	7.0	7.5	8.0	8.5
Emersion PO ₂ (torr)	49.5±18.0	46.0±18.3	51.8±21.3	54.0±6.5	50.8±9.0
Shutdown PO ₂ (torr)	23.4± 2.3	23.2± 2.6	21.9± 3.2	24.1±4.8	24.4±5.9

Table 5. Effects of pH on H₂S-induced (at 50mg/L concentration) changes in the PO₂ levels (mean ± SD) at which emersion and aerobic shutdown occurred in *Carcinus maenas*, N = 10

Variable	pH levels		
	6.5	7.5	8.5
Emersion PO ₂ (torr)	59.7±9.5	68.6±9.2	70.3± 9.8
Shutdown PO ₂ (torr)	43.9±9.8	38.4±8.9	44.1±10.2

ments, sulfide was introduced as the sodium salt (50 mg Na₂S • 9H₂O • l⁻¹). In aqueous solution, H₂S acts as a weak acid and Na₂S acts as a weak base. Therefore, it would be reasonable to expect a pH effect on the response to sulfide and either high or low pH shifts to either basic or acidic conditions. However, no such effect was found, possibly because the threshold level inducing the differential shutdown level was so low (50 mg • l⁻¹) that the pH related shifts in equilibrium species still left S⁼ well above the threshold level. It could be that the crabs are so sensitive to S⁼ that any exposure may elicit a threshold response.

Little is known of the mechanisms that enable *Carcinus* to survive sulfide exposure during aerobic shutdown. Under these conditions, there is no immediate danger from H₂S as a metabolic poison since the anaerobic metabolic pathways are insensitive to S⁼ (Theede et al, 1969). Although no studies have been conducted on *Carcinus*, many detoxification mechanisms have been described for other anaerobic invertebrates, such as the turbellarians *Archiloba silaoni*, *Solenofilomorpha funilis*, and *Pseudohaplo gonaria* which produce sulfite, thiosulfate, and elemental sulfur, respectively. Also, the gastrotrich, *Dolichodasys carolinensis* produces elemental sulfur (Powell et al, 1980). Therefore it could be possible for more organisms to rely on H₂S-insensitive anaerobic metabolic pathways during sulfide exposure, while simultaneously detoxifying the sulfide.

Conclusions

1. Exposure to various chemical substances were associated with significant alterations in the respiratory response to hypoxia in *C. maenas*.

2. During exposure to pentachlorophenol during hypoxia there was a significant decrease in the PO_2 level at which emersion behavior of *C. maenas* occurred. This decrease may be related to narcosis or to decreased metabolic costs resulting from increased activity levels of Na^+ and K^+ ATPases.

3. When *C. maenas* was exposed to H_2S during hypoxia there was a significant increase in the PO_2 level at which aerobic shutdown occurred. This increase may have been due to the relative insensitivity of the anaerobic metabolic pathways as compared to the aerobic pathways.

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Psychology and Education Section

The Activity of West Virginia High School Seniors in the Westinghouse Science Talent Search 1942-1986

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Abstract

This report describes the procedures of the Westinghouse Science Talent Search and then focuses on West Virginia's participation throughout the history of the competition. A brief comparison of West Virginia's activity with that of other states is presented.

Since 1942, West Virginia has produced 16 finalists, and has had 108 contestants place in the Honor's Group (top 300 in the nation). A complete listing of Honor's Group recipients and their high schools is given. Another table lists all the finalists from West Virginia along with their high schools and awards received.

The West Virginia State Science Talent Search is conducted among the West Virginia participants in the national search. A list of winners of the State Science Talent Search from 1970 to 1986, along with their awards, is also given in this report.

The Westinghouse Science Talent Search was started in 1942 and is the oldest and largest competition of its type (Science Service, 1942-1986).¹ Many science educators also consider it to be the most prestigious. Finalists have gone on to win five Nobel Prizes and two Field Medals. The program is administered throughout the 50 States, Possessions, and American Overseas Schools by Science Service of Washington, DC. Awards are provided by the Westinghouse Electric Corporation and the Westinghouse Educational Foundation.

¹All data and information on the Westinghouse Science Talent Search was provided by Science Service, 1719 N Street, N.W., Washington, DC. 20036.

Any boy or girl who is in the last year of secondary school (public, private, or parochial) is eligible to enter the Westinghouse Science Talent Search. Students entering the competition must be expected by the certifying school official to complete college entrance qualifications before October 1 of the year following their entry. Each student must submit a report of about 1,000 words on an independent research project. In addition to the report, each entrant must fill out a Personal Data Blank, and include a high school transcript along with standardized test scores. Judging is done by highly qualified committees designated by Science Service, and is based on the research report, scholastic and personal records, and any other information assembled by the judges, with greatest weight being given to the project report.

In recent years, approximately 15,000 units of application materials have been requested each year, with about 1,200 students actually completing their entries and becoming contestants (Science Service, 1942-1986). From these the judges first select an Honors Group of 300 who show the greatest talent in science and engineering. The names and addresses of the Honors Group members are sent to every college and university in the country recommending them for offers of scholarships and other financial assistance.

The top forty from the Honors Group are invited to Washington, DC for an all-expense paid trip to the Science Talent Institute. Here 10 winners are selected by the board of judges to receive one of the following four-year Westinghouse Science Scholarships: One \$20,000 (\$5,000 per year), two \$15,000 (\$3,750 per year), three \$10,000 (\$2,500 per year), four \$7,500 (\$1,875 per year). The remaining 30 contestants are given Westinghouse Science Awards of \$1,000 each. This group of the top forty makes up the Winners Group of the Westinghouse Science Talent Search.

West Virginia has had 16 contestants place in the Winners Group since 1942. Table 1 lists these winners, their high schools and awards. It should be noted that the amounts of the scholarships and awards have changed through the years. Of these 16 winners, only 3 placed in the top 10 and received scholarships. These are Thomas Quermann (1943), Linda Sue Powers (1966), and Julia Elizabeth Little (1979). A total of \$10,300 in scholarships and awards has been received by West Virginia winners.

West Virginia ranks 23rd among the states in number of winners, tied with Colorado, District of Columbia, and Nebraska. New York is 1st with 567 winners, followed by Illinois with 129, and California with 127. The number of winners from West Virginia appears extremely low when compared with these top three states. However, on the basis of number of winners per total number of applicants, the figures are nearly proportionate.

Table 2 is the list of West Virginia participants receiving Honorable Mention in the Westinghouse Science Talent Search from 1942 through 1986. A total of 108 West Virginia high school seniors have been named to the Honors Group in this period. West Virginia ranks 29th among the states in number of Honorable Mentions. New York is first with 3631,

**Table 1. Westinghouse Science Talent Search
Winners from West Virginia*
1942-1966**

YEAR	WINNERS FROM WV	HIGH SCHOOL	AWARD
1942	Lynch, Robert L.	Weston	\$ 200
1942	Smith, Harlan J.	Wheeling	200
1943	Quermann, Thomas	Washington Irving	100
1944	Davis, Nancy S.	Williamson	100
1946	Spalding, Jean J.	South Charleston	100
1947	Simmons, Gustavus J.	Sissonville	100
1951	Dennison, John M.	Keyser	100
1954	Berry, George B., Jr.	South Charleston	100
1955	Hawkins, Carol	South Charleston	100
1955	Stone, John W.	Stonewall Jackson	100
1957	Greenlee, Donald R.	Stonewall Jackson	100
1960	Thompson, Joyce	Stonewall Jackson	250
1966	Powers, Linda Sue	Woodrow Wilson	3000
1976	Wargo, Natalie K.	Morgantown	250
1979	Little, Julia E.	St. Albans	5000
1981	Piehl, Forrest L.	Keyser	500

*Information provided by Science Service, Inc., 1719 N Street, N.W., Washington, DC 20036.

followed by California with 790, and Illinois with 760. Again, when one compares the number of Honorable Mentions with the total number of applicants from these states, the percentages are not far apart. In fact, in recent years, West Virginia has had a higher percentage of honors per applicants than some of the leading states. In 1986, both West Virginia and New York had 28.5 per cent of their applicants named to the Honors Group. The big difference, of course, is in the number of yearly entries. In 1986, West Virginia with only 7 entries ranked 28th (tied with North Carolina and Oregon) among the states in number of completed entries. Again, New York led the list with 522 entries, followed by Florida with 76 and California with 50. While there is not a consistent correlation between population size of the states and the number of entries, most of the states with small populations have a low number of entries in the Talent Search. Table 3 shows the number of entries, honors, winners, and participating high schools from West Virginia over the past 20 years.

It can be seen from Table 3 that participation by West Virginia students has dropped off in the last ten years, while the number of winners and honorable mentions has slightly increased. The author can offer no explanation for the lower number of entries. With every high school in the state receiving publicity and forms for applications from Science Service, one would think that more students from more high schools would take advantage of competing in the Westinghouse Science Talent Search.

**Table 2. Westinghouse Science Talent Search
Honors Group from West Virginia*
1942-1986**

YEAR	HONORS GROUP FROM WV	HIGH SCHOOL
1942	Lynch, Robert L.	Weston
1942	Smith, Harlan J.	Wheeling
1943	Hood, Virginia D.	Washington Irving
1943	Quermann, Thomas	Washington Irving
1944	Davis, Nancy S.	Williamson
1945	Fields, Wileta N.	Williamson
1945	Grover, John W.	Moorefield
1945	Slaven, Katharine H.	Williamson
1946	Davitian, Evelyn	Williamson
1946	Hickman, Frances M.	Williamson
1946	McEuen, Marshall M.	Williamson
1946	Spalding, Jean J.	South Charleston
1947	Corbin, Harold S.	Washington Irving
1947	Cunningham, Orpha R.	Lumberport
1947	Felton, John W.	Williamson
1947	Kurtz, Kenneth R.	Weston
1947	Marsh, John M.	Philippi
1947	Mayerchak, Julia	Williamson
1947	Simmons, Gustavus J.	Sissonville
1948	Bornmann, John A.	Stonewall Jackson
1949	Flippen, Richard B.	Williamson
1949	Kiessling, Francis J.	St. Joseph's
1949	McCullough, Edgar J., Jr.	South Charleston
1950	Wells, Charles J.	South Charleston
1951	Day, Nancy L.	South Charleston
1951	Dennison, John M.	Keyser
1951	Gibson, William A.	Morgantown
1951	Jones, Edwin C.	Morgantown
1951	Stott, Charles W.	St. Joseph's
1952	Anderson, Louise E.	Morgantown
1952	Massau, Jack L.	Stonewall Jackson
1953	Anderson, James B.	Morgantown
1953	Hicks, Donald L.	South Charleston
1954	Berry, George B.	South Charleston
1954	Powell, William R.	Charleston
1954	Walliser, Virginia L.	Follansbee
1955	Hawkins, Carol	South Charleston
1955	Stone, John W.	Stonewall Jackson
1956	Hunneshagen, Joanne I.	South Charleston
1956	Nasser, Yvonne	Huntington

*Information from Science Service, Inc., 1719 N Street, N.W., Washington, DC 20036.

Table 2—Continued

YEAR	HONORS GROUP FROM WV	HIGH SCHOOL
1956	Newman, John C.	Huntington
1956	Whitman, Dale A.	Stonewall Jackson
1956	Yeager, Leland R., Jr.	Charleston Catholic
1957	Crawford, Margaret E.	Stonewall Jackson
1957	Greenlee, Donald R.	Stonewall Jackson
1957	Houchins, Ray C.	Stonewall Jackson
1957	Meisel, David D.	Fairmont
1957	Villinger, Frederic W.	Fairmont
1957	Winter, John E.	Charleston
1958	Lang, Roger M.	Stonewall Jackson
1958	Lovejoy, William D.	Stonewall Jackson
1958	Singer, June W.	Stonewall Jackson
1959	Bowman, Henry W.	Charleston
1959	Galinsky, Norman M.	Charleston
1959	Gibbard, Allan F.	Morgantown
1959	Jarrett, Eugene L.	St. Albans
1959	Johnston, George T. III	Charleston
1960	Brock, Sabra E.	Stonewall Jackson
1960	Harper, George R.	St. Albans
1960	McCormick, Joseph A. III	Stonewall Jackson
1960	Saliga, Suzanne B.	Notre Dame
1960	Thompson, Joyce A.	Stonewall Jackson
1960	Vande Linde, Vernon D.	St. Albans
1961	Brooks, Thomas H.	Buffalo
1961	Gwynne, John T.	Morgantown
1961	Hills, Henry M.	Stonewall Jackson
1961	Ingram, Doris A.	Stonewall Jackson
1961	Meredith, Jamison, Jr.	Fairmont
1961	Wojdyla, John R.	Madonna
1962	McGrew, Frank A. III	South Charleston
1962	Zacks, Leonard H.	Charleston
1963	Frame, Brenda K.	Stonewall Jackson
1964	Arnett, Carroll D.	Rowlesburg
1964	Melott, Adrian L.	Moundsville
1966	Childs, James E.	Morgantown
1966	Powers, Linda S.	Woodrow Wilson
1967	Harley, Dorcas J.	Morgantown
1967	Samples, Michael W.	Stonewall Jackson
1968	Hoback, Mary H.	Huntington East
1968	Reyer, Bette A.	Morgantown
1969	Justice, Penniford L.	Parkersburg
1969	Stonestreet, Vickie L.	Herbert Hoover
1971	Woelfel, George F.	St. Joseph Central
1973	Chen, Jane	Morgantown
1973	Collins, Marshall S.	Woodrow Wilson

1974	Clarkson, George R.	Morgantown
1976	Wargo, Natalie K.	Morgantown
1977	Malcomb, Cecil D.	Richwood
1978	Coffield, James E.	John Marshall
1978	Rasicci, Gina M.	Weir
1979	Little, Julie E.	St. Albans
1979	Wydoski, Richard G.	Jefferson
1980	Hake, Lisa E.	Morgantown
1980	Gelderman, Richard F.	Union
1980	Thomas, Michael J.	Morgantown
1981	Miller, Debra L.	Wheeling Park
1981	Piehl, Forrest L.	Keyser
1981	Pritt, Mark D.	Greenbrier East
1981	Smith, W. Garth	Parkersburg South
1982	Lewine, Michael A.	Wheeling Park
1982	Mott, William R., Jr.	East Fairmont
1982	Wydoski, Duane S.	Jefferson
1983	Dzmura, Thomas A.	Linsly Institute
1983	Klemm, Robert B., Jr.	Linsly Institute
1985	Robert, John E.	Parkersburg South
1985	Wilcox, William E., Jr.	Parkersburg
1986	Heck, Michael A.	North Marion
1986	Kolaja, Thomas A.	Morgantown

Since 1946 State Science Talent Searches have been held concurrently with the national competition by special arrangement with Science Service. In 1985, 34 states and the District of Columbia cooperated with Science Service in this plan to increase recognition and assistance to young scientists. West Virginia has held a State Science Talent Search since 1946 (Williamson, 1963).

The West Virginia State Science Talent Search is sponsored by the West Virginia Academy of Science. It is administered by a Director appointed by the Academy, who conducts the search and raises money for the awards. The state search is conducted each year among those students who have successfully entered the Westinghouse Science Talent Search. As soon as the national judging is completed, Science Service sends all the materials of the West Virginia contestants to the state director. A committee of three judges, appointed by the director, selects the winners. Each winner receives an appropriately engraved brass on walnut plaque and a modest monetary award. The amounts of the awards are suggested by the director for approval by the Academy Executive Committee. The awards are presented at the Awards Banquet of the West Virginia State Science and Engineering Fair in April of each year. An estimated \$13,000 has been awarded by the Academy since 1946 (West Virginia, 1970-1986). Table 4 is a list of the winners of the West Virginia State Science Talent Search, along with their awards, from 1970 to 1986.

**Table 3. Westinghouse Science Talent Search
Number of entries, high schools, honors,
and winners from West Virginia*
1967-1986**

YEAR	WV ENTRIES	HIGH SCHOOLS	HONORS	WINNERS
1967	22	12	2	
1968	26	10	2	
1969	30	14	2	
1970	19	9		
1971	3	3	1	
1972	12	8		
1973	12	7	2	
1974	4	4	1	
1975	4	4		
1976	7	4	1	1
1977	5	4	1	
1978	8	4	2	
1979	8	6	2	1
1980	14	7	3	
1981	11	6	4	1
1982	7	6	3	
1983	3	2	2	
1984	6	5		
1985	7	5	2	
1986	7	4	2	

*Data provided by Science Service, Inc., 1719 N Street, N.W., Washington, DC 20036.

**Table 4. Winners of the West Virginia State Science
Talent Search** 1970-1986**

YEAR	WINNERS STATE SCI. TAL. SEARCH 1970-1986	AWARD
1970	Baker, Winton A.	\$ 100
1970	McDaniel, David H.	100
1970	Sommerville, Lawrence P.	100
1971	Roy, Verna M.	50
1971	Wade, Stephen E.	100
1971	Woelfel, George F.	300
1972	Cremer, Steven A.	100
1972	Darich, Michalene	100
1972	Selip, Gregory J.	100
1973	Chen, Jane	200
1973	Collins, Marshall S.	100

1974	Clarkson, George R.	200
1974	Knight, Dale K.	25
1974	Taler, Ted A.	25
1975	Blott, Guy	25
1975	Knotts, Donna	25
1975	Snyder, Edward	25
1975	Stern, Mont	25
1976	Lemley, Donald G., Jr.	25
1976	Mounce, Adrian P.	25
1976	Wargo, Natalie K.	300
1977	Davidson, Bruce J.	25
1977	Malcomb, Cecil D.	150
1978	Bundy, Thomas A.	15
1978	Coffield, James E.	70
1978	Davies, Greg S.	15
1978	Rosicci, Gina	70
1979	Little, Julia E.	100
1979	Wydoski, Richard G.	75
1980	***	
1983	Dzmura, Thomas A.	50
1983	Klemm, Robert B., Jr.	25
1984	Adams, John Boyd	Award Plaque Only
1984	Groves, Leonora J.	Award Plaque Only
1984	Rahall, George G.	Award Plaque Only
1985	Robert, John E.	50
1985	Wilcox, William E., Jr.	100
1985	Wydoski, Susan	25
1986	Heck, Michael A.	50
1986	Kolaja, Thomas A.	100
1986	Albert, Shelly S.	25

**Information from the Treasury Records of the West Virginia Academy of Science, and the records of the Director of the West Virginia State Science Talent Search.

***Information for the years 1980 through 1982 not available.

Acknowledgments

The author expresses his appreciation to Ms. Dorothy Schriver of Science Service for providing information from the files of the Westinghouse Science Talent Search, and to Dr. Roy B. Clarkson, Treasurer of the West Virginia Academy of Science, for allowing him to examine the Treasury Records.

Literature Cited

1. Science Service, Inc. 1942-1986. File data and information of the Westinghouse Science Talent Search. Science Service, 1719 N Street, N.W., Washington, DC.
2. West Virginia Academy of Science. 1970-1986. Treasury Records of the West Virginia Academy of Science. Dr. Roy B. Clarkson, Treasurer. Morgantown, WV.

3. Williamson, D. B. 1963. Science Talent Search. In *A History of the West Virginia Academy of Science 1924-1963*. Nelle Ammons, Editor. Published by the West Virginia Academy of Science. Morgantown, WV. 52 pp.

Minutes of the Annual Business Meeting
West Virginia Academy of Science
61st Annual Meeting
Room 134 Harris Hall
Marshall University
Huntington, West Virginia 25701

April 5, 1986

The meeting called to order by President Kuryla.

The minutes of 1985 Business Meeting were distributed and approved.

The Treasurer's report was distributed and approved.

There was a discussion about the general concern of getting the Proceedings published more quickly after the Annual Meeting.

Dr. Taylor, as President-elect, commented that he would, as President, be encouraging new and young members to become active in the Academy.

He will also be working on speeding the publication of the Proceedings.

Dr. Das Sarma reiterated that quicker publication of the Proceedings would encourage membership and the members' participation in the Annual Meetings.

The question of a home for the Junior Academy was raised and whether the State Science Fair should be moved yearly to perhaps the same location as the Academy meeting site. President Kuryla left these questions for consideration during 1986-87.

1987 Annual Meeting site and date not yet determined.

Election of new officers: President-elect: Dr. John Chisler; Secretary: Dr. Gary Snyder; Treasurer: Dr. Roy B. Clarkson.

Dr. Pauley noted the deaths of: Gene Frum, Marshall University; Mo Coover, West Virginia University; Bertil Anderson, West Virginia University. A moment of silence was observed in their memory.

In his final remarks: President Kuryla noted: The renewal of IRS Tax Exempt Status for the Academy. The receipt of two grants.

Commended Dr. Taylor and Marshall University for their sponsorship of the Industry and Environment Symposium which was held in conjunction with the Academy Meeting.

Reviewed High School Science Teacher recognition award and expressed hope that this award could be made soon.

Recognized the opening of the new section of archaeology in the Academy.

The gavel was passed to Dr. Taylor.

The meeting was adjourned.

John A. Chisler
Secretary

**WEST VIRGINIA ACADEMY OF SCIENCE
ANNUAL TREASURER'S REPORT
1985**

April 4-5, 1986
WVAS Annual Meeting
Marshall University
Huntington, West Virginia

January 1, 1985 to December 31, 1985

CASH RECEIPTS

Balance on hand January 1, 1985	\$12,355.27
Dues	\$ 700.00
Institutional Membership	1,000.00
Proceedings (Libraries)	1,040.00
Contributions (Talent Search)	180.00
Annual Meeting	490.00
Page Charges	671.50
Interest on Savings	324.41
Advertisements	195.00
Abstract Charges	98.00
Grant from Union Carbide Co.	\$2,000.00
TOTAL RECEIPTS FOR YEAR	\$6,698.91
TOTAL RECEIPTS & BALANCE ON HAND	\$19,054.18

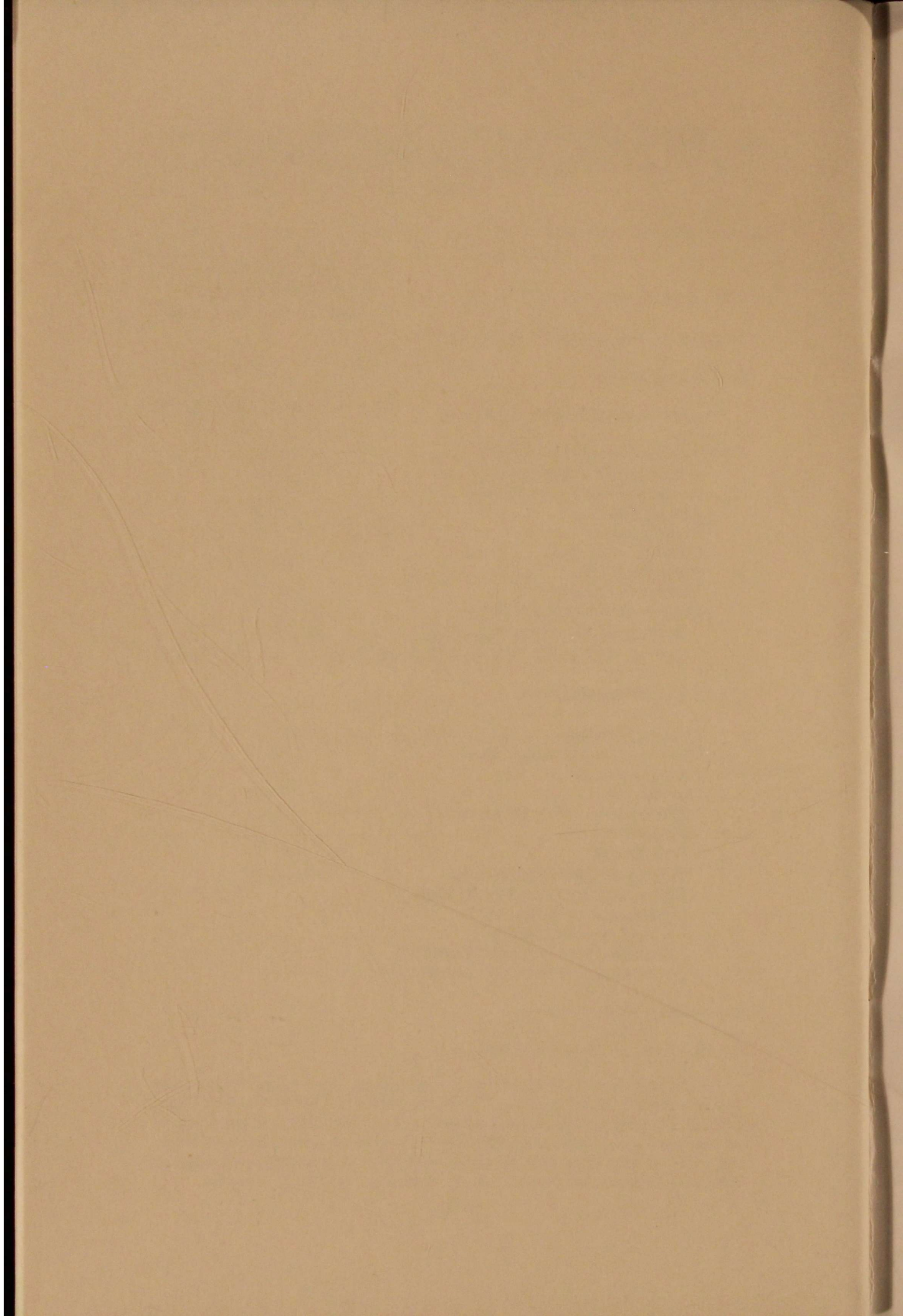
CASH DISBURSEMENTS

Printing (McClain)	\$4,954.25
Contributions (Jr. Acad. Sci.)	750.00
Annual Meeting	342.00
Postage	23.58
National Assoc. of Academy of Science (dues) .	30.00
Miscellaneous	289.37
Talent Search	250.00
TOTAL DISBURSEMENTS	\$6,639.20
BALANCE DECEMBER 31, 1985	\$12,414.98*
(Savings—\$552.05)	
(Checking—\$3,270.70)	
(Certificate of Deposit—\$4,000.00)	
(Certificate of Deposit—\$4,592.23)	

Respectfully submitted,

Roy B. Clarkson, Treasurer, WVAS

*It must be noted that printing Vol. 56 Nos. 2, 3 & 4 of the Proceedings would ordinarily have been paid for during the year, 1985. Also the Union Carbide Co. grant of \$2,000 will be paid out of this. This balance needs to be reduced by that amount (\$6000.00+) in order to reflect our true financial picture. The Financial Records of WVAS were audited on April 1, 1986, and found to be correct.—E. C. Keller, Jr. and D. F. Blaydes.



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