Volume 78, Number 2

WVAS

West Virginia
ACADEMY of SCIENCE

Founded 1924

Proceedings of West Virginia Academy of Science

2006

Submitted Papers of the Eighty-First Annual Session

WVAS
West Virginia
ACADEMY of SCIENCE
Founded 1924

Member of the American Association of Academies of Science

Proceedings of the
West Virginia Academy of Science
2006

Vol. 78 No. 2
SUBMITTED PAPERS OF THE EIGHTY-FIRST ANNUAL SESSION

Shepherd University
Shepherdstown, WV
April 22, 2006

Printed by
Ralston Press
July 2007

West Virginia Academy of Science, Inc.

The West Virginia Academy of Science was founded at Morgantown, November 28, 1924. The Academy was incorporated under the Code of West Virginia on May 9, 1959 as a "nonstock corporation, which is not organized for profit but for the advancement of learning and scientific knowledge."

Officers 2006

President ..... Mark Flood, Fairmont
President-Elect ..... Donald Gray, Morgantown
Past President ..... John Landolt, Shepherdstown
Treasurer ..... Roger Seeber, West Liberty
Assistant Treasurer ..... John Warner, Buckhannon
Secretary ..... Jim Rentch, Morgantown
Proceedings Editor ..... John Warner, Buckhannon
Associate Editors ..... Darla Wise, Athens; G. Paul and Marjorie Richter, Buckhannon
Marcia Harrison, Huntington

Chair of the WV Junior Academy of Science ..... Marcia Harrison, Huntington
Chair of the Annual Meeting at Shepherdstown ..... Burt Lidgerding, John Landolt,
and Diane Shewbridge, Shepherdstown

Dues Structure

Student $10.00
Regular $25.00
Contributing $30.00
Sustaining $60.00
Life $150.00
Institutional $200.00
Corporate $200.00
Library subscription to proceedings $25.00

Institutional Members

Fairmont State University
NASA
Shepherd State University
WVCEMR
West Liberty State College
West Virginia State College
West VirginiaWesleyan College

Proceedings of the West Virginia Academy of Science
John Warner, Editor
Darla Wise, G. Paul and Marjorie Richter,
and Marcia Harrison, Associate Editors

Published as a peer-reviewed journal at the West Virginia Academy of Science, Inc. Manuscripts for publication should be sent to the editor, Dr. John Warner, Box 129, West Virginia Wesleyan College, Buckhannon, WV 26201. Manuscripts must be sent electronically (email or compact disk) in Microsoft WORD to warner@wvwc.edu. Three hardcopies should also be sent to the address above. Proofs, edited manuscripts, and all correspondence regarding papers for publication should be directed to the editor. For additional information, call (304) 472-1907.

Application for membership in the Academy and dues should be sent to Roger Seeber, Box 139, West Liberty State College, West Liberty, WV, 26074. Changes of address should be sent to Dr. Seeber. Libraries or others wishing to purchase the ***Proceedings*** should direct requests to the editor. Correspondence concerning library exchanges should be directed to the Director of Libraries, West Virginia University, Morgantown, WV 26506.

Proceedings of the
West Virginia Academy of Science 2006
Submitted Papers
Volume 78:2

TABLE OF CONTENTS

ANDREA CHANGURIS, TRAVIS SMITH, ELIJAH TICE, JUSTIN ELLIS, and JASON BEST. The Development of an Initial Light Pollution Map for the Area Surrounding the Shepherd University Observatory. 1

JUDITH SILVER and ERIK STOKES. A Great Circle Metric. 8

MARK B. WATSON and THOMAS K. PAULEY. Spatial Distribution of Turtles in the Great Kanawha River, West Virginia. 14

CLIFFORD E. STARLIPER. Imperiled Freshwater Mussels (Unionidae): Disease Prevention as Part of the Conservation Efforts. 26

TAMARA LUSHNIKOVA, VICTOR FET, and W. IAN TOWLER. Phylogenetic Analysis and Genomic Structure of the cone Photoreceptor Gene, NCKX2 (SLC24A2). 33

RAMSEY MILLER, DANIEL FELBAUM, and DEBRA HULL. Students' Sex Role Perceptions of Themselves and Scientists. 48

Poem by BASUDEB DASSARMA. Common Sense Chemistry. 52

The Development of an Initial Light Pollution Map for the Area Surrounding the Shepherd University Observatory

Andrea Changuris, Travis Smith, Elijah Tice, Justin Ellis, and Jason Best
Shepherd University Observatory, Shepherd University, Shepherdstown, WV 25443

ABSTRACT

Shepherd University has recently built a campus astronomical observatory that will be used for teaching, research, and outreach projects. The observatory has been erected on the roof of the Robert C. Byrd Center for Legislative Studies, which is located at the heart of campus. This location allows for convenient travel from classrooms and labs to the observatory. However, having the observatory located directly on campus has one potentially serious drawback: the effects of campus lighting can have a major impact on the quality of observations made at the site. The purpose of this research was to measure the effect of campus lighting on observational conditions. We present the results of our measurements in the form of a quantitative map of light pollution on the Shepherd University Campus, allowing for visual and quantitative analyses of the effects of campus lighting on the observatory. If the campus lighting has a significant impact on the long-term efficiency of the observatory, further studies will then be conducted in order to safely, effectively, and economically reduce the impacts of campus lighting so that the observatory can be used to its full potential.

INTRODUCTION

The Shepherd University Observatory was built in the summer and fall of 2005 (the World Year of Physics) and the winter of 2006, and was made possible by a Research Challenge Fund Science and Technology Award from the West Virginia Experimental Program to Stimulate Competitive Research (WV EPSCoR). This award, in the form of an Innovation Grant, was obtained by associate professor Dr. Jason Best of Shepherd University in October 2004. The Innovation Grants Program targets comprehensive classroom or laboratory innovations, and encourages direct student involvement, interdepartmental and interdisciplinary collaborations, and the development of opportunities for West Virginia students to enter careers in the sciences or mathematics. This grant was supplemented with funds from Shepherd University and private donations.

The observatory consists of numerous telescopes (the primary instrument has a 14-inch diameter), along with dedicated research charge-coupled device (CCD) cameras, spectrographs, and filters for data acquisition. The facility is housed in a 15-foot diameter dome that is located on the roof of the Robert C. Byrd Center for Legislative Studies, in the academic and administrative center of campus. Current research facilities in place at the institution will serve as data reduction and processing centers.

Once the observatory is fully operational, a primary use of the observatory will be for coursework in astronomy, physics, physical science, and science education courses. In addition, research on variable stars, minor planets, and star clusters will be conducted by both students and faculty. Finally, public outreach efforts to the University community, area K-12 schools, and the general public will be expanded to incorporate the new instrumentation.

Light Pollution: Bane of the Astronomer

Light pollution is a growing problem for observatories all over the world, as any extraneous light negatively impacts astronomical observations. The more light that is projected into the atmosphere by artificial sources, the more limited observations become (Luginbahl *et al.* 2002). It is important to understand that large cities are not the only sources of this pollution, although major population centers certainly contribute to the problem. One of the most significant demonstrations of this is illustrated in Figure 1, which shows the Earth at night.

Figure 1: The Earth's city lights. Data courtesy Marc Imhoff of National Aeronautics and Space Administration (NASA) Goddard Space Flight Center (GSFC) and Christopher Elvidge of National Oceanic and Atmospheric Administration (NOAA) National Geophysical Data Center (NGDC). Image by Craig Mayhew and Robert Simmon, NASA GSFC. Courtesy NASA.

According to NASA 2006, Figure 1 was "created with data from the Defense Meteorological Satellite Program (DMSP) Operational Linescan System (OLS). Originally designed to view clouds by moonlight, the OLS is also used to map the locations of permanent lights on the Earth's surface." It should be noted that the lights represent urbanization, as opposed to population. Nevertheless, the difficulty that such lights would cause for astronomical observation is obvious. One can see the general outline of major urban centers without the need for a geographical map underneath, especially in North America, Europe, and Asia.

The work of Cinzano *et al.* (2001) is one of the more significant studies on light pollution. The resulting "World Atlas of the Artificial Night Sky Brightness" is an attempt to quantify light pollution using satellite data and accurate models of atmospheric light propagation. Such maps, as in Figure 2, illustrate relationships between natural processes (e.g. atmospheric chemical reactions, zodiacal light, stellar light) and artificial sources.

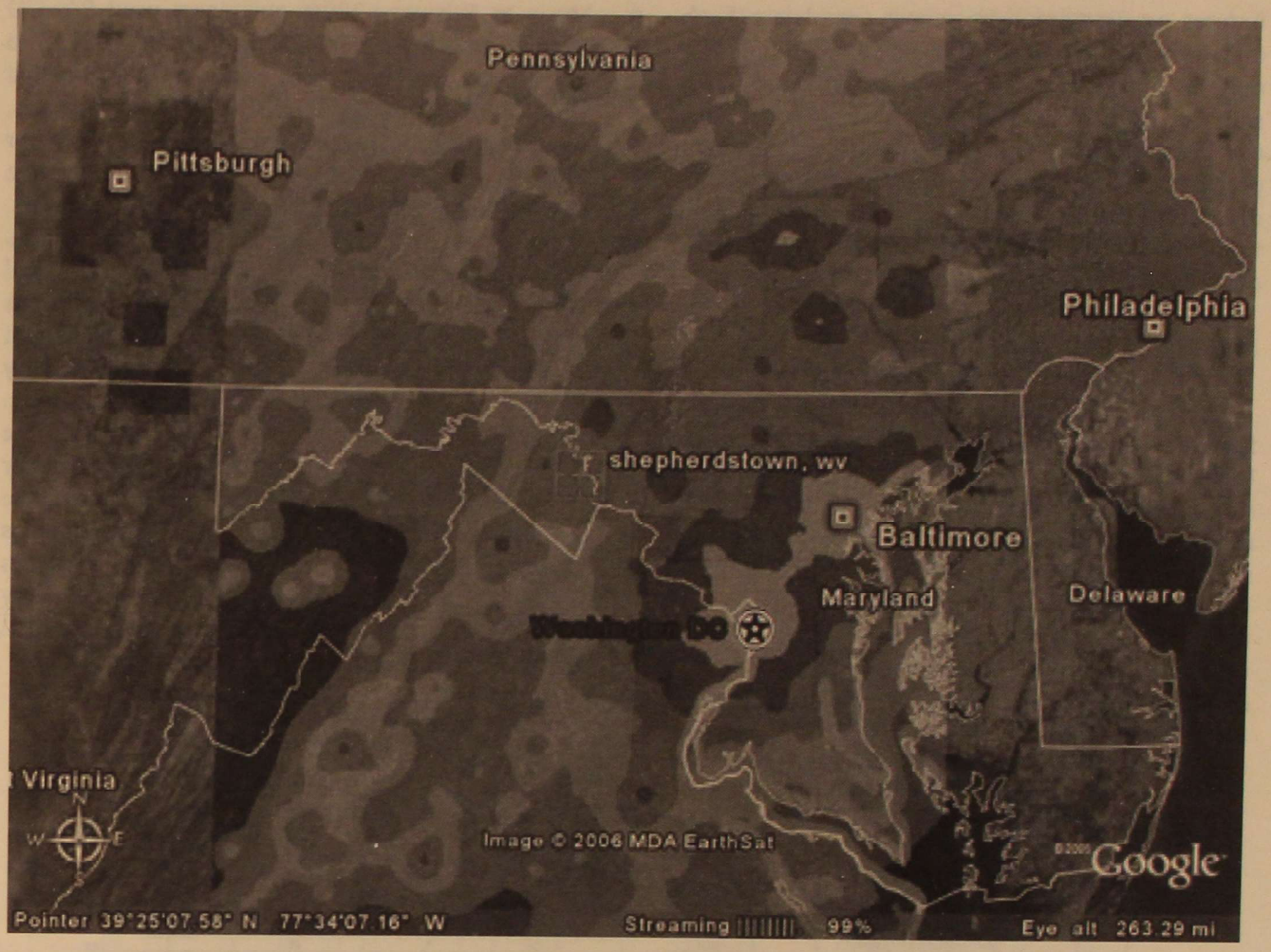


Figure 2: Artificial night sky brightness map of the area centered on Shepherdstown, WV. Credit: P. Cinzano, F. Falchi (University of Padova), C. D. Elvidge (NOAA National Geophysical Data Center, Boulder). Copyright Royal Astronomical Society. Reproduced from the Monthly Notices of the Royal Astronomical Society by permission of Blackwell Science.

The area around Washington D.C. has an artificial night sky brightness that is over 27 times greater than that of the natural sky. Even a relatively rural area such as Shepherdstown has an artificial night sky brightness that is approximately the same level as the natural sky. The obvious potential for impact on astronomical activities has led to cities such as Tucson, Arizona (near which the Kitt Peak National Observatory is located) enacting significant light control legislation.

The Shepherd University Observatory was placed at its specific location for both access and security reasons. The Byrd Legislative Center is located very near the building in which the science classrooms are housed, as well as near the building housing the astrophysics research lab. The ability to have students travel quickly between these buildings and the observatory facilitates greater use of all resources. Furthermore, the roof of the Byrd Legislative Center is one of the most secure on campus. A rooftop location also increases the horizon available for observations compared to the possible ground-based locations considered. However, this placement makes the observatory susceptible to the effects of campus lighting. While the Artificial Night Sky Brightness Atlas provides significant data from satellite imagery, no light pollution map specific to the Shepherdstown area (to our knowledge) has been constructed on the scale of the campus.

There are numerous modifications that can be made to address light pollution, such as installing broadband filters on telescopes, shielding light

Table 1: Light measurements at specified grid points of the light pollution map (measured in lux). Cells with no entries correspond to locations not directly under the control of the University, or areas inaccessible due to construction.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A			20.10	4.30												
B			0.70	14.20												
C		4.90	2.90	2.10	1.70											
D	0.43	6.00	2.60	9.00	34.70	17.37										
E	0.13	6.70	22.70	0.50		0.14	0.05									
F	36.70	0.03	0.04	7.70		16.13	0.10	0.03	0.01					0.19	1.25	
G	1.52	0.04	0.51	2.41	1.70	7.30			0.04		0.19			2.05	0.34	
H	4.00	0.01	0.02		0.01	0.31	0.20		2.57	1.12	0.34	3.48	80.00	1.55	0.81	
I					7.80	0.84	0.14			2.62	0.41	1.97	1.35	12.21	6.51	
J											4.72	8.71	7.02	11.85	4.07	7.61
K												0.04	5.03	37.20	1.78	
L												11.62	1.88	0.50	18.18	
M												7.25	9.33	15.5		
N												2.20	2.80			

RESULTS AND DISCUSSION

The light pollution map is presented as Figure 3, and the data in Table 1. As one can see on the light pollution map (Figure 3), there are indeed areas on campus that may severely impact research and observations performed at the Shepherd University Observatory. The observatory is located directly within one of the most highly light-polluted areas: specifically, the observatory's immediate eastern horizon (grid areas I14, J14, K14) is directed towards a very bright area of campus, with lux values ranging from 11.85 to 37.20. It should be noted that many of these strongly lit areas are parking lots with unshielded light fixtures. The immediate western horizon (grid areas I13, J13, K13), with values between 1.35 and 7.02 lux, is less light-polluted, showing a greater promise for observations.

The west side of the campus (columns 1 through 8, inclusive) has areas such as the West Woods Residence Halls (grid areas A3, B4) with light values of 20.10 and 14.20 lux, which are comparable to the area directly around the observatory. Most of these areas are associated with parking: however, not all parking areas on the west campus are equally illuminated. For example, the commuter parking lot (grid areas E7, F7, and F8) has values between 0.03 and 0.10 lux. By way of comparison, some of the least lit areas on campus, such as the Softball Field and the Butcher Center (F2, F3, G2, G3, H2, H3) have values less than 0.51 lux, with most values between 0.01 and 0.04 lux. To study the consistency of our measurements, several points were measured again during the March 2006 New Moon. Values taken during the March 2006 New Moon at selected grid points were not significantly different from the February 2006 measurements at those same points, falling within instrumental variation. This result strengthened confidence in the February 2006 measurements.

Future Directions

The light pollution map of campus is the first step in a series of projects analyzing light pollution that the observatory staff will be conducting. A greater number of grid points and finer GPS resolution are two potential improvements to the current methodology. Statistical analyses of the data collected at various points in time will yield more accurate light pollution maps. Quantitative observations from the observatory site will allow a substantive link to the contribution of the campus to overall skyglow. Computational modeling of the efficiency of current lighting will also be undertaken. In addition, the map will need to be expanded in accordance with the continual development of the campus, as its potential

impact on the observatory will need to be quantified. The outcomes of these projects will allow economical and observatory-friendly lighting possibilities to be presented to the University as alternatives to the current systems.

This study afforded a unique opportunity to work with campus personnel to safely and economically design lighting for new construction projects (see King 2001 and Luginbahl *et al.* 2002 for examples), as well as to educate the campus community about the potential scientific and economic costs of wasteful lighting.

REFERENCES CITED

Cinzano, P. *et al.* 2001. The first world atlas of the artificial night sky brightness. Monthly Notices of the Royal Astronomical Society. 328:689-707.

King, B. 2001. Is It Possible? SkyNews. Volume VII, Issue 2 [Electronic version].<http://www.skynewsmagazine.com/pages/light_pollution.html>. Accessed March 1, 2006.

Luginbuhl, C. *et al.* 2002. International Dark Sky Association Outdoor Lighting Code Handbook Version 1.14. <http://www.darksky.org/handbook/lc-hb-v1-14.html>. Accessed January 10, 2006.

National Aeronautics and Space Administration (NASA). 2006. Visible Earth: Earth's City Lights. <http://visibleearth.nasa.gov/view_rec.php?id=1438>. Data accessed March 24, 2006.

West Virginia GIS Technical Center (WVGIS). 2006. SAMB Orthophotos (2003). <http://wvgis.wvu.edu/>. Data accessed January 24, 2006.

A GREAT CIRCLE METRIC

Dr. Judith Silver
Marshall University

Erik Stokes
University of Kentucky

ABSTRACT

For many years the equation for distance on a sphere has been known, though not widely publicized. Using only trigonometry, this paper presents a unique proof that the formula is indeed a metric.

INTRODUCTION

The shortest distance between two points in $\Re^n$ is a straight line, but on a sphere it is a *great circle.* For example, a plane flying from San Francisco (latitude $37.4°$) to London (latitude $39.9°$) won't fly due east; it will fly northeast over the Hudson Bay and Greenland (Figure 1). Great circle flight paths and mileage between other airports can be found at several places on the internet, such as the *Great Circle Mapper* [1].

Figure 1: Great circle flight path between San Francisco and London [1].

Great circles also have practical applications in naval navigation and in astronomy. But, what is a *great circle*? It is essentially a transformed equator that passes through the two points of interest. Great circles and lines are both examples of *geodesics*, length-minimizing curves on a surface.

In discussing great circles, this paper will always be dealing with points on the surface of a sphere. Thus spherical coordinates are the natural ones to use except on the occasion where conversion to rectangular coordinates simplifies computations. We define ρ to be the distance from the origin to the given point, θ to be the angle in the xy-plane measured from the x-axis toward the y-axis with $0 \le \theta < 2\pi$, and ϕ to be the angle in the yz-plane measured from the z-axis toward the y-axis with $0 \le \theta \le 2\pi$ (Figure 2).

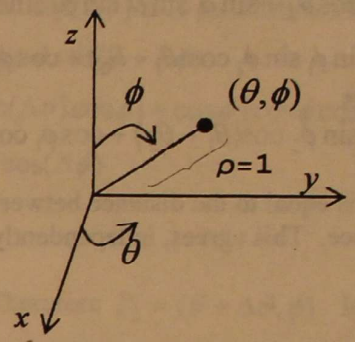


Figure 2: Spherical coordinates on the unit sphere.

The radius of a sphere is constant. Thus, to determine a formula for the great circle distance between two surface points, we will direct our attention to just the unit sphere (where $\rho = 1$). This also allows us to emphasize that the surface of a sphere is a two-dimensional manifold. Therefore, rather than representing a random point on the sphere as (ρ, θ, ϕ), we will denote it more simply as (θ, ϕ). In rectangular coordinates, a random point will be written as $\langle x, y, z \rangle$. To convert from spherical to rectangular coordinates, or vice versa, refer to Table 1 below.

Conversion from Spherical to Rectangular Coordinates	Conversion from Rectangular to Spherical Coordinates
$x = \sin\phi \cos\theta$	$\rho = 1$
$y = \sin\phi \sin\theta$	$\theta = \arctan\left(\frac{y}{x}\right)$
$z = \cos\phi$	$\phi = \arccos(z)$

Table 1: Coordinate Conversion on the Unit Sphere

1. The Spherical Metric Formula

We know that distance in $\Re$ is typically defined by $d(x,y) = |x - y|$, and in $\Re^2$ distance is defined by $d(P_1, P_2) = \sqrt{(x_2 - x_1)^2 + (y_2 - x_2)^2}$. Both of these distance formulas describe metrics. In other words, for all P_1, P_2, and P_3:

(i) $d(P_1, P_2) = d(P_2, P_1)$;
(ii) $d(P_1, P_2) \ge 0$ while $d(P_1, P_2) = 0$ if and only if $P_1 = P_2$; and
(iii) $d(P_1, P_2) + d(P_2, P_3) \ge d(P_1, P_3)$.

Using only trigonometry, we will develop a similar formula for great circle distance and we will then give a supporting proof showing that it is also a metric.

To find the great circle distance between two points $P_1 = (\theta_1, \phi_1)$ and $P_2 = (\theta_2, \phi_2)$ on a unit sphere, we first convert our spherical coordinates to rectangular coordinates using Table 1. Then we will have:

$$\mathbf{u} = (\theta_1, \phi_1) = \langle \sin\phi_1 \cos\theta_1, \sin\phi_1 \sin\theta_1, \cos\phi_1 \rangle$$

$$\mathbf{v} = (\theta_2, \phi_2) = \langle \sin\phi_2 \cos\theta_2, \sin\phi_2 \sin\theta_2, \cos\phi_2 \rangle$$

Using the dot product we can find the angle between **u** and **v**.

$$\begin{aligned}\mathbf{u} \cdot \mathbf{v} &= \sin\phi_1 \cos\theta_1 \sin\phi_2 \cos\theta_2 + \sin\phi_1 \sin\theta_1 \sin\phi_2 \sin\theta_2 + \cos\phi_1 \cos\phi_2 \\ &= \sin\phi_1 \sin\phi_2 \cos(\theta_1 - \theta_2) + \cos\phi_1 \cos\phi_2\end{aligned}$$

So the measure of the angle between **u** and **v** is

$$\arccos[\sin\phi_1 \sin\phi_2 \cos(\theta_1 - \theta_2) + \cos\phi_1 \cos\phi_2].$$

Since the radian measure of the central angle is equal to the distance between two points on a unit sphere, we can now write a formula for the great circle distance. This agrees, independently, with the great circle metric formula cited in other places [2].

2. The Great Circle Metric

The following theorem shows that the three identifying characteristics of a metric are satisfied by the great circle distance formula. Note that, for non-unit spheres, the actual distance would be $\rho \cdot d(P_1, P_2)$.

Theorem: *Let* $P_1 = (\theta_1, \phi_1)$ *and* $P_2 = (\theta_2, \phi_2)$ *be arbitrary points on the unit sphere* S^2. *Define*

$$d(P_1, P_2) = \arccos[\sin\phi_1 \sin\phi_2 \cos(\theta_1 - \theta_2) + \cos\phi_1 \cos\phi_2] \qquad (1)$$

as the distance between the two points. This formula determines a metric.

Proof:

(i) For all $P_1, P_2 \in S^2$,

$$\begin{aligned}d(P_1, P_2) &= \arccos[\sin\phi_1 \sin\phi_2 \cos(\theta_1 - \theta_2) + \cos\phi_1 \cos\phi_2] \\ &= \arccos[\sin\phi_2 \sin\phi_1 \cos(\theta_2 - \theta_1) + \cos\phi_2 \cos\phi_1] \\ &= d(P_2, P_1)\end{aligned}$$

(ii) $d(P_1, P_2) = \arccos[\sin\phi_1 \sin\phi_2 \cos(\theta_1 - \theta_2) + \cos\phi_1 \cos\phi_2] \geq 0$ for all P_1, P_2 since $0 \leq \arccos(x) \leq \pi$ for all $x \in \Re$.

Let $P_1 = P_2 = (\theta, \phi)$. Then we need to show that $d(P_1, P_2) = 0$.

$$\begin{aligned}d(P_1, P_2) &= \arccos[\sin\phi_1 \sin\phi_2 \cos(\theta_1 - \theta_2) + \cos\phi_1 \cos\phi_2] \\ &= \arccos[\sin^2\phi + \cos^2\phi] \\ &= 0.\end{aligned}$$

Let $d(P_1, P_2) = 0$. We need to show $P_1 = P_2$. Suppose $P_1 = (\theta, \phi)$ and $P_2 = (\theta + \Delta\theta, \phi + \Delta\phi)$.

Then $d(P_1,P_2) = \arccos[\sin\phi\sin(\phi+\Delta\phi)\cos[\theta-(\theta+\Delta\theta)]+\cos\phi\cos(\phi+\Delta\phi)]$

$\cos(0) = 1 = \sin\phi\sin(\phi+\Delta\phi)\cos(\Delta\theta)+\cos\phi\cos(\phi+\Delta\phi)$.

Since $0 \le \phi \le \pi$ and $0 \le \phi+\Delta\phi \le \pi$ this implies that $\sin\phi \ge 0$ and $\sin(\phi+\Delta\phi) \ge 0$. We also know that $\cos(\Delta\phi) \le 1$, so it follows that

$1 \le \sin\phi\sin(\phi+\Delta\phi)(1)+\cos\phi\cos(\phi+\Delta\phi)$

$\le \sin\phi\,[\sin\phi\cos(\Delta\phi)+\sin(\Delta\phi)\cos\phi]+\cos\phi\,[\cos\phi\cos(\Delta\phi)-\sin\phi\sin(\Delta\phi)]$

$\le \sin^2\phi\cos(\Delta\phi)+\cos^2\phi\cos(\Delta\phi)$

$\le \cos(\Delta\phi)$

Hence $\cos(\Delta\phi) = 1$ and $\Delta\phi = 0$. Therefore $P_2 = (\theta+\Delta\theta,\phi)$. It remains to show that $\Delta\theta = 0$, and thus $P_1 = P_2$.

$0 = d(P_1,P_2) = \arccos\{\sin\phi\,\sin\phi\,\cos[\theta-(\theta+\Delta\theta)]+\cos\phi\,\cos\phi\}$

$1 = \sin^2\phi\cos(\Delta\theta)+\cos^2\phi$

$0 = \sin^2\phi\,[\cos(\Delta\theta)-1]$.

Either we have case 1, where $\sin^2\phi = 0$, or we have case 2, where $\cos(\Delta\theta)-1 = 0$.

If we have case 1, then either $\phi = 0$ or $\phi = \pi$. If $\phi = 0$, then $P_1 = (\theta,0)$ and $P_2 = (\theta+\Delta\theta,0)$. Both of these must be at the north pole, due to lack of declination, and thus $P_1 = P_2$. The rotation is irrelevant.

If $\phi = \pi$, then $P_1 = (\theta,\pi)$ and $P_2 = (\theta+\Delta\theta,\pi)$. These points must both be at the south pole, and again $P_1 = P_2$.

If we have case 2, then $\cos(\Delta\theta) = 1$ so $\Delta\theta = 0$, giving $P_1 = P_2$. Thus we note that in all cases $P_1 = P_2$ if $d(P_1,P_2) = 0$.

(iii) All that remains to complete the proof is to show that $d(P_1,P_3)+d(P_2,P_3) \ge d(P_1,P_2)$ for all P_1, P_2, and $P_3 \in S^2$. Without loss of generality, we can let $P_1 = (0,\phi_1)$, $P_2 = (\theta,\phi_2)$, and $P_3 = (0,0)$ since any three points can be transformed to these positions while preserving the distances between them (Figure 2).

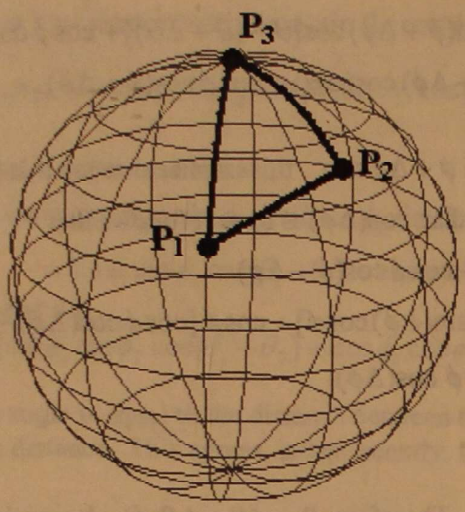


Figure 2: Transformed location of 3 arbitrary points.

We need to show

$$d[(0,\phi_1),(\theta,\phi_2)] \le d[(0,\phi_1),(0,0)] + d[(\theta,\phi_2),(0,0)] \quad \text{or}$$

$$\arccos\left[\sin\phi_1 \sin\phi_2 \cos\theta + \cos\phi_1 \cos\phi_2\right] \le \phi_1 + \phi_2. \qquad (2)$$

Since $0 \le \phi_1, \phi_2 \le \pi$, $\sin\phi_1 \sin\phi_2 \ge 0$. Also, $\cos\theta + 1 \ge 0$. Therefore,

$$\sin\phi_1 \sin\phi_2 [\cos\theta + 1] \ge 0$$

$$\sin\phi_1 \sin\phi_2 \cos\theta + \cos\phi_1 \cos\phi_2 \ge \cos\phi_1 \cos\phi_2 - \sin\phi_1 \sin\phi_2$$

$$\sin\phi_1 \sin\phi_2 \cos\theta + \cos\phi_1 \cos\phi_2 \ge \cos(\phi_1 + \phi_2)$$

But the inverse cosine is a decreasing function and reverses an inequality, so formula (2) is true.

Parts (i), (ii), and (ii) together show that (1) is a metric.

3. Further Research Topics

A sphere of constant radius is essentially a two-dimensional manifold with a well-defined metric. Considering it in this manner allows analogies to be developed for many well known geometric objects and theorems. We are currently applying this to the study of spherical conics.

References

[1] Karl L. Swartz. "Great Circle Mapper." http://gc.kls2.com/.
[2] Eric W. Weisstein. "Great Circle." From *Math World* – A Wolfram Web Resource. http://mathworld.wolfram.com/GreatCircle.html.

SPATIAL DISTRIBUTION OF TURTLES ALONG THE GREAT KANAWHA RIVER, WEST VIRGINIA

Mark B. Watson, Department of Natural Sciences and Mathematics, University of Charleston, Charleston, WV 25304 and Thomas K. Pauley, Department of Biological Sciences, Marshall University, Huntington, WV 25755

ABSTRACT

This study was the first systematic study of turtle populations in the Great Kanawha River, West Virginia. Multiple trapping methods and direct observations were employed to inventory the navigable portion of the Kanawha River and its major tributaries and slack water areas. Distribution and morphometric data were collected from turtles captured. We observed over 890 turtles comprising six different species. Turtle species were not evenly distributed along the length of the river. Navigational dams have created a variety of habitats and these habitats contained different assemblages of turtles. This study provides important information on the distribution and abundance of turtles in an imperiled high-order stream. We have shown that the Kanawha River has a diverse turtle population and that embayments along the river should be conserved as turtle habitat.

Introduction

Turtle populations are declining over much of North America and many of these declines can be directly attributed to human impacts (Ernst et al. 1994). Because most turtles require over eight years to reach maturity, they are extremely vulnerable to habitat destruction, collection for pet trade, and over-harvesting (Gibbons and Selmlitch 1982; Gibbons 1997; Dodd 1990). This is especially important in states such as West Virginia that provide only weak protection, e.g. high possession limits for turtle harvest (Levell 1995).

The present study provides important information on the distribution and abundance of turtles in the Great Kanawha River, an imperiled high-order stream. The Great Kanawha River forms from the confluence of the New and Gauley rivers in Fayette County. It drains much of the central section of the state as it flows northwest to the Ohio River. Navigational dams along the river slow the river and inundate the mouths of many streams, creating a wide diversity of habitats as the river flows from the mountainous upper reaches to the wide river valley at the lower reaches. Large portions of the Great Kanawha River are close to industrialized areas, resulting in riparian habitat that is being negatively impacted by urban sprawl and development. Although the Great Kanawha River is one of the two largest rivers in West Virginia and is an important commercial and recreational avenue, little is known about the turtle fauna of the Great Kanawha River or in West Virginia in general. Distribution records for turtle species in the Great Kanawha River were previously taken from museum records of specimens that were randomly collected i.e., without systematic inventories (Green 1969; Major 1975; Green and Pauley 1987). This is the first intensive turtle inventory for West Virginia or the Kanawha River.

Methods

The study area was the navigable or impounded portion of the Kanawha River mainstem and its larger embayments and tributaries (Figure 1). All tributaries navigable by small boat were inventoried upstream a distance of at least 2 km from the mouth.

Five types of turtle traps were used during the study: three sizes of funnel and two floating (basking) traps. Funnel traps included a mini-hoop net (51 cm diameter and 140 cm length), hoop net (91 cm diameter and 180 cm length) baited with canned or fresh fish, and a Fyke net, an unbaited funnel type trap with one to three 4.5 to 9 m leads that are 1.2 m deep. Floating traps were designed to be free or attached to basking logs. Spotting scopes or binoculars were used to identify basking turtles. The position of each trap or observation was recorded with a handheld global positioning system (GPS) receiver (Garmin GPS12). Funnel or hoop traps were spaced equally along tributaries (approximately 160 m apart) and traps on the Kanawha River mainstem were set each 1.6 km (1 mile) starting at the mouth of the river (Point Pleasant, WV) and continuing upstream to kilometer 158 (mile 98) at the mouth of Loop Creek near the town of Deepwater. Traps were located at mile points designated by the "Great Kanawha River Navigational Charts" (U.S. Army Corps of Engineers, 1997). Km "0" is designated at the mouth of the Kanawha River at Point Pleasant, WV. Traps were usually set mid-afternoon to evening and left overnight. Whenever possible, traps were set with the open end facing downstream to allow turtles to follow the scent of the bait and the trap opening.

Morphometric data collected from each turtle captured included: carapace length, carapace width, plastron length, plastron width, dorso-ventral height, precloacal distance (distance from base of tail to anterior edge of the cloaca), length of longest claw and mass. All measurements were taken in the field and turtles were released at site of capture. Carapace length measurements were taken from the cervical scute to the midline of the posterior marginal scute. Carapace width measurements were taken from the edge of the left and right marginal scutes across the seam of the middle vertebral scutes. Plastron length was taken from the anterior end of the intergular plastral seam to the posterior midline of the anal plate (notch). Plastron width was measured from the outer edges of the seam between the femoral and abdominal plastral scutes. Dorso-ventral distance was measured from the seam between the middle vertebral scutes to the abdominal plastral scutes. Claw measurements were taken along a straight line from the tip of the longest claw to its base. Precloacal distance was measured from the posterior edge of the anal plastral scute to the anterior edge of the cloaca. This measurement was taken after gentle pressure was exerted to extend the tail and the turtle had relaxed its tail muscles. Mass was measured by placing the turtle in a pre-weighed container with hand held spring-balance scale (Pesceola). Sex of the turtles was determined by observing secondary sex traits of adult males such as length of front claws and length of precloacal distance in relation to body size (Mosimann and Bider 1960; Ernst et al. 1994).

Results

We observed 894 turtles over a two-year period from March 1999 to September 2000 and of the turtles observed, 831 were captured and measured. Most turtles were adults (776 of 831), and males outnumbered females (455 vs. 321). Species recorded were the Midland Painted Turtle (*Chrysemys picta marginata*), Eastern Snapping Turtle (*Chelydra s.serpentina*), Red-eared Slider (*Trachemys scripta elegans*), Stinkpot (*Sternotherus odoratus*), Eastern Spiny Softshell (*Apalone s. spinifera*), Northern Map Turtle (*Graptemys geographica*), and Ouachita Map Turtle (*Graptemys ouachitensis*). Turtle species were not evenly distributed in the river valley. The majority of turtles captured or observed were found in the shallow embayments of the Winfield pool below kilometer 64 (mile 40). Turtles were also abundant in embayments in the middle to lower sections of the Robert C. Byrd pool of the river.

The Midland Painted Turtle, *C. picta marginata*, occurred along the lower half of the river and was not found upstream of the mouth of the Coal River close to mile 44 (Figure 2). This subspecies showed signs of intergradations with the Eastern subspecies of Western Painted Turtle, *C. p. picta* in the Great Kanawha River. The two subspecies differ in the alignment of the carapace scutes and the amount of figure on the plaston. The joints of carapace scutes of the eastern subspecies are aligned where the joints of the midland subspecies do not. The plastron of the midland subspecies is highly figured, where the plaston of the eastern subspecies lacks figure (Ultsch et al. 2001). Many painted turtles in this study showed varying degrees of carapace scute alignments and varying amounts of figure on the plastron. This species was the most frequently observed (377 individuals) in the Winfield Pool of the river (Figure 2). Most of these turtles were observed in shallow embayments; few individuals were captured in the mainstem or tributaries.

Chelydra s. serpentine was the most widespread species. It was observed from the mouth of the river, upstream to the head of navigation (Figure 3). One hundred and ninety-five individuals were recorded in a variety of habitats including embayments, tributaries, and the main river. This was the only turtle species observed in portions of the river immediately adjacent to industrial and urban areas of Charleston.

Trachemys s. elegans was the third most abundant species captured. Most Red-eared Sliders were found between river kilometers 56-72 (miles 35 – 45), that corresponds to the locations of three large embayments (Figure 4). No Red-eared Sliders were captured or observed upstream of the Coal River. The abundance of this species decreased, with only occasional individuals recorded along the main river below Winfield Locks and Dam.

The fourth most abundant species was the *Sternotherus odoratus*. Eighty-seven individuals were observed from the mouth of the Great Kanawha River upstream to the head of navigation (km 158), but it was not observed from river kilometer 72 (mile 45) up stream to above kilometer 129 (mile 80), an area that industrial and urban development has degraded the habitats (Figure 5). Degradation was apparent by such activities as stabilizing the river bank with large piles of rocks or steel pilings and removing riparian vegetation.

Seidel, M.E. and N.B. Green 1981. Geographic distribution – *Psuedemys scripta elegans*. Herpetological Review 12:64.

Seidel, M.E. and N.B. Green 1982. On the occurrence of cooter turtles (subgenus Pseudemys) in the upper Ohio River Valley. Herpetological Review 13: 133.

Ultsch, G.R., G. M. Ward, C.M. LeBerte, B.R. Kuhajda, and E.R. Stewart. 2001. Intergradation and origins of subspecies of the turtle *Chrysemys picta*: morphological comparisons. Canadian Journal of Zoology 79: 485-498.

Army, Corps of Engineers. 1997. Great Kanawha River Navigation Charts: Mouth to Head of Navigation. Defense Dept., Army, Corps of Engineers. Huntington District. 88p.

Wright, D.M.D, M.A., Bozek, J.R. Probst, and E.M. Anderson. 1999. Responses of turtle assemblage to environmental gradients in the St. Croix River in Minnesota and Wisconsin, USA. Canadian Journal of Zoology. 77: 989-1000.

Figure Legends:

Figure 1. Study area of the Great Kanawha River Drainage in West Virginia.

Figure 2. Distribution of locations where Painted Turtles (*C. picta*) were observed or trapped along the Great Kanawha River.

Figure 3. Distribution of locations where Eastern Snapping Turtles (*C. serpentina*) were observed or trapped along the Great Kanawha River.

Figure 4. Distribution of locations where Red-eared Sliders (*T. scripta*) were observed or trapped along the Great Kanawha River.

Figure 5. Distribution of locations where Stinkpot Turtles (*S. odoratus*) were observed or trapped along the Great Kanawha River.

Figure 6. Distribution of locations where Eastern Spiny Softshells (*A. spinifera*) were observed or trapped along the Great Kanawha River.

Figure 7. Distribution of locations where map turtles (*G. ouachitensis* and *G. geographica*) were observed or trapped along the Great Kanawha River.

Table 1 Morphometric data of turtles of the Kanawha River*

	Css		Gg		Cpm		Tse		So		Ass	
Sex	M	F	M	F	M	F	M	F	M	F	M	F
n	102	47	2	7	197	142	81	86	52	27	21	12
Sex Ratio (M/F)	2.2:1		0.3:1		1.4:1		0.9:1		1.9:1		1.7:1	
ave CL	27.5	23.7	17.3	20.6	12.8	14.7	18.7	20.6	10.7	10.3	16.8	23.3
max CL	38.0	30.2	20.7	25.2	15.9	17.4	23.2	27.4	13.5	12.2	19.3	34.3
S.E. CL	0.5	0.5	na	1.1	0.1	0.1	0.3	0.4	0.2	0.1	0.3	1.5
ave CW	23.5	19.8	13.2	15.4	9.2	10.5	13.7	15.6	7.2	7.2	14.5	20.0
max CW	32.0	25.3	15.4	18.1	11.5	12.6	17.2	19.8	8.3	8.2	16.4	28.3
S.E. CW	0.43	0.5	na	0.7	0.06	0.1	0.2	0.3	0.1	0.1	0.26	1.3
ave PL	20.5	18.3	14.9	17.4	11.6	13.5	16.4	19.1	7.5	7.6	12.0	16.1
max PL	30.0	23.7	18.1	20.5	14.4	16.4	20.7	25.4	8.8	8.8	13.4	24.6
S.E. PL	0.4	0.5	3.2	0.8	0.1	0.1	0.3	0.4	0.1	0.1	0.2	1.1
ave PW	8.7	7.7	7.9	9.4	6.0	7.0	8.2	9.6	3.1	3.2	5.7	8.2
max PW	13.1	10.9	9.7	11.2	9.9	10.5	10.1	19.7	3.7	3.8	6.8	13.3
S.E. PW	0.2	0.2	na	0.5	0.04	0.1	0.1	0.2	0.04	0.1	0.1	0.6
ave DV	11.3	10.5	6.0	7.1	4.3	5.3	6.6	8.0	4.0	3.9	4.1	5.4
max DV	17.7	13.7	7.4	8.4	5.7	6.4	8.7	10.7	5.1	4.6	5.1	8.4
S.E. DV	0.2	0.2	na	0.4	0.03	0.04	0.1	0.2	0.05	0.1	0.1	0.4
ave PreClo	10.9	7.0	3.6	1.9	1.6	0.9	2.9	1.3	na	na	6.8	6.7
max PreClo	16.9	9.5	3.73	2.5	2.65	4.2	4.2	2.5	na	na	8.4	11.7
S.E. PreClo	0.3	0.2	na	0.2	0.02	0.04	0.1	0.05	na	na	0.1	0.6
ave Claw	2.4	2.0	1.5	1.0	1.1	0.7	1.7	1.0	0.6	0.6	0.7	0.9
max Claw	3.8	2.8	2.1	1.2	1.8	1.75	2.2	2.8	0.75	0.85	0.9	1.3
S.E. Claw	0.1	0.05	na	0.05	0.02	0.01	0.03	0.03	0.01	0.03	0.02	0.1
ave Mass (kg)	5.9	3.7	0.6	1.0	0.26	0.4	0.8	1.4	0.2	0.2	0.5	1.1
max Mass (kg)	14.9	7.2	1.1	1.7	0.5	0.7	1.6	4.5	0.3	0.3	2.7	3.3
S.E. Mass	0.3	0.2	na	0.2	0.004	0.008	0.03	0.1	0.007	0.01	0.1	0.3

*all lengths are centimeters

Abbreviations: Css, *Chelydra s. serpentina*; Gg, *Graptemys geographica*; Cpm, *Chrysemys picta marginata*; Tse, *Trachemys scripta elegans*; So, *Sternotherus odoratus*; Ass, *Apalone s. spinifera*; CL, carapace length; CW carapace width; PL, plastron length; PW, plastron width; DV, dorso-ventral length; PreClo, precloacal distance; Claw, length of longest claw.

Figure 1. Study area of the Great Kanawha River Drainage in West Virginia

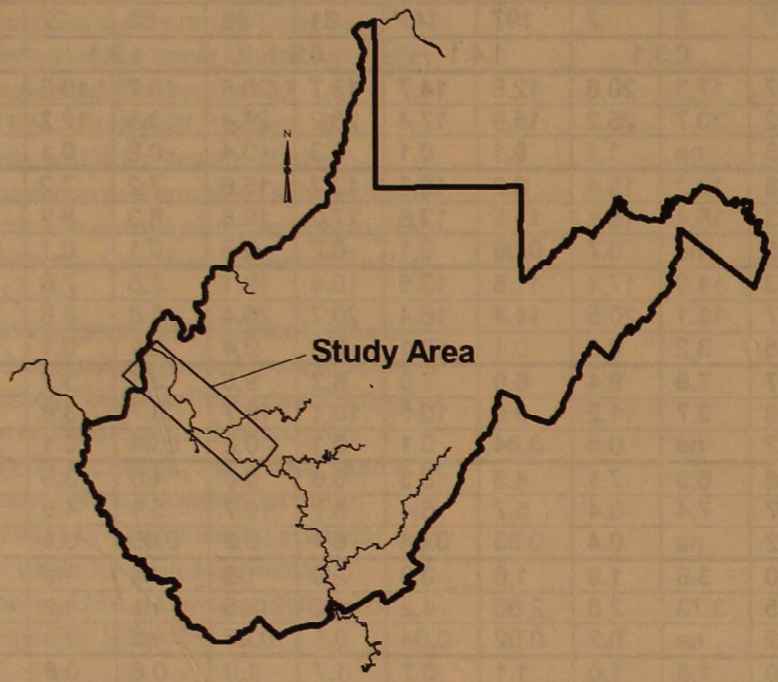


Figure 2. Distribution of locations where Painted Turtles (*C. picta*) were observed or trapped along the Great Kanawha River.

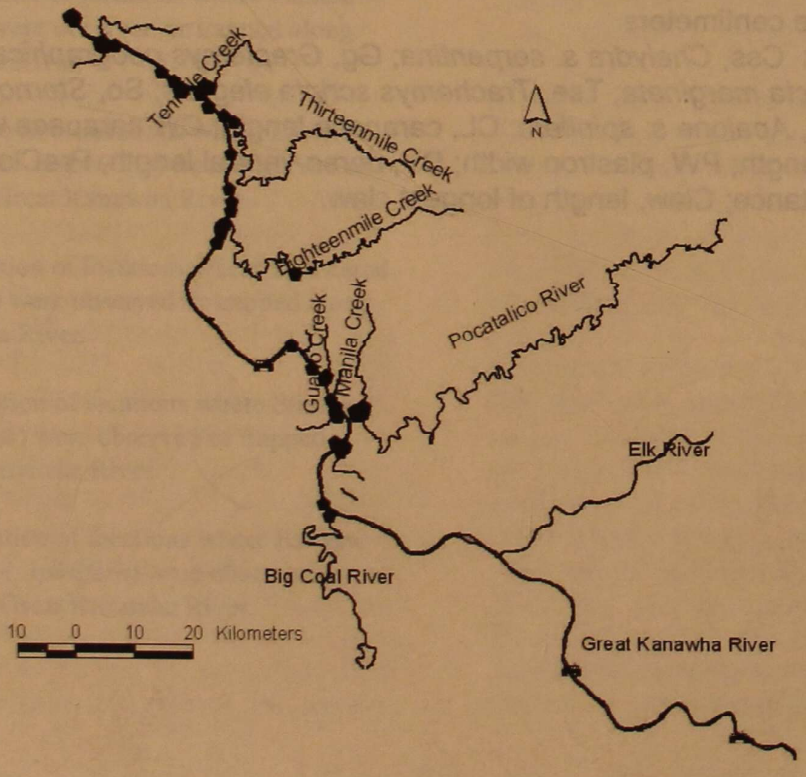


Figure 3. Distribution of locations where Eastern Snapping Turtles (*C. serpentina*) were observed or trapped along the Great Kanawha River.

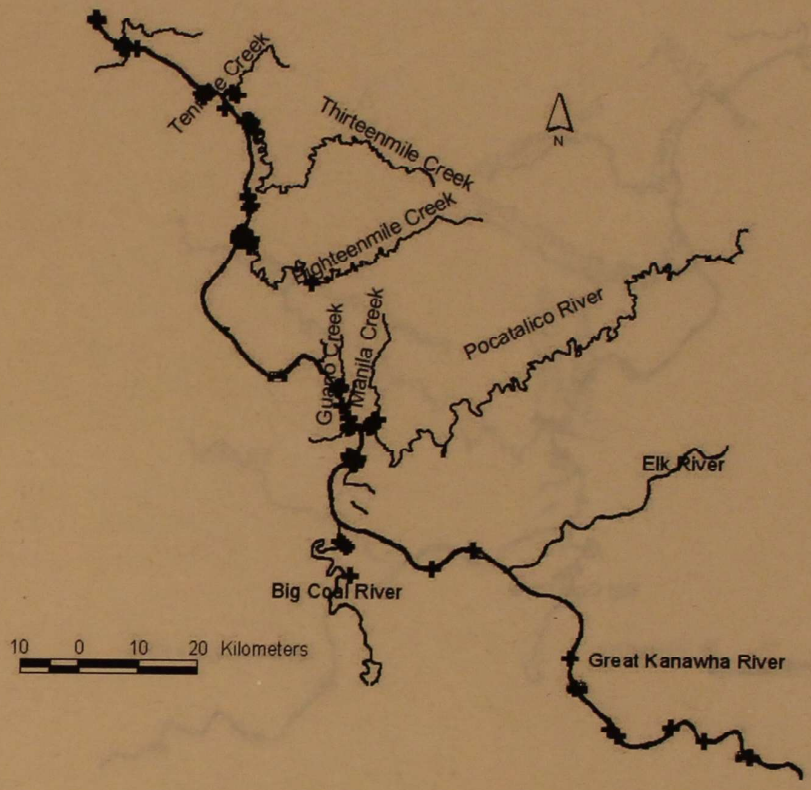


Figure 4. Distribution of locations where Red-eared Sliders (*T. scripta*) were observed or trapped along the Great Kanawha River.

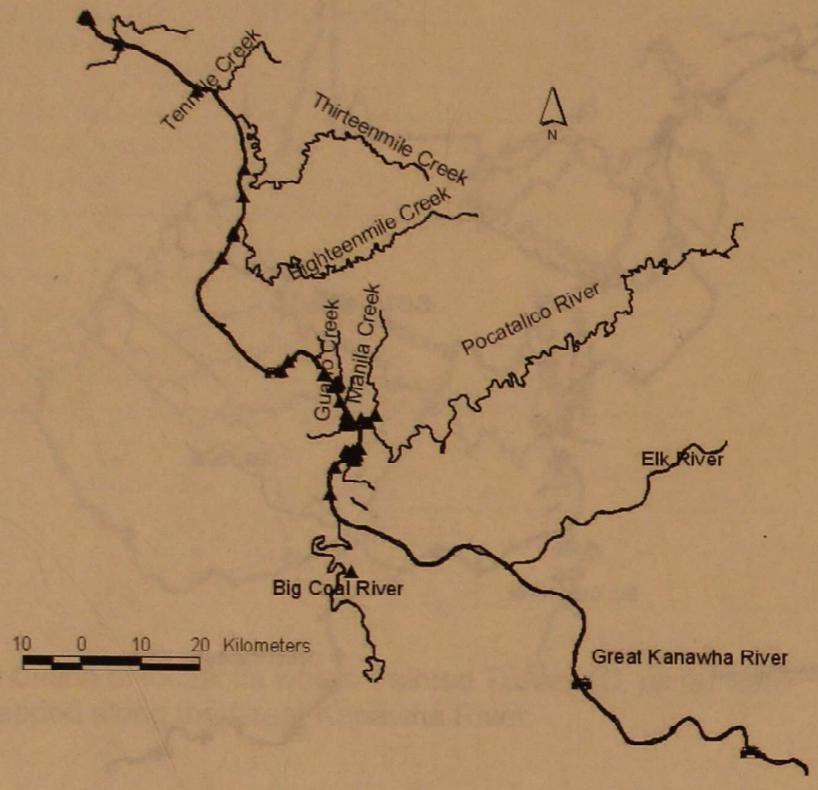


Figure 5. Distribution of locations where Stinkpot Turtles (*S. odoratus*) were observed or trapped along the Great Kanawha River.

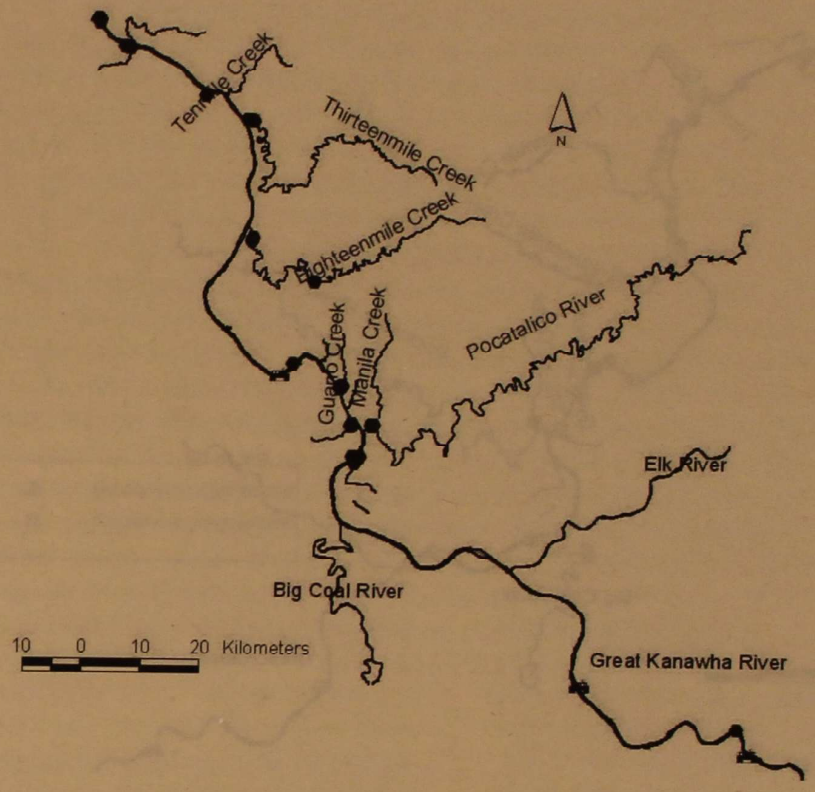


Figure 6. Distribution of locations where Eastern Spiny Softshells (*A. spinifera*) were observed or trapped along the Great Kanawha River.

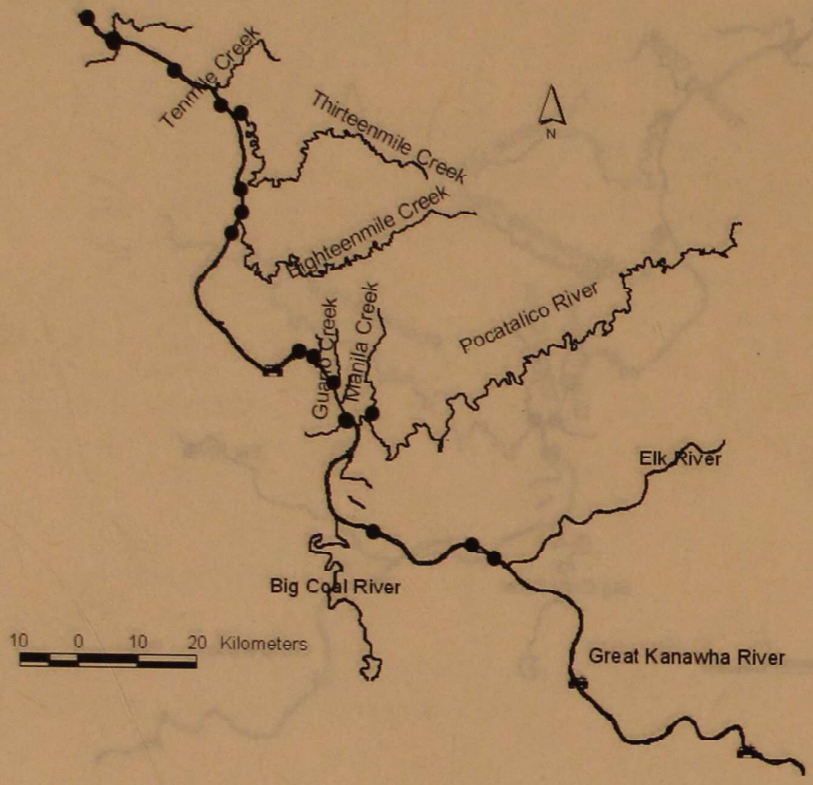
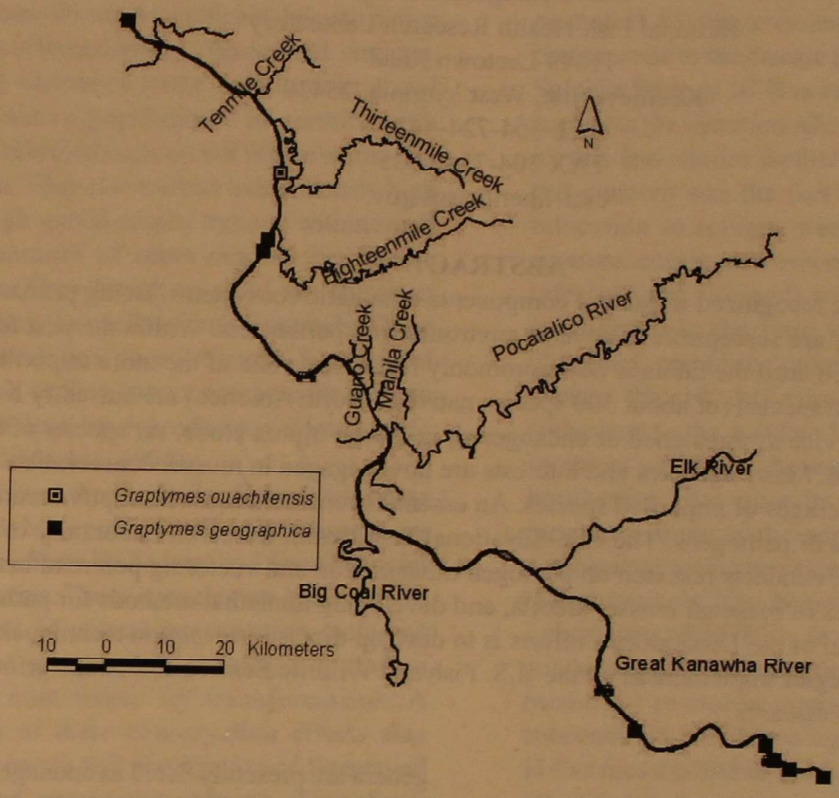


Figure 7. Distribution of locations where map turtles (*G. ouachitensis* and *G. geographica*) were observed or trapped along the Great Kanawha River.



IMPERILED FRESHWATER MUSSELS (UNIONIDAE): DISEASE PREVENTION AS PART OF THE CONSERVATION EFFORTS.

Clifford E. Starliper
U.S. Geological Survey
National Fish Health Research Laboratory
11649 Leetown Road
Kearneysville, West Virginia. 25430
TEL 304-724-4433
FAX 304-724-4435
cstarliper@usgs.gov

ABSTRACT

Freshwater mussels are recognized important components of aquatic ecosystems. Being primarily sedentary, filter-feeding animals, they are susceptible to adverse environmental parameters. Within the past few decades, their numbers have declined until the fauna is now commonly regarded as one of the more imperiled in the United States. Seventy-two species (of about 300 species native to North America) are currently listed by the U.S. Fish and Wildlife Service as threatened or endangered; in West Virginia alone, six species of endangered mussels are currently listed. Many agencies and interests are now engaged in mussel conservation efforts, including propagation at refuges of imperiled species. An essential consideration with captive rearing at refuges is preventing introductions of pathogens. The USGS National Fish Health Research Laboratory in Leetown, WV, with its many cooperators, conducts research on pathogen transmission and vectoring potential between fish and mussels, identifying causes of seasonal mussel dieoffs, and developing nonlethal methods for pathogen screening. The overall goal of the Laboratory's efforts is to develop disease-prevention techniques that could be used in management strategies implemented by the U.S. Fish and Wildlife Service and others prior to the onset of large-scale problems with diseases.

INTRODUCTION

There are approximately 300 species and subspecies of freshwater mussels native to North America. This fauna is particularly rich in species diversity and in overall numbers of animals in the southeastern United States where favorable water temperatures, food availability, and presence of the required host fishes are abundant. Mussels are recognized important components of aquatic ecosystems. As filter-feeding animals, mussel beds with dense populations are capable of filtering substantial amounts of water, affecting nutrient cycling, and providing improved environments for benthic plants and animals (Vaughn and Spooner, 2006).

Within the past few decades, historical numbers have significantly declined until the fauna is now recognized as one of the more imperiled in the United States (Neves et al., 1997). In fact, approximately 70 % of the native species are imperiled (Williams et al., 1993), and 72 species are currently federally listed by the U.S. Fish and Wildlife Service as endangered or threatened (Code of Federal Regulations, 2006). In West Virginia alone, six species representing five genera are presently listed as endangered (Table 1). A suite of causes, either individually or in combination have been identified as reasons for their decline. Furthermore, the life cycle of unionid mussels requires a host fish for transformation of embryonic glochidia into juveniles. Therefore, any adverse impact to the host fish affects the sustainability of mussel populations. Factors affecting the fish hosts include diseases, impoundments that prevent or limit their movements, and other environmental impacts.

Mussels have often been referred to as important sentinels for the overall health of aquatic ecosystems. This is in large part based on their relatively sedentary existence and their filtering of water for respiration and food. However, this also renders them susceptible to compromised environmental conditions, such as those caused by pollutants or increased water temperatures and reduced dissolved oxygen concentrations often associated with decreased water flows. Disturbances to land adjacent to bodies of water that result in soil runoff and disturbances to river and lake bottoms (e.g. altered flows, siltation, etc.) can be detrimental to mussels (Ellis, 1936; Fuller, 1974).

Likely the most serious imperilment to native mussel populations in recent years has been the introduction of the nonnative zebra mussel (*Dreissena polymorpha*) in the mid 1980's (Herbert et al., 1991; Nalepa, 1994). Since its introduction, this invasive species has become the most significant threat to native populations in affected rivers, lakes, and streams. Zebra mussel colonies have resulted in local extirpation of native populations. In contrast with native species, zebra mussels do not utilize a host fish for reproduction. They also tolerate tremendously high numbers (i.e. high spatial density indices) within small areas, with thousands of zebra mussels per square meter possible. They attach to solid structures or surfaces, including the shells of native animals. These colonizations result in a poor prognosis for survival of native animals as they cannot compete for oxygen and food, much less for reproduction (Haag et al., 1993; Gillis and Mackie, 1994).

These combined threats to the future well-being of native mussels have precipitated an increased focus on conservation efforts by Federal and State agencies and private interests. These agencies are involved in a number of facets of mussel conservation including descriptions of geographic ranges, population densities, and host fishes for transformation. A significant part of these conservation efforts also involves maintenance and propagation of threatened and endangered species at refuges. Therefore, husbandry and captive rearing techniques are the current focus for much research. The ultimate goal for captive propagation is reintroductions into natural watersheds or augmentations of existing populations after peak numbers of zebra mussels subside.

CONSERVATION EFFORTS FOR NATIVE MUSSELS

Refuges for mussels include fish hatcheries that have been modified to accommodate both the mussels and host fishes. Typically, these hatcheries (refuges) rear a suite of sport and restoration fish species and an even greater variety of mussel species. One of the larger hatcheries in the U.S. Fish and Wildlife Service rearing both fish and mussels is the White Sulphur Springs National Fish Hatchery (NFH) in White Sulphur Springs, West Virginia. This has for many years served as one of a few national broodstock production hatcheries for trout for the U.S. Fish and Wildlife Service's fish hatchery system. Millions of eggs are produced and shipped annually to other facilities. This hatchery maintains an "A-1" classification within the Service's health inspection program, meaning the facility has a closed, specific pathogen-free water supply and fish have been determined to be pathogen- and disease-free for at least a number of years. Maintaining this classification is essential to the mission of this hatchery. Any compromise to the disease prevention strategy at White Sulphur Springs NFH is cause for great concern, and such was the situation when mussels were introduced at this and similar facilities. The primary reason for this concern was the fact that mussels collected for relocation to refuges were taken from open-water environments known to contain fish that are potentially infected (and diseased) with any of a variety of fish pathogens. At the time when refuges were being identified, one of the more important questions was: what is the risk that mussels will act as vectors of pathogens to the resident-hatchery fish populations? In recent years, and because of the large number of hatcheries that now have mussel propagation programs, there is the additional consideration for pathogen vectoring to resident populations of hatchery-reared mussels. The National Fish Health Research Laboratory (NFHRL), in Leetown, WV has been conducting research to address these serious concerns raised by resource managers. The research has concentrated on addressing the following questions: 1) Can mussels harbor a fish pathogen? 2) Can mussels act as fish pathogen vectors? 3) What is the risk that fish could become infected with a pathogen introduced via mussels, and more importantly, what can be done to prevent or significantly reduce the risk for pathogen vectoring?

CAN MUSSELS HARBOR A FISH PATHOmENu RECOVERY OF BACTERIA

The risks and consequences for introductions of pathogens through movements of fish (or fish eggs) from open water systems (e.g. rivers) to fish hatcheries, and among hatcheries, are well documented (Wedemeyer, 2001). Much is known about the primary diseases of cultured fishes, such as pathogenicity and transmission, geographic and host ranges, and prevention and treatment strategies (Noga, 1996; Woo and Bruno, 1999; Wedemeyer, 2001). Fish husbandry involves spawning the brood fish and rearing the progeny all within the same facility. This negates that constant need for shipments of eggs or fry, which eliminates an important source for pathogen introductions. Careful fish culture practices ensure maintenance of the genetic integrity of particular fish

strains, and maintains a disease-free status. However, the self-sustaining fish propagation model is not without problems. Indirect effects of intensive fish culture such as overcrowding, reduced dissolved oxygen, increased detritus and ammonia can predispose fish to diseases. Compromised husbandry is relevant when considering the potential for pathogen introductions when fish from open waters are moved to hatcheries. Because both fish and mussels exist in aquatic environments, it is reasonable to anticipate that some of these same disease issues with fish will become increasingly important as mussel propagation activities escalate.

To facilitate the study of the bacterial flora of freshwater mussels, a reliable procedure to recover the bacteria was developed (Starliper et al., 1998). This procedure involves aseptic techniques that allow for enumeration of viable bacteria and it yields isolated single colonies that can be picked for characterization. To perform the procedure, after morphometric data is recorded, the external surfaces of the valves (shells) were decontaminated and pried open so the adductor muscles could be cut. Fluid, which is inside the valves, but outside of the soft tissues, was clean-caught in a sterile Petri dish. With subsequent studies, we have shown that fluid samples are representative of the bacterial flora within the mussels' tissues. The soft tissues were excised and the outer surfaces decontaminated by swirling in 200 mg/L sodium hypochlorite for 30 seconds. This step ensures that the resulting bacteria were from within the tissues. The soft tissues may be processed in toto, as tissue homogenates, or they can be separated and processed individually. Results have shown that the total bacteria from tissues does not vary significantly among individuals within a population, or between species collected at the same time and from the same location (Starliper et al., 1998). The bacteriological media that can be used for primary isolation can be tailored to a specific need or to the suspected bacteria present. General growth media, which are commonly used at fish diagnostics laboratories, including brain heart infusion and R2A (Becton Dickinson and Company, Sparks, MD), and differential or selective media for isolation of specific bacteria are suitable for use for mussels. As with the bacteria cultured from fish, growth temperatures are lower and incubation times will generally be longer, relative to bacteria cultured from mammals. We have found that our procedure to isolate bacteria from mussels avoids nearly all fungal contamination on primary isolation plates, which is often a confounding problem in bacteriology of field samples. Fungal overgrowth renders plate media useless.

When the concept of cohabiting mussels and fish at hatcheries was introduced, mussels were suspected to be fish pathogen vectors; however, there were no previous reports to demonstrate this. Studies were designed in an attempt to demonstrate presence of fish pathogens in feral mussels (Starliper et al., 1998; Starliper and Morrison, 2000). Starliper and Morrison (2000) used 13 bacteriological media, including differential and selective media specifically designed for recovery of recognized fish pathogens. Media for Gram-positive bacterial pathogens *Renibacterium salmoninarum* and *Carnobacterium piscicola*, and for Gram-negatives including *Aeromonas salmonicida*, *Yersinia ruckeri*, and *Flavobacterium columnare* were employed. Six collections, representing six species of mussels were made throughout one sampling season. The conditions for diving dictate the seasons, which vary geographically, and is typically June through November in the Ohio River, adjacent to West Virginia. Although no pathogens were recovered during this intensive effort, the study provided useful data on the total bacteria present in tissues from this variety of hosts and covering the range in water temperatures. On average, the soft tissue homogenates contained about 1.0 ! 10 ^{5} cfu bacteria per g and the predominant bacteria isolated were motile *Aeromonas* spp. and *Pseudomonas* spp. Although these two genera include species that are opportunistic or secondary pathogens to fishes, none are recognized primary pathogens.

Flavobacterium columnare, the cause of columnaris disease to many cool and warmwater fishes, was isolated from a threeridge mussel *Amblema plicata* from the Ohio River, adjacent to Wood County, WV (Starliper et al., 1998). This isolation confirmed that feral mussels collected from open waters could indeed harbor fish pathogens. Since, *F. columnare* was isolated from a rainbow mussel *Villosa iris* from the Clinch River, Virginia (Starliper et al. 2007, In Review).

CAN MUSSELS ACT AS FISH PATHOGEN VECTORS? THE BACTERIAL FLORA OF MUSSELS AND DEPURATION

Since the introduction of zebra mussels, the number of large rivers affected by this invasive species has increased. The prognoses for survival of native mussel populations ahead of the advancing spread of zebra mussels is poor, not only for numbers of

individuals, but also for historic community structure (Haag et al., 1993; Gillis and Mackie, 1994). Often, what appears to be the only option to salvage local native populations is to relocate them to refugia. It was not known whether these relocated mussels could be exposed to pathogens when placed at the refuges. A study was done to determine if the resident bacteria in water or on fish at a major refuge could be pathogenic to mussels (Starliper and Morrison, 2000). The predominant bacteria were isolated from the mucus of 100 rainbow trout *Oncorhynchus mykiss* reared at the White Sulphur Springs NFH. Thirteen different bacteria were isolated, and each was grown in pure culture and used for waterborne (bath) challenges to six species of mussels. Each group of mussels was exposed to a minimum of 3.53 ! 10^5 cfu bacteria per mL of tank water and for up to 24 hr duration. No mortality or adverse effects, i.e. weak and slow valve closures in response to stimuli, were noted in the three-week observation periods that followed the challenges.

Additional waterborne exposures to mussels were done using two important fish pathogens, *A. salmonicida* and *R. salmoninarum*. Following the exposures, brook trout, *Salvelinus fontinalis,* were placed in the tanks to cohabit with the mussels. Although the mussels were unaffected, the fish cohabiting with the *A. salmonicida*-infected mussels began to die after eight days of cohabitation, and *A. salmonicida* was confirmed as the causal agent of mortality to the fish. It was noted that eight days was only slightly longer than the mortality expected if the fish were exposed directly to the pathogen. This was a pivotal observation because it led to the eventual development of a model to study pathogen transmission between fish and mussels, and pathogen depuration dynamics in mussels. These challenge and cohabitation studies also demonstrated how readily *A. salmonicida* was transmitted between fish and mussels. The previously described isolation of *F. columnare* from a feral mussel and the ease with which bacterial transmission through simple cohabitation occurred are good indications that pathogen vectoring via wild-caught mussels to fish maintained at refuges is a viable threat.

The response of the indigenous bacterial flora of mussels to a change in their water was evaluated (Starliper et al., 1998). The total bacterial cfu/g in soft tissues was shown to be stable across the relocation; however, the profiles of the bacterial species present in the mussels were very responsive to the water change. Significant changes to the bacterial profiles occurred within 24 hours. This was important because it showed the mussels' ability to depurate pathogens (e.g., during a quarantine). Another indication of depuration activity was shown with *F. columnare.* This pathogen was isolated from the *A. plicata* sampled directly upon collection from the Ohio River. At the same time, groups of cohorts were collected and relocated to flow-through, pathogen-free spring water at the Leetown laboratory. The first group of 15 mussels was sampled after 24 hr and *F. columnare* was not isolated. No additional isolations of *F. columnare* were made beyond the initial (day 0) isolation.

WHAT IS THE RISK FOR VECTORING BY MUSSELS, AND WHAT CAN BE DONE TO PREVENT PATHOGEN INTRODUCTIONS?

Native mussels that are taken from rivers for relocation to refuges must first undergo a minimum 30-day quarantine (Chafee, 1997; Gatenby et al., 1998). This requirement ensures that they are free from zebra mussels before being relocated to a locale not affected by zebra mussels. Thirty days allows zebra mussel veligers to develop to the stage when they can be seen with the naked eye upon inspection. At the end of the quarantine and if at least one zebra mussel is found, an additional 30-day quarantine is done. Ideally, this quarantine presents an opportunity for the native mussels to depurate pathogens. Studies were done to evaluate the 30-day duration for depuration of *A. salmonicida* (Starliper, 2001; 2005). A model was developed to evaluate the effect of quarantine in pathogen-free water to eliminate that risk to vector pathogens. To date, two species of mussels have been evaluated, *A. plicata* and *Fusconaia ebena* (Starliper, 2005; 2006). Mussels are unaffected by *A. salmonicida*, but certain fish species are highly susceptible, which made for a sensitive bioindicator for depuration. To use the model, an artificially established epizootic in either brook trout or Arctic char, *Salvelinus alpinus,* was begun by injection with *A. salmonicida*. When these fish commenced dying, non-injected fish were placed in the tank to become horizontally (i.e., naturally) infected. By the time the first horizontally infected fish began to die, all of the injected fish had succumbed. At this point, the mussels were added to the tank to cohabit with the infected fish. Within 2-4 weeks, a 100 % incidence of *A. salmonicida* in the mussels was determined by bacterial culture. The mussels were then moved from

the infection tank to "clean" tanks supplied with pathogen-free water to initiate depuration. Mussels were sampled through 30 days to determine if the pathogen was depurated. Depuration was considered complete when *A. salmonicida* was not isolated from the mussels or the tank water effluent (i.e., from shedding into the water), and bioindicator fish did not become infected or diseased during a subsequent observation period. After only one day of depuration, the prevalence of *A. salmonicida* in mussels had decreased to 30 % or less, and depuration was complete in both *A. plicata* and *F. ebena* within half of the required 30-day quarantine duration.

The results showing effective depuration of *A. salmonicida* well within the established quarantine duration are very encouraging for resource managers. However, caution should be exercised in interpreting these results to mean depuration of all pathogens would be expected to be completed similarly. *Aeromonas salmonicida* is not a pathogen to mussels, therefore it does not "parasitize" and stage disease in mussels in a fashion similar to what primary pathogens of mussels would do, particularly viruses. Some of the studies described here will need to be repeated using recognized, primary pathogens to mussels to determine if depuration in quarantine remains as effective as for *A. salmonicida*. One characteristic of the model is it resulted in a worst-case scenario because of the maximum pathogen (*A. salmonicida*) uptake by the mussels. It is highly unlikely that mussels would be exposed to this high experimental challenge load (cfu) in their natural environments because of dilution, etc. In these situations, the quarantine might be even more effective.

CURRENT RESEARCH

Our initial studies on mussels focused primarily on the risks of pathogen introductions to fish. There is an increasing need for development of mussel disease prevention tools that can be used as part of a broad conservation strategy. These tools will serve propagated mussels and will be important to prevent pathogen dissemination to feral populations through stocking of hatchery-reared animals. Two current projects at the NFHRL are addressing the issue of disease prevention. One is an attempt to identify the cause(s) of natural mussel dieoffs that have been observed in some rivers, and the other is to develop non-destructive sampling techniques.

In recent years, the number of mussel population surveys done has increased. Because of this, natural mussel dieoffs in certain rivers have been observed. Causes for these dieoffs have not been identified; however, some of the same observations from these dieoffs are known to be associated with dieoffs of fish. For example, mussel dieoffs have occurred seasonally at certain geographic locales, and this is typically related to a change in water temperature. Varying degrees of host specificity have been observed and with at least one dieoff, mortality was primarily affecting gravid females of a predominant mussel species, the slabside pearlymussel, *Lexingtonia dolabelloides*. Gravidity, more specifically the associated stressors with spawning are thought to be compromising the host; this is known to be a possible predisposing factor to diseases in fishes. The main focus of our study to investigate a suspected role of infectious agents in these dieoffs is to conduct periodic sampling of healthy mussels in disease-affected parts of the river to determine the normal bacterial flora. This baseline data will allow us to recognize bacterial pathogens present in moribund animals during an active dieoff. When the primary bacterial isolation plates from diseased mussels are evaluated, we will be looking for pure cultures of the same bacterium from most of the mussels examined. These data will be combined with observations of the previously mentioned parameters (i.e., seasonality, host specificity, etc.) to establish a cause-effect relationship. Once specific pathogens to mussels are described, periodic health inspections can be conducted, similar to the U.S. Fish and Wildlife Service's fish health inspection program.

The current procedure for primary isolation of bacteria from mussels requires sacrificing the animals. Clearly, this is unacceptable for threatened and endangered species. This can only be done on species that occur abundantly, and are not currently recognized candidates for propagation at refuges. We recently began to assess selected (biopsy) sites that could be taken from mussels using non-destructive procedures. Two criteria must be met for these procedures to be accepted and implemented. First, the procedures themselves to collect the samples must not cause obvious detrimental affects to mussels, not only for their survival, but also their ability to reproduce. Second, the prevalence of isolation of a pathogen from sites collected using non-destructive procedures must be at least as effective in recovering the pathogens as

standard lethal assays. In our current study, hemolymph, mantle and fluid are the candidate non-destructive sites. Preliminary results show that all three can be collected without causing mortality, and fluid yields a percentage of *A. salmonicida* isolation recovery equal to, or greater than those from lethal fluid and soft tissue homogenates.

ACKNOWLEDGEMENTS

Funding has been from the U.S. Geological Survey and the U.S. Fish and Wildlife Service. Many colleagues have contributed to our research, including (in no order): Rita Villella and Glenn Nelson II, USGS Aquatic Ecology Laboratory, Leetown, WV; Patricia Morrison and Dean Rhine, USFWS Ohio River Islands NWR, Parkersburg, WV; Janet Clayton and Craig Stihler, WV Division of Natural Resources, Elkins, WV; Richard Neves, Bill Henley, and Jesse Jones, USGS, Freshwater Mollusk Conservation Center, USFWS, (respectively) at Virginia Tech, Blacksburg, VA; Shane Hanlon, USFWS SW Virginia Field Office, Abingdon, VA; Jeff Powell, USFWS Daphne, AL; Jeff Garner, Alabama Department of Conservation and Natural Resources, Florence, AL; John Coll and Patricia Barbash, USFWS Lamar Fish Health Unit, Lamar, PA; and Catherine Gatenby and Julie Devers, USFWS White Sulphur Springs National Fish Hatchery, White Sulphur Springs, WV.

LITERATURE CITED

Chafee, C. 1997. Ohio River valley ecosystem mollusk conservation plan: strategic action plan-phase II. Appendix H: Draft freshwater mussel quarantine protocol. U.S. Fish and Wildlife Service. Bloomington, IN. 13 pp.

Code of Federal Regulations. 2006. Title 50, Wildlife and Fisheries; Part 17, Endangered and Threatened Wildlife and Plants. Department of the Interior, U. S. Fish and Wildlife Service, Washington, DC. (http://www.access.gpo.gov/nara/cfr/waisidx_06/50cfr17_06.html)

Ellis, M.M. 1936. Erosion silt as a factor in aquatic environments. Ecology. 17:29-42.

Fuller, S.L.H. 1974. Clams and Mussels (Mollusca: Bivalvia). In: C.W. Hart, Jr. and S.L.H. Fuller (Editors) Pollution Ecology of Freshwater Invertebrates. Academic Press, New York, NY. pp. 215-273.

Gatenby, C.M., M.A. Patterson, B.C. Parker, P. Morrison, and R.J. Neves. 1998. A protocol for the salvation and quarantine of mussels from zebra-infested waters. Conservation, captive care, & propagation. Freshwater Mussel Symposium, Columbus, OH., March 6-8, p. 18-19.

Gillis, P.L., and G.L. Mackie. 1994. Impact of the zebra mussel, *Dreissena polymorpha*, on populations of Unionidae (Bivalvia) in Lake St. Clair. Can. J. Zool. 72:1260-1271.

Haag, W.R., D.J. Berg, D.W. Garton, and J.L. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Can. J. Fish. Aquat. Sci. 50:13-19.

Herbert, P.D.N., C.C. Wilson, H.H. Murdoch, and R. Lazar. 1991. Demography and ecological impacts of the invading mollusk *Dreissena polymorpha*. Can. J. Zool. 69:405-409.

Nalepa, T.F. 1994. Decline of native unionid bivalves in Lake St. Clair after infestations by the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 51:2227-2233.

Neves, R.J., A.E. Bogan, J.D. Williams, S.A. Ahlstedt, and P.W. Hartfield. 1997. Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. In: Benz, G.W., and D.E. Collins (Editors) Aquatic fauna in peril: the Southeastern prospective. Southeast Aquatic Research Institute, Lenz Design and Communications, Decatur, GA. pp. 43-86.

Noga, E.J. 1996. Fish Disease: Diagnosis and Treatment. Mosby-Year Book, Inc., St. Louis, MO. 367 pp.

Starliper, C.E., R.J. Neves, S. Hanlon, and P. Whittington. 2007. In Review. A survey of the bacterial flora in three species of mussels from the Holston and Clinch Rivers, Virginia. J. Moll. Stud.

Starliper, C.E. 2006. Final Report, Imperiled freshwater mussels: The need for research to prevent the introduction and transmission of pathogens and diseases. U. S. Geological Survey, Leetown Science Center, Leetown, WV. 23 pp.

Starliper, C.E. 2005. Quarantine of *Aeromonas salmonicida*-harboring ebonyshell mussels (*Fusconaia ebena*) prevents transmission of the pathogen to brook trout (*Salvelinus fontinalis*). J. Shellfish Res. 24:573-578.

Starliper, C.E. 2001. The effect of depuration on transmission of *Aeromonas salmonicida* between the freshwater bivalve *Amblema plicata* and Arctic char. J. Aquat. Anim. Health. 13:56-62.

Starliper, C.E., and P. Morrison. 2000. Bacterial pathogen contagion studies among freshwater bivalves and salmonid fishes. J. Shellfish Res. 19:251-258.

Starliper, C.E., R. Villella, P. Morrison, and J. Mathias. 1998. Studies on the bacterial flora of native freshwater bivalves from the Ohio River. Biom. Lett. 58:85-95.

Vaughn, C.C., and D.E. Spooner. 2006. Unionid mussels influence macroinvertebrate assemblage structure in streams. J. N. Am. Benthol. Soc. 25(3):691-700.

Wedemeyer, G.A. (Editor) 2001. Fish Hatchery Management, Second Edition. American Fisheries Society, Bethesda, MD. 733 pp.

Williams, J.D., M.L. Warren, Jr., K.S. Cummings, J.L. Harris, and R.J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries. 18:6-22.

Woo, P.T.K., and D.W. Bruno. 1999. Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections. CABI Publishing, New York, NY. 874 pp.

Table 1. Current list of native freshwater mussels of West Virginia that are identified as federally endangered, and their citations for listing (Office of the Federal Register, The National Archives and Records Administration. College Park, MD).

Common Name	Scientific Name	Federal Register Citation
Clubshell	*Pleurobema clava*	1993. 58: 5638-5642
Fanshell	*Cyprogenia stegaria*	1990. 55: 25591-25595
Pink mucket (pearlymussel)	*Lampsilis abrupta*	1976. 41: 24062-24067
Northern riffleshell	*Epioblasma torulosa rangiana*	1993. 58: 5638-5642
Ring pink (mussel)	*Obovaria retusa*	1989. 54: 40109-40112
James spinymussel	*Pleurobema collina*	1988. 53: 27689-27693

PHYLOGENETIC ANALYSIS AND GENOMIC STRUCTURE OF THE CONE PHOTORECEPTOR GENE, NCKX2 (SLC24A2)

TAMARA LUSHNIKOVA, VICTOR FET and W. IAN TOWLER, Department of Biological Sciences, Marshall University, Huntington, West Virginia 25755-2510, Office: (304) 696-3116 ; Fax (304) 696-3243, Email: fet@marshall.edu

ABSTRACT

The exchanger gene NCKX2 (SLC24A2) codes for a protein involved in regulation of Ca^{2+} flow in cone photoreceptor. We refined the genomic structure of Na^{+}/Ca^{2+}–K exchanger gene of cone photoreceptor (NCKX2) not determined previously by Sharon et al. (2002; Invest. Ophthalmol. Vis. Sci. 43). The full length of the gene was 335,162 bp. Alignment of human NCKX2 protein with related proteins revealed non-conserved residues in H0 transmembrane domain of NCKX in rod-dominated animals. In cone-dominated vertebrates NCKX2 proteins share the conserved isoleucine[49] and serine[53] in H0 domain. In NCKX2 proteins from rod-dominated animals these amino acid residues are changed to valine[49] and proline[53]. Critical residues in the ! 1- and ! 2- repeats, comprising parts of transmembrane domains H2-H3 and H8-H9 were highly conserved in all exchanger transporters except NCKX6. Human NCKX6 shows 30% and 39% homology to the !1 - and !2- repeats, of human NCKX2. Phylogeny of fourteen published nucleotide and inferred amino acid sequences that were extracted from the GenBank database was reconstructed. The cone photoreceptor NCKX2 (SLC24A2) gene forms a sister group to the rod NCKX1 while NCKX3 and NCKX4 form another branch. NCKX5 and NCKX6 are related to the NCKX1-NCKX4. The 12.6-12.7 % nucleotide sequence distance between primates and rodents and the ca. 8% sequence distance within rodents make the cone photoreceptor NCKX2 gene a good evolutionary marker for order- and family-level phylogenetic analysis. NCKX2 gene may be considered a candidate gene for color vision mechanism study.

INTRODUCTION

The exchanger gene NCKX2 (SLC24A2) codes for an important protein involved in the regulation of Ca^{2+} flow in cone photoreceptor. Prinsen et al. (2000) reported molecular cloning, intraretinal localization by *in situ* hybridization, and initial functional characterization of cone-specific Na^{+}/Ca^{2+}–K exchangers. They identified NCKX transcripts in both human and chicken cones. Similar proteins of NCKX family are expressed in rod photoreceptors (Kang and Schnetkamp, 2003) as well as in other organs such as brain and kidney (Tucker et al., 1998; Cooper et al., 1999; Kraev et al., 2001; Li et al., 2002). In rod and cone photoreceptors, NCKX function to extrude Ca^{2+} that enters photoreceptors in darkness through the light-sensitive, cGMP-gated channels (CNG). Both NCKX1 and NCKX2 associate with the CNGA subunits of both the rod and cone cGMP-gated channels (Kang et al., 2003). The mammalian retina has two kinds of photoreceptors, rods and cones. Rods contain the visual pigment rhodopsin and are responsible for vision under conditions of low ambient light. Cones bestow high visual acuity under bright light conditions, and their different subtypes (each with unique visual pigment) constitute the basis of color vision. Rods dominate the mammalian retina, while cones represent only 3-5% of all photoreceptors in rodent and primate retina. Three mammalian groups (simian primates, squirrels, and tree shrews) have drastically reduced the rod mosaic and established cone-dominant or pure cone retinas, or pure cone regions (foveas) of retinas. Squirrel and tree shrew visual systems are also retinotectal in organization compared to the retinal-geniculate-cortical of primates (Ahnelt and Kolb, 2000). Direct OFF cone bipolar connections to rods could be a characteristic specific to the smaller eyes of rodents, but absent in the larger eyes of cats, monkeys, and humans (Sharpe and Stockman, 1999). Understanding the design of the sensory cells and their mosaic arrangement in the retina provides the basis for the study of the organization of visual pathways and visual perception in vertebrate species. Mammalian photoreceptors are uniform and it is difficult to distinguish between rods and cones. Studying the mechanism of chromatic differentiation may lead to future treatment of some forms of color blindness.

Although the overall sequence lengths of the rod and cone NCKX proteins are quite different, hydropathy analysis and sequence alignment indicate two sets of putative transmembrane-spanning

segments with a high degree of conservation (Prinsen et al., 2000). All members of Na^+/Ca^{2+}–K exchanger family, NCKX1 (Cooper et al., 1999), NCKX2 (Prinsen et al., 2000), NCKX3 (Kraev et al., 2001), NCKX4 (Li et al., 2002), and NCKX5 (Schnetkamp, 2004) share sequence similarity in two hydrophobic and internally homologous domains, commonly referred to as !-repeats (Kraev et al., 2001; Kraev and MacLennan, 2002; Li et al., 2002; Szerencsei et al., 2002; Kinjo et al., 2005). The !1- and !2- repeats, comprising parts of transmembrane domains H2-H3, and H8-H9 (Fig. 3) form a portion of the ion translocation pathway and are believed to have originated from an ancient gene-duplication event (Nicoll et al., 1996; Schwarz and Benzer, 1997; Prinsen et al., 2000; Philipson et al., 2002; Winkfein et al., 2003). The difference in length in Na^+/Ca^{2+}–K exchanger family is accounted for by the difference of the two large hydrophilic loops at the N-terminus and between the two sets of 5-6 putative transmembrane-spanning segments (H0,1-11) (Fig. 3; Kinjo et al., 2003). Human NCKX3 encodes a protein that displayed a high level of sequence identity with the other family members, NCKX4, rod NCKX1, and cone/neuronal NCKX2, in the hydrophobic regions surrounding the !-repeat sequences thought to form the ion-binding pocket for transport. Outside of these regions, NCKX3 showed no significant identity to other known properties (Kraev et al., 2001). NCKX3 and NCKX4 transcripts are most abundant in brain and, at lower level, in many other tissues (Kraev et al., 2001; Li et al., 2002).

The recently discovered mouse NCKX6 protein shares more sequence similarity with previously identified exchangers in the !-repeat regions but has little primary sequence similarity outside these regions (Cai & Lytton, 2004b). Expression of NCKX6 in various tissues suggests a key role for this molecule in regulating intracellular Ca^{2+} homeostasis in mammalian cells and tissue. However, functional roles of all NCKX exchangers are not well understood.

The human cone exchanger gene SLC24A2 (NCKX2) has been assigned to chromosome region 9p23 as a result of refinement of the physical map of the area surrounding the ribosomal protein S6 gene (Pata et al., 1992; Povey et al., 1997; Chadwick et al., 1999). To refine the area of human chromosome 9p22-23, which is the most commonly deleted in various types of human cancers (Knuutila et al., 1999), Reigo et al. (2000) constructed a 4.2 Mb uninterrupted double-linked YAC contig to enable a detailed and reliably marker-related mapping of any chromosomal rearrangements or new genes in this particular region. The gap was closed by constructing a cosmid map within YAC 804 B9. The order of the markers was supported by fluorescent *in situ* hybridization with cosmids on human metaphase chromosomes. The Sanger Centre (UK) used cosmids selected by Reigo et al. (2000) to produce 17 new STS markers, which led to BAC sequencing covering this 1.2 Mb gap. In BLAST search, the sequences of BACs in this 1.2 Mb gap matched the cloned cDNA of SCL24A2 (NCKX2) cone photoreceptor gene (Prinsen et al., 2000).

Our goals in this study were to refine the genomic structure of NCKX2 and to compare this gene with other known NCKX exchanger genes through a phylogenetic analysis.

MATERIALS AND METHODS

The GenBank database (www.ncbi.nlm.nih.gov/genome) was used for genomic searches. In BLAST search, four STS markers (stc 112F8T3, stc 112F8T7, stc 239E12T3, stc 252E11T3) were used to find three BACs carrying sequences corresponding to cDNA of SLC24A2 gene, or NCKX gene of cone photoreceptor (Fig. 1, Table 2). These genomic sequences were used to refine NCKX2 gene exon-intron structure (Fig. 2).

For phylogenetic comparison of NCKX genes, 14 published nucleotide DNA sequences corresponding to mRNAs, and also the inferred amino acid sequences, were extracted from the GenBank database. Of these, 11 genes belonged to five mammalian species: human (*Homo sapiens*), mouse (*xus mus culus*), rat (*Rattus norvegicus*), dolphin (*Tursiops truncatus*), and cow (*9os taurus*) (Table 1). As outgroups to mammalian genes, we used NCKX of a nematode (*Caenorhabditis elegans*), NCKX of sea urchin (*Strongylocentrotus purpuratus*), and NCKX2 of chicken (*Gallus gallus*). The DNA and amino acid sequences were aligned using ClustalX 1.81 (Thompson et al., 1997).

Phylogenies (gene trees) were reconstructed, and genetic distance matrix was generated using PAUP* 4.0b10 (Swofford, 1998) using standard Maximum Parsimony (unweighted) and Neighbor Joining algorithms under different weighting assumptions. Bootstrap support values were obtained by 100 pseudoreplicates. Cladograms for the molecular sequences from PAUP* were generated by TreeView 1.6.6 (Page, 2001) and edited using Metafile Companion (Companion Software).

RESULTS

In BLAST search, four STS markers (stc 112F8T3, stc 112F8T7, stc 239E12T3, stc 252E11T3) were used to find three BACs carrying sequences corresponding to cDNA of SLC24A2 gene, or NCKX2 gene of cone photoreceptor (Fig. 1). The sequences of BACs RP11-471J7, RP11-25202, RP11-363E7 matched the cloned cDNA of SCL24A2 (NCKX2) cone photoreceptor gene (Prinsen et al., 2000). Using two BLAST sequence comparisons between each BAC and cDNA of human NCKX2 (AF097366), we revealed most of genomic structure of Na^{+}/Ca^{2+}–K exchanger gene (NCKX) of cone photoreceptor (NCKX2) not determined previously by Sharon et al. (2002) (Table 2, Fig. 2). The full length of the gene was 335,162 bp. The gene consists of 11 exons ranging in size from 41 to 992 bp separated by 10 introns from 2.6 to 158 kb. Comparison of the second exon of 992 bp with homologs revealed that it is conserved among the exchanger genes in vertebrates and other animal groups.

Alignment of the human NCKX2 protein with related 14 exchange transporter proteins revealed conserved amino acid sequences in the !1- and !2-repeat areas, which comprise parts of transmembrane domains H2-H3, and H8-H9 (Figs. 3 and 5). These areas are believed to form a portion of the ion translocation pathway and could have arisen from an ancient gene duplication event (Nicoll et al., 1996; Prinsen et al., 2000; Philipson et al., 2002; Winkfein et al., 2003). Analysis of human NCKX2 for conserved amino acids (Fig. 4) confirms high similarity between chicken and human NCKX2 proteins in H0-H1 domains and revealed non-conserved residues in H0 transmembrane domain of NCKX2 in rod-dominated animals (Fig. 5). In two cone-dominated vertebrates NCKX2 proteins show the conserved amino acids isoleucine[49] and serine[53] in H0 domain. In rod-dominated NCKX2 proteins these amino acid residues are changed to valine[49] and proline[53]. Hydrophobic segments at the N-terminus (H0) of NCKX2 that are thought to function as cleavable signal peptides target NCKX2 in rod and cone photoreceptors (Kang et al., 2003). NCKX6 protein shows 30% and 39% homology to the !1- and !2-repeats of human NCKX2, respectively.

We constructed a phylogenetic tree (Fig. 4) for all 14 published amino acid sequences of translated mRNA from NCKX calcium transporter superfamily (See Table 1 for GenBank accession numbers). The saved Clustal X alignment had 1255 amino acid residue positions, with 527 parsimony-informative characters. Maximum Parsimony (MP) analysis yielded a single maximum parsimonious tree with length 3,055 steps, which had high consistency index (CI = 0.78907) and retention index (RI = 0.7607), and low homoplasy index (HI = 0.1093). The branches of the cladogram were extremely well supported by bootstrap resampling test, most branches with 90 to 100 % support. With nematode NCX being an outgroup, all remaining NCKX genes clearly fall into two very closely related groups (bootstrap supports 100 % and 95 %). The first group includes the rod NCKX1 and the cone NCKX2 (SLC24A2), each 100 % supported. All four known NCKX1 genes (mouse, human, cow, and dolphin) group together (100 % support). All four known NCKX2 genes (chicken, mouse, rat, and human) also group together (100 % support). The second group includes a clade of very closely related human NCKX3 and NCKX4 (99 % support), and a sister clade of human NCKX6 and sea urchin NCKX (88 % support). The human NCKX5 stands alone within the second group without close clustering to any other genes (bootstrap support 57 %). The identical tree topology was revealed by the Neighbor Joining analysis.

SISOUSSION

In this study, we refined the genomic structure of Na^{+}/Ca^{2+}–K exchanger gene (NCKX) of cone photoreceptor (NCKX2) not determined previously by Sharon et al. (2002) (Table 2, Fig. 2). The NCKX2 gene has an unusually long exon II, length of 992 bp that contains the translational start site and codes for more than half of the protein, a property also found in NCKX1 and NCKX3 genes (Sharon et al., 2002). The size difference between NCKX genes makes a comparison of their overall identity difficult to interpret because the sequences coding for the two large hydrophilic loops are of a considerably different length and are quite variable, even when comparing rod NCKX sequences in mammals. The NCKX2 protein has a high degree of sequence identity with NCKX1 in the hydrophobic regions and shares a significant functional similarity as well (Sheng et al., 2000; Dong et al., 2001). Similarly, NCKX3 and NCKX4 genes also share virtually identical exon boundaries whereas the two pairs have arrangements quite different from one another. This pattern similarity/dissimilarity seen for exon boundaries is

also evident in amino acid identity between proteins, which is about 60 % within each pair (NCKX1/ NCKX2 and NCKX3/NCKX4) and only 35 % between pairs. These comparisons suggest that the four members of the NCKX branch of the Na^+/Ca^{2+}–K exchanger gene superfamily have arisen from two distant, sequential, symmetrical duplication events. NCKX4 is most closely related to NCKX3, sharing an overall amino acid identity of 60% that increases to 80 % within the hydrophobic regions, as well as addicted extracellular C-terminus (Li et al., 2002). NCKX6, which is similar to NCKX3 and NCKX4 in gene arrangement, does not have the unusually long coding exon found as a conserved feature in the NCKX1, NCKX2, NCKX3 and NCKX4 genes. However, the exon boundaries in NCKX6 do not match those of NCKX3 and NCKX4.

In our phylogeny, which is based on entire known amino acid sequences, we show that all known vertebrate NCKX genes clearly fall into two groups. The cone NCKX2 is very closely related to the rod NCKX1. All four known NCKX2 genes group together, as well as all four known (mammalian only) NCKX1 genes. The more distantly related sister group includes a very closely knit branch of human NCKX3 and NCKX4, as well as human NCKX6 (with closely related sea urchin NCKX) and the human NCKX5.

Comparison of these genes offers a possibility to rebuild and reinterpret a conceptual framework for the data. In the previously published data, regions containing the two clusters of hydrophobic domains, corresponding to amino acids 84-249 and 804-955 of human NCKX1 were used for further sequence alignment and phylogenetic analysis (Cai and Lytton, 2004a, b). When sequence comparison is limited to the proposed transmembrane spanning segments for all vertebrate NCKX described to date, NCKX genes fall clearly into two groups: the rod NCKX, and the cone and brain NCKX. In the cation/Ca^{2+} exchanger superfamily phylogenetic comparison, two absolutely conserved acidic residues were at positions 65 and 246 (one in each of two !-repeats). Such two key acidic residues could be involved in neutralizing the two positive charges on Ca^{2+}, and thus help to overcome the energy barrier required to transport a divalent cation.

The previously published phylogeny of the NCKX family showed that two distant and sequential gene replication events gave rise to the branches of the NCKX family: one branch containing NCKX1 and NCKX2, another branch consisting of NCKX3, NCKX4, and a third branch with NCKX6 (Cai and Lytton, 2004a, b).

In our phylogenetic tree (Fig. 4), the cone photoreceptor NCKX2 gene forms a sister group to the rod NCKX1 while NCKX3 and NCKX4 form another branch, clearly joining NCKX5 and NCKX6. Thus, in our opinion, NCKX3-NCKX6 genes are not closely related to NCKX1 and NCKX2.

In our opinion, the NCKX genes, including NCKX2, could be also useful for studies of mammalian evolution. Our genetic distance matrix based on mRNA sequences (Table 3) shows 12.6-12.7% overall sequence distance in NCKX2 between primates and rodents and ca. 8% sequence distance within rodents (rat and mouse; Table 3). This makes the cone photoreceptor NCKX2 (and other NCKX genes) another good evolutionary marker for order- and family-level phylogenetic analysis in mammals. Note that the NCKX1 clade of the tree (Fig. 4), although based only on a few species, recovers with strong support (88%) the currently well-confirmed close phylogenetic relationship between the orders Cetacea (dolphin) and Artiodactyla (cow), as opposed to primates and rodents.

In the patients with a cone dysfunction or degeneration the serious mutations in NCKX2 gene could be connected to color vision mechanism (Prinsen et al., 2000). NCKX2 sequence contains eleven hydrophobic segments (1-11) as well as a hydrophobic segment at the N terminus (0), that functions as a cleavable signal peptide. The arrangement of hydrophobic segments contains two clusters of five transmembrane spanning helices each (Fig 3; Kang et al., 2005). Sharon et al. (2002) found that six mutations affected residues in transmembrane domains H5, H6, H8, H10 and three of six possibly pathogenic mutations were missense changes located in conserved regions. However, the rod and cone NCKX genes were analyzed for the mutations in patients with hereditary retinal disease, and pathogenicity could not be established with certainty. There is no reported evidence of patients whose disease can be completely explained by cone exchanger mutations. Failure to find patients whose disease can be completely explained by rod or cone exchanger mutations may be due to a very low frequency of exchanger mutations.

However, defects in photoreceptor development and function are the major cause of inherited retinal degenerative diseases, which constitute a clinically and genetically heterogeneous group (RetNet; http://www.sph.uth.tmc.edu/Retnet/home.htm). Winkfein et

al. (2002) conducted scanning mutagenesis of the core of the two sets of TM's (H2-3 and H8-9) of NCKX2 that make up the !-1 and !-2 repeats that contain the only sequence elements conserved between the Na^+/Ca^{2+} and Na^+/Ca^{2+}–K gene families. The four of five residues located within membrane spanning helices H2 (E188), H3 (D258), and H8-H9 (D548, and D575), constitute the key residues involved in Ca^{2+} binding and transport (Winkfein et al., 2002; Kang et al., 2005; Kinjo et al., 2005).

In a study of the evolution of vertebrate color vision, it would be highly desirable to work with the transmembrane domain H0-H1 of NCKX2 gene. Comparison of conserved sequences (Fig. 3) confirmed high similarity of chicken and human NCKX2 genes in H0-H1 domains. In these two cone-dominated vertebrates isoleucine[49] and serine[53] are the conserved amino acids in H0 domain. In rod-dominated mammals, such as mouse and rat, a region encoding transmembrane domain H0 in the NCKX2 gene is modified identically. Our data indicate that the NCKX2 gene in rod-dominated animals should not be used as a model for studies of disease pathogenesis and the evaluation of treatment strategies in humans. To understand how primates use visual signals, it is interesting to compare primates to other cone-dominated animals. One approach to delineate mechanisms of disease pathogenesis involves the generation and characterization of animal models. Many of the genes found to be mutant in animals that result in a retinal degeneration (RD) have been found to be similarly abnormal in the human. The chicken has the advantage of having a relatively large proportion of cone photoreceptor cells, somewhat similar to that the human retina. There are no exact models for many of the rare forms of RD. Small inexpensive animal models can be useful in the early phases of investigation where larger numbers of subjects are generally needed (Chader, 2002). The possible connection of transmembrane domains H0 and H1 in NCKX2 protein to color vision mechanism has to be studied in a new candidate animal model.

ACKNOWLEDGMENTS

We thank the Department of Biological Sciences of Marshall University (Huntington, West Virginia, USA) for support of our studies. V.F. was a host for a visiting Fulbright research fellowship No. 24597 for T.L. at Marshall University.

REFERENCES

Ahnelt, P.K., Kolb, H., 2000. The mammalian photoreceptor mosaic-adaptive design. Prog. Retin. Eye Res. 19(6), 711-777.

Cai, X., Lytton, J., 2004a. The cation/Ca(2+) exchanger superfamily: phylogenetic analysis and structural implications. Mol. Biol. Evol. 21, 1692-1703.

Cai, X., Lytton, J., 2004b. Molecular cloning of a sixth member of the K+ -dependent Na+/Ca2+ exchanger gene family, NCKX6. J. Biol. Chem. 279, 5867-5876.

Chader, G.J., 2002. Animal models in research on retinal degenerations: past progress and future hope. Vision Res. 42(4), 393-399.

Chadwick, B.P., Campbell, L.J., Jackson, C.L., Ozelius, L., Slaugenhaupt, S.A., Stephenson, D.A., Edwards, J.H., Wiest, J., Povey, S., 1999. Report on Sixth International Workshop on Chromosome 9. Ann. Hum. Genet. 63, 101-117.

Cooper, C.B., Winkfein, R.J., Szerencsei, R.T., Schnetkamp, P.P., 1999. cDNA cloning and functional expression of the dolphin retinal rod Na-Ca+K exchanger NCKX1: comparison with the functionally silent bovine NCKX1. Biochemistry 38, 6276-6283.

Dong, H., Light, P.E., French, R.J., Lytton, J., 2001. Electrophysiological characterization and ionic stoichiometry of the rat brain K(+)-dependent Na(+)/Ca(2+) exchanger, NCKX2. J. Biol. Chem. 276, 25919-25928.

Kang, K., Schnetkamp, P.P., 2003. Signal sequence cleavage and plasma membrane targeting of the retinal rod NCKX1 and cone NCKX2 Na+/Ca2+-K+ exchangers. Biochemistry 42, 9438-9445.

Kinjo, T.G., Szerencsei, R.T., Winkfein, R.J., Kang, K., Schnetkamp, P.P., 2003. Topology of the retinal cone NCKX2 Na/Ca-K exchanger. Biochemistry 42, 2485-2491.

Knuutila, S., Aalto, Y., Autio, K., Bjorkqvist, A.M., El-Rifai, W., Hemmer, S., Huhta, T., Kettunen, E., Kiuru-Kuhlefelt, S., Larramendy, M.L., Lushnikova, T., Monni, O., Pere, H., Tapper, J., Tarkkanen, M., Varis, A., Wasenius, V.M., Wolf, M., Zhu, Y., 1999. DNA copy number losses in human neoplasms. Am. J. Pathol. 155, 683-694.

Kraev, A., MacLennan, D., 2002. Toward comparative genomics of calcium transporters. Ann. N Y Acad. Sci. 976, 63-59.

Kraev, A., Quednau, B.D., Leach, S., Li, X.F., Dong, H., Winkfein, R., Perizzolo, M., Cai, X., Yang, R., Philipson, K.D., Lytton, J., 2001. Molecular cloning of a third member of the potassium-dependent sodium-calcium exchanger gene family, NCKX3. J. Biol. Chem. 276, 23161-23172.

Li, X.F., Kraev, A.S., Lytton, J., 2002. Molecular cloning of a fourth member of the potassium-dependent sodium-calcium exchanger gene family, NCKX4. J. Biol. Chem. 277, 48410-48417.

Nicoll, D.A., Hryshko, L.V., Matsuoka, S., Frank, J.S., Philipson, K.D., 1996. Mutation of amino acid residues in the putative transmembrane segments of the cardiac sarcolemmal Na+-Ca2+ exchanger. J. Biol. Chem. 271(23), 13385-13391.

Page, R.D.M., 2001. TreeView 1.6.6. Glasgow, Scotland.

Pata, I., Hoth, S., Kruppa, J., Metspalu, A., 1992. The human ribosomal protein S6 gene: isolation, primary structure and localization in chromosome 9. Gene 121, 387-392. Philipson, K.D., Nicoll, D.A., Ottolia, M., Quednau, B.D., Reuter, H., John, S., Qiu, Z., 2002. The Na+/Ca2+ exchange molecule: an overview. Ann. N Y Acad. Sci. 976, 1-10.

Povey, S., Attwood, J., Chadwick, B., Frezal, J., Haines, J.L., Knowles, M., Kwiatkowski, D.J., Olopade, O.I., Slaugenhaupt, S., Spurr, N.K., Smith, M., Steel, K., White, J.A., Pericak-Vange, M.A., 1997. Report on the Fifth International Workshop on Chromosome 9. Ann. Hum. Genet. 61, 183-206.

Prinsen, C.F., Szerencsei, R.T., Schnetkamp, P.P., 2000. Molecular cloning and functional expression of the potassium-dependent sodium-calcium exchanger from human and chicken retinal cone photoreceptors. J. Neurosci. 20, 1424-1434.

Reigo, A., Lushnikova, T., Laanpere, I., Ilisson, R., Metspalu, A., 2000. Refined mapping of human chromosomal region 9p22 distal to the interferon gene cluster and construction of a transcript map of 1.2 Mb surrounding RP S6 gene. Program Nr: 2364 www.faseb.org/genetics/ashg99/f2364.htm.

Schnetkamp, P.P., 2004. The SLC24 Na+/Ca2+ -K+ echnager family: Vision and beyond. Pflugers Arch. 447, 683-688.

Schwarz, E.M., Benzer, S., 1997. Calx, a Na-Ca exchanger of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA 94, 10249-10254.

Sharon, D., Yamamoto, H., McGee, T.L., Rabe, V., Szerencsei, R.T., Winkfein, R.J., Prinsen, C.F., Barnes, C.S., Andreasson, S., Fishman, G.A., Schnetkamp, P.P., Berson, E.L., Dryja, T.P., 2002. Mutated alleles of the rod and cone Na-Ca+K-exchanger genes in patients with retinal diseases. Invest. Ophthalmol. Vis. Sci. 43, 1971-1979.

Sharpe, L.T., Stockman, A., 1999. Rod pathways: the importance of seeing nothing. Trends Neurosci. 22(11), 497-504.

Sheng, J.Z., Prinsen, C.F., Clark, R.B., Giles, W.R., Schnetkamp, P.P., 2000. Na(+)-Ca(2+)-K(+) currents measured in insect cells transfected with the retinal cone or rod Na(+)-Ca(2+)-K(+) exchanger cDNA. Biophys. J. 79, 1945-1953.U S A. 1997 Sep 16;94(19):10249-54.

Swofford, D.L., 1998. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0. Sinauer Associates, Sunderland, Mass.

Szerencsei, R.T., Winkfein, R.J., Cooper, C.B., Prinsen, C., Kinjo, T.G., Kang, K., Schnetkamp, P.P., 2002. The Na/Ca-K exchanger gene family. Ann. N Y Acad. Sci. 976, 41-52.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 24, 4876–4882.

Tucker, J.E., Winkfein, R.J., Murthy, S.K., Friedman, J.S., Walter, M.A., Demetrick, D.J., Schnetkamp, P.P.M., 1998. Chromosomal localization and genomic organization of the human retinal rod Na-Ca+K exchanger. Hum. Gen. 103, 411-414.

Winkfein, R.J., Szerencsei, R.T., Kinjo, T.G., Kang, K.J., Perizzolo, M., Eisner, L., Schnetkamp, P.P.M., 2003. Scanning mutagenesis of the alpha repeats and of the transmembrane acidic

residues of the human retinal cone Na/Ca-K exchanger. Biochemistry 42, 543-552.

TABLE 1. A list of known genes of the NCKX family (*Caenorhabditis* NCX4 used as an outgroup).

Species	gene name	accession number	map position	gene size, Kb	mRNA size, bp	Reference
Caenorhabditis elegans	NCX4	NM_060203			2067	GenBank
Strongylocentrotus	NCKX	NM_214625			3673	Su and Vacquier (2002)
Gallus gallus	NCKX2	AF177986			2182	Prinsen et al. (2000)
Rattus norvegicus	NCKX2	NM_031743			8942	Tsoi et al. (1998)
Mus musculus	NCKX1	NM_144813			5244	Strausberg, R. (unpublished, GenBank)
Mus musculus	NCKX2	NM_172426			3830	Tsoi et al. (1998)
Homo sapiens	NCKX1	AF062922	15q22		5374	Tucker et al. (1998)
Homo sapiens	NCKX2	AF097366	9p23	335	2221	Prinsen et al. (2002)
Homo sapiens	NCKX3	AF169257	20p13	510	3905	Kraev et al. (2001)
Homo sapiens	NCKX4	NM_153648	14q32.13	174	4169	Li et al. (2002)
Homo sapiens	NCKX5	NM_205850			1617	Cai and Lytton (2002)
Homo sapiens	NCKX6	NM_024959			2993	Cai and Lytton (2002)
Bos taurus	NCKX1	X66481			3906	Reilander et al. (1992)
Tursiops truncatus	NCKXr	AF059031			3312	Cooper et al. (1999)

TABLE 2. Human Na-Ca+K- exchanger gene for retinal cones, NCKX2 (SLCA242). Original exon-intron structure annotated using advanced BLAST search and data from Sharon et al. (2002)*

exon	exon size, bp	BAC clone position, bp	intron size, bp
1	154	RACE*	1,700
2	992	RP11-471J7, 33669-32678	158,000
3	41	RP11-25202, 25739-25698	2,600
4	109	RP11-25202, 23022-23131	22,000
5	53	RP11-25202, 719-666	19,000
6	100	RP11-363E7, 143904-143804	3,400
7	119	RP11-363E7, 140350-140231	23,000
8	134	RP11-363E7, 117151-117017	22,000
9	91	RP11-363E7, 95018-94927	70,000
10	169	RP11-363E7, 87942-87773	4,500
11	250 + >7,000 noncoding	RP11-363E7, 83283-82860 RACE *	

TABLE 3. Nucleotide distance matrix for NCKX family mRNAs (see Table 2).

Uncorrected ("p") distance matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Mus NCKX2	-												
2 Rattus NCKX2	0.073	-											
3 Homo NCKX2	0.126	0.127	-										
4 Gallus NCKX2	0.287	0.290	0.281	-									
5 Caenorhab_N	0.472	0.472	0.478	0.460	-								
6 Bos NCKX1	0.453	0.444	0.428	0.424	0.505	-							
7 Tursiops_NCKXr	0.460	0.446	0.431	0.430	0.496	0.107	-						
8 Mus NCKX1	0.552	0.552	0.432	0.423	0.521	0.223	0.219	-					
9 Homo NCKX1	0.559	0.556	0.440	0.436	0.524	0.169	0.158	0.309	-				
10 Homo NCKX4	0.510	0.501	0.513	0.507	0.551	0.532	0.518	0.529	0.524	-			
11 Homo NCKX3	0.613	0.610	0.521	0.517	0.573	0.529	0.526	0.620	0.628	0.322	-		
12 Homo NCKX5	0.551	0.544	0.544	0.546	0.536	0.537	0.551	0.548	0.533	0.513	0.527	-	
13 Strongyl	0.620	0.619	0.574	0.564	0.616	0.584	0.577	0.642	0.637	0.547	0.629	0.595	-
14 Homo NCKX6	0.639	0.638	0.623	0.625	0.668	0.646	0.634	0.668	0.656	0.648	0.660	0.675	0.672

Figure Legends:

Figure 1. Physical map of human chromosome 9p23 around NCKX2 (=SLC24A2) gene.

Figure 2. Exon-intron structure of human NCKX2 (=SLC24A2) gene.

Figure 3. Topology of NCKX2 and location of transmembrane domains (after Kang et al., 2005). NCKX2 sequence contains eleven hydrophobic segments (1-11), as well as a hydrophobic segment at the N terminus (0), that functions as a cleavable signal peptide. The arrangement of hydrophobic segments contains two clusters of five transmembrane-spanning helices each.

Figure 4. Phylogenetic tree of known NCKX genes based on amino acid sequences. Numbers under the branches indicate values of bootstrap support.

Figure 5. Alignment of human retinal cone NCKX2 amino acid sequence with 13 selected transmembrane domains (H0, H1-H3, H8-H9) of NCKX1-NCKX6 proteins from six vertebrate species and NCX/NCKX from two invertebrate species. The putative transmembrane segments are underlined. Black boxes indicate identity to human NCKX2 in at least two proteins; gray boxes, an amino acid substitution. The !-repeat motifs as originally identified by Schwartz and Benzer (1997) are doubly underlined. Bold italics indicate critical residues in !-repeats which show <20% of wild-type activity when mutated (Winkfein et al., 2003)

Figure 1

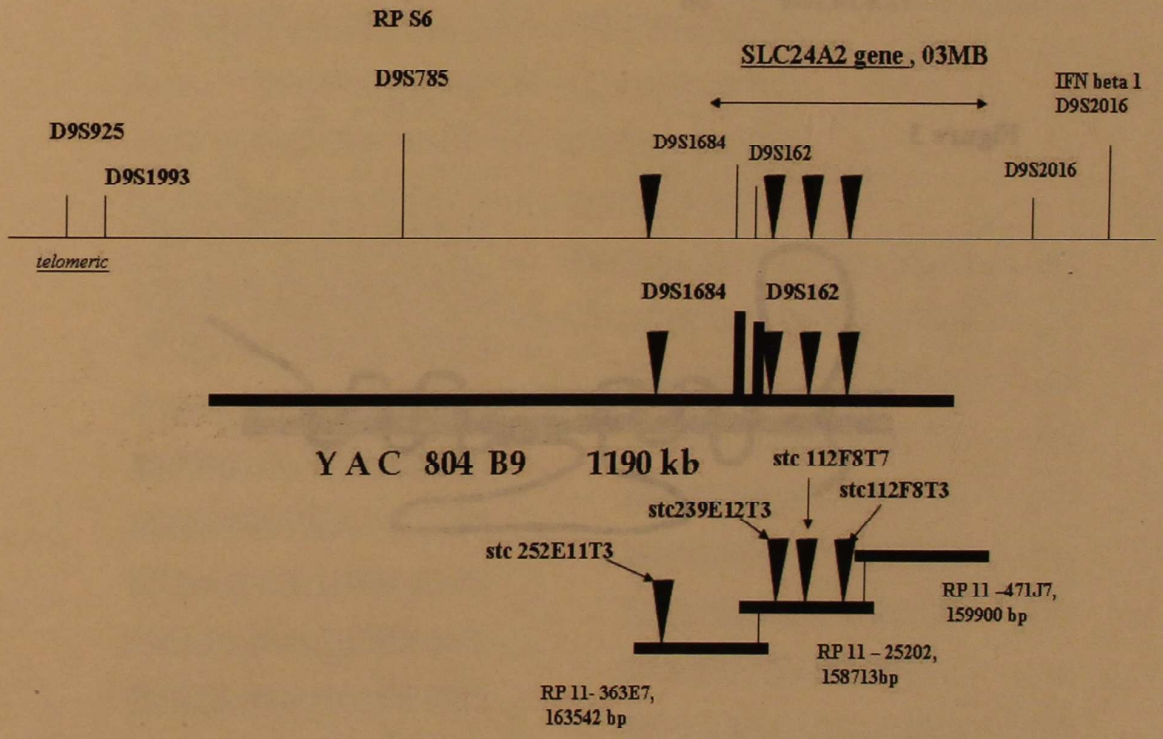


Figure 2

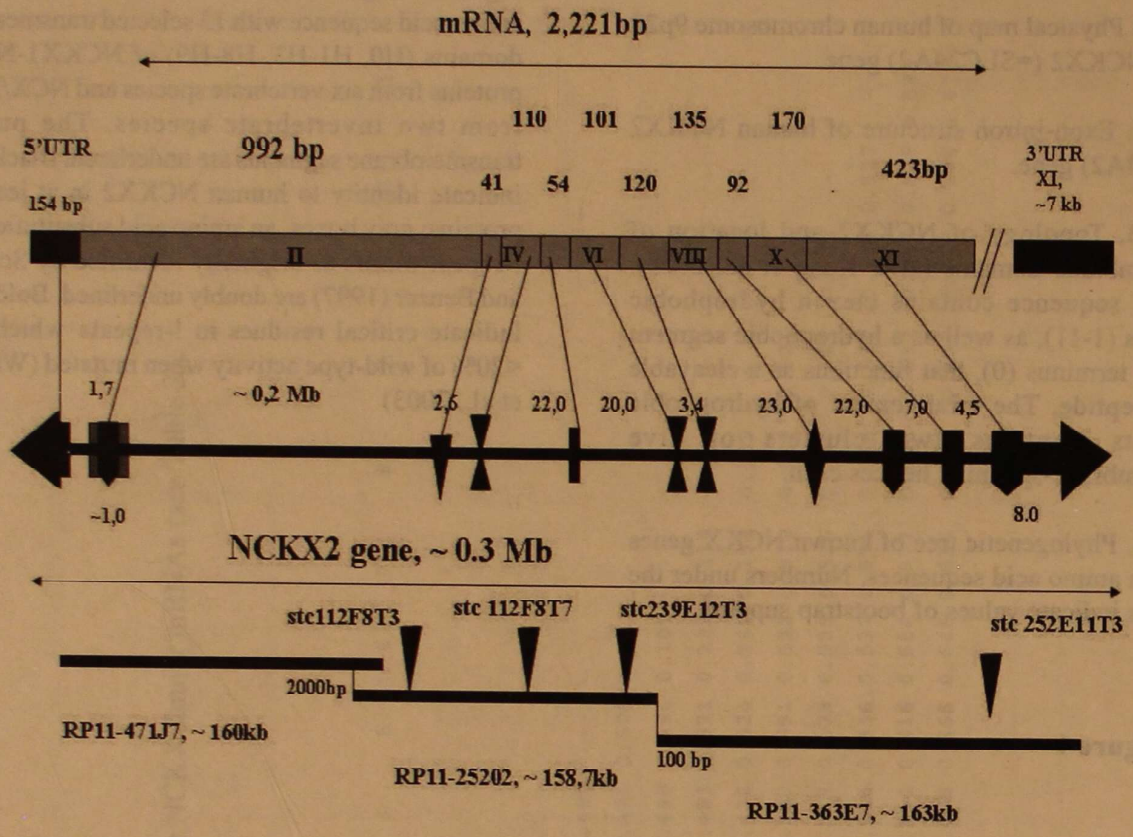


Figure 3

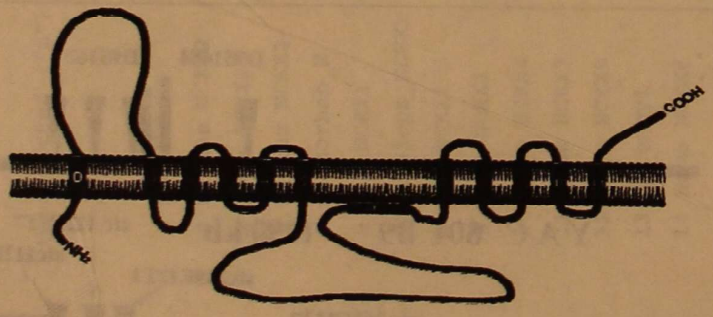


Figure 4

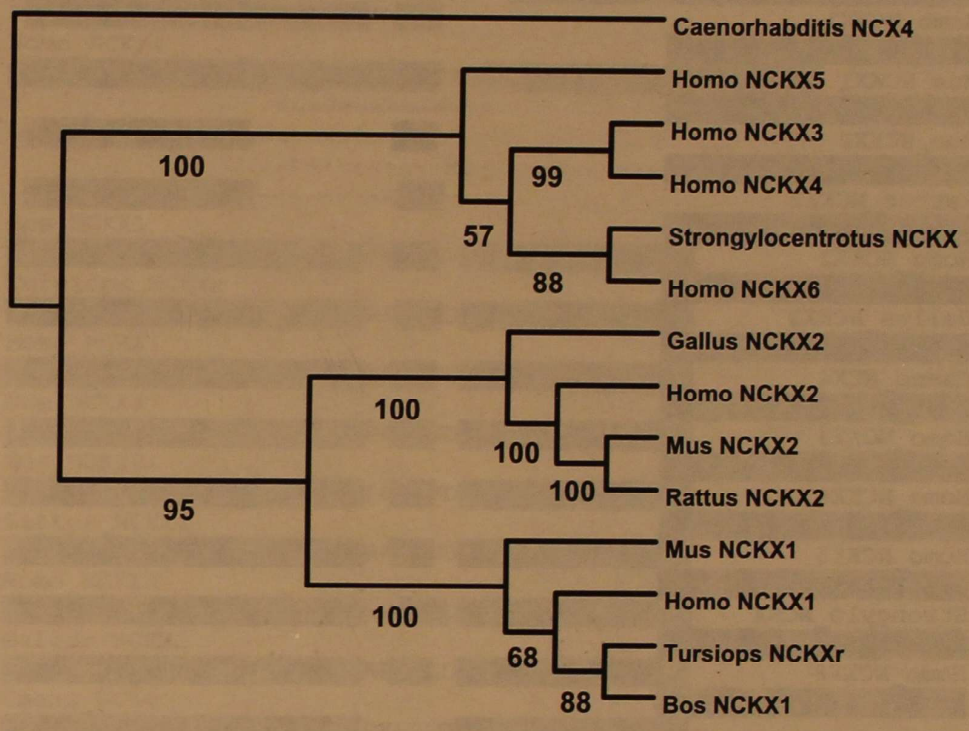
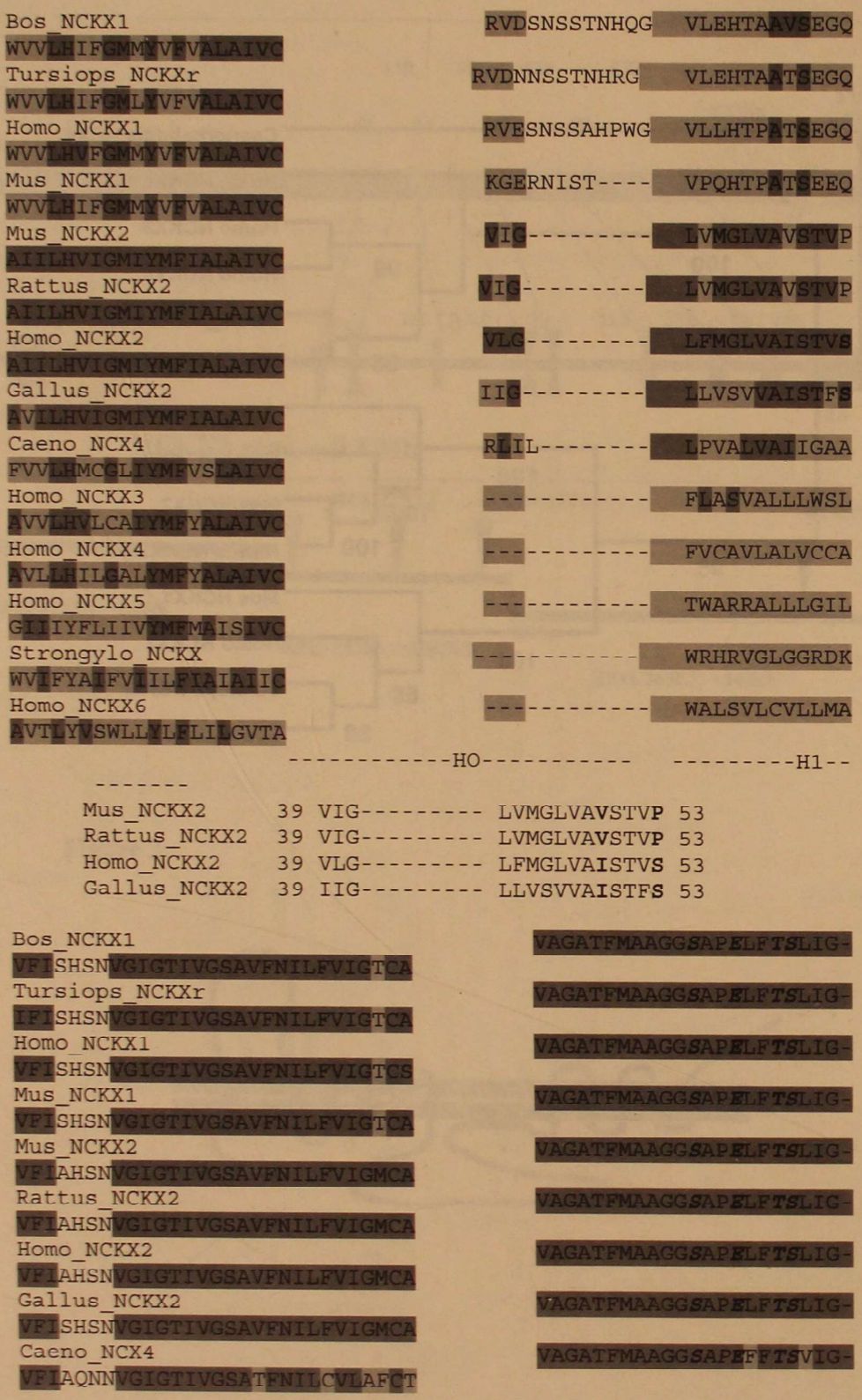

Caenorhabditis NCX4
Homo NCKX5
Homo NCKX3
Homo NCKX4
Strongylocentrotus NCKX
Homo NCKX6
Gallus NCKX2
Homo NCKX2
Mus NCKX2
Rattus NCKX2
Mus NCKX1
Homo NCKX1
Tursiops NCKXr
Bos NCKX1
100
99
57
88
100
100
100
95
100
68
88

Figure 5


Bos_NCKX1
RVDSNSSTNHQG VLEHTAAVSEGQ
WVVLHIFGMMYVFVALAIVC
Tursiops_NCKXr
RVDNNSSTNHRG VLEHTAATSEGQ
WVVLHIFGMLYVFVALAIVC
Homo_NCKX1
RVESNSSAHPWG VLLHTPATSEGQ
WVVLHVFGMMYVFVALAIVC
Mus_NCKX1
KGERNIST---- VPQHTPATSEEQ
WVVLHIFGMMYVFVALAIVC
Mus_NCKX2
VIG--------- LVMGLVAVSTVP
AIILHVIGMIYMFIALAIVC
Rattus_NCKX2
VIG--------- LVMGLVAVSTVP
AIILHVIGMIYMFIALAIVC
Homo_NCKX2
VLG--------- LFMGLVAISTVS
AIILHVIGMIYMFIALAIVC
Gallus_NCKX2
IIG--------- LLVSVVAISTFS
AVILHVIGMIYMFIALAIVC
Caeno_NCX4
RLIL-------- LPVALVAIIGAA
FVVLHMCGLIYMFVSLAIVC
Homo_NCKX3
---------- FLASVALLLWSL
AVVLHVLCAIYMFYALAIVC
Homo_NCKX4
---------- FVCAVLALVCCA
AVLLHILGALYMFYALAIVC
Homo_NCKX5
---------- TWARRALLLGIL
GIIIYFLIIVYMFMAISIVC
Strongylo_NCKX
---------- WRHRVGLGGRDK
WVIFYAIFVIILFIAIAIIC
Homo_NCKX6
---------- WALSVLCVLLMA
AVTLYVSWLLYLFLILGVTA
----------HO---------- ---------H1--
Mus_NCKX2 39 VIG--------- LVMGLVAVSTVP 53
Rattus_NCKX2 39 VIG--------- LVMGLVAVSTVP 53
Homo_NCKX2 39 VLG--------- LFMGLVAISTVS 53
Gallus_NCKX2 39 IIG--------- LLVSVVAISTFS 53
Bos_NCKX1
VAGATFMAAGGSAPELFTSLIG-
VFISHSNVGIGTIVGSAVFNILFVIGTCA
Tursiops_NCKXr
VAGATFMAAGGSAPELFTSLIG-
IFISHSNVGIGTIVGSAVFNILFVIGTCA
Homo_NCKX1
VAGATFMAAGGSAPELFTSLIG-
VFISHSNVGIGTIVGSAVFNILFVIGTCS
Mus_NCKX1
VAGATFMAAGGSAPELFTSLIG-
VFISHSNVGIGTIVGSAVFNILFVIGTCA
Mus_NCKX2
VAGATFMAAGGSAPELFTSLIG-
VFIAHSNVGIGTIVGSAVFNILFVIGMCA
Rattus_NCKX2
VAGATFMAAGGSAPELFTSLIG-
VFIAHSNVGIGTIVGSAVFNILFVIGMCA
Homo_NCKX2
VAGATFMAAGGSAPELFTSLIG-
VFIAHSNVGIGTIVGSAVFNILFVIGMCA
Gallus_NCKX2
VAGATFMAAGGSAPELFTSLIG-
VFISHSNVGIGTIVGSAVFNILFVIGMCA
Caeno_NCX4
VAGATFMAAGGSAPEFFTSVIG-
VFIAQNNVGIGTIVGSATFNILCVLAFCT

```
Homo_NCKX3                                    VAGATFMAAGSSAPELFTSVIG-
VFITKGDVGVGTIVGSAVFNILCIIGVCG
Homo_NCKX4                                    VAGATFMAAGSSTPELFASVIG-
VFITHGDVGVGTIVGSAVFNILCIIGVCG
Homo_NCKX5                                    VAGTTFMAAGSSAPELVTAFLG-
VFITKGDIGISTILGSAIYNLLGICAACG
Strongylo_NCKX                          VAGATFMAAGSSAPELFTAVIG-VAFES-
DVGVGTIVGSAVFNILIIIALTA
Homo_NCKX6
VAGVTFLAFGNGAPDIFSALVAFSDPHTAGLALGALFGAGVLVTTVVAGGIT
                  ==============          α1-          repeat
=========================
                  -----------H2------------      ----------H3-
---------
Bos_NCKX1
IMGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDIT
Tursiops_NCKXr
IMGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDIT
Homo_NCKX1
IMGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDIT
Mus_NCKX1
IMGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDIT
Mus_NCKX2
IMGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDIT
Rattus_NCKX2
IMGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDIT
Homo_NCKX2
IMGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDIT
Gallus_NCKX2
IMGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDIT
Caeno_NCX4
IIGLTILAAGTSIPDLITSVIVARKGLGDMAVSSSVGSNIFDVC
Homo_NCKX3
IMGITFLAAGTSVPDCMASLIVARQGMGDMAVSNSIGSNVFDIL
Homo_NCKX4
IMGITFLAAGTSVPDCMASLIVARQGLGDMAVSNTIGSNVFDIL
Homo_NCKX5
VMGLTLLAAGTSIPDTIASVLVARKGKGDMAMSNIVGSNVFDML
Strongylo_NCKX
TMGLVIVAVGTSVPDALSSILVARDGYGDMAVSNAIGSNVFDIN
Homo_NCKX6
VLGLTLLAWGNSIGDAFSDFTLARQGYPRMAFSACFGGIIFNIL
                  ========== α2- repeat ====================
```

STUDENTS' SEX ROLE PERCEPTIONS OF THEMSELVES AND SCIENTISTS

Ramsey Miller, Daniel Felbaum, and Debra Hull
Wheeling Jesuit University

ABSTRACT

Undergraduate students completed the Bem Sex Role Inventory twice—once describing themselves and once describing a typical scientist—in a reverse counterbalancing sequence. The inventory measures the degree to which one expresses personality traits characteristic of a particular gender role—either masculine, near masculine, androgynous, near feminine, or feminine. The students' Bem scores for their perceptions of themselves and a typical scientist were compared. Results showed that, on average, students rated themselves in the androgynous range, but rated scientists in the masculine range. Students perceive the typical scientist to have significantly more stereotypically masculine characteristics (such as being independent, assertive, forceful, analytical, dominant, individualistic, competitive, and ambitious) than they perceive themselves as having. The difference between self and scientist ratings was much larger for women than for men. These results may help explain why historically, relatively few students, especially women students, have chosen to pursue careers in science.

Students' Gender Role Perceptions of Themselves and Scientists

For a number of years, psychologists have been concerned about public perceptions of and attitudes towards those working in the profession. Webb and Speer (1986) report that in his 1982 presidential address to APA, William Bevan voiced his concerns about the way the public understands the science of psychology, saying that the public is ignorant and distrustful toward science in general. Subsequently, four candidates for the APA presidency felt that psychology's public image was a major issue for the profession.

However, in their research, Webb and Speer (1986) found generally positive attitudes towards psychologists. They compared six professions– psychiatrist, physician, counselor, teacher, psychologist, and scientist– to determine what qualities the public attributed to each profession. Their goal was to see if psychologists were viewed negatively. Using a prototype model, the researchers asked a group of undergraduate students to write a descriptive paragraph about typical members of these professions. A second group of undergraduates read the paragraphs and grouped the descriptions by common characteristics. A third and fourth group rated people in the six professions based on the characteristics they believed a person in that field would have. When compared to the other five professions, psychologists were rated above the means on rich, patient, inquisitive, understanding, psychological, and helpful. Except for inquisitiveness, which might be considered a trait of scientists, the other characteristics appear more related to the practice of psychology than to the science of psychology.

Another line of research looks at the perceptions people hold of scientists. Constantinos and Elena Papanastasiou (2004) thought that studying youth's perceptions of scientists was important because of the potential for students' attitudes to influence their educational achievement. They believed that a more positive attitude toward science could increase achievement in a science classroom. The researchers gave participants questionnaires that asked them to indicate abilities they thought were necessary to succeed in science, to describe what motivates them to do well in science, and to rate their overall attitude towards science. Results showed that the biggest influence on attitudes toward science were teachers. They concluded that negative attitudes about science might be diminished if science teachers and their methods were positive and encouraging.

In a cross-cultural study, Yvonne Fung (2002) assessed the perceptions of Hong Kong children towards scientists by using the Draw-A-Scientist Test, which calls for children to draw a scientist. Many of the children depicted the scientist as a cartoon figure or a figure that resembled an alien. Some drew scientists as robots or monsters. Sixty percent of the children drew the scientist as a man and only fourteen percent (all of whom were girls) drew the scientist as a woman. Many of the drawings included glasses, a lab coat, or lab equipment. Overall, the perceptions that the children had about scientists seemed to be very similar, negative, and stereotypical.

To test the question of whether parents stereotype science as gender-specific, Tenenbaum and Leaper (2003) interviewed families, then asked them to perform science-related tasks. The researchers found evidence that parents tended to think that their sons were more interested in science than their daughters. The parents treated their children differently—using more science-related terms while performing the tasks with sons than with daughters and acting as if sons were better able to comprehend science than daughters were.

In a study designed to elicit more positive attitudes towards scientists from children, Palmer (1997) found that children aged six and ten describe a scientist as a man of middle to old age who is usually bald or has messy hair and a beard. He is imagined to be wearing a lab coat and glasses. When he asked children if scientists could do anything about endangered species, he found that most of the students believed that a scientist could do something—showing that children also have a positive views about scientists, at least when asked directly.

Our study was designed to determine the way that college students rate themselves on a gender role scale compared to the way that they rate a typical scientist. We hypothesized that students would rate a scientist as more masculine than they would rate themselves. To take this one step further, we also wanted to compare the ratings given by men and women. We expected that the difference in ratings between students and their view of a typical scientist would be much larger for women than for men.

Methods

Participants

Thirty-nine students (18 females and 21 males) from a small liberal arts university took part in the study. The mean age for the participants was 20, and a convenience sample was used. Each participant received one extra credit participation point and 5 dollars. Participation in the experiment took approximately 10 minutes. The participants took the surveys in groups according to their availability, yet the surveys were completed individually without any discussion. The study met the requirements of the IRB.

Materials

Participants used the Bem Sex Role Inventory (BSRI), a test created by Sandra Bem that measures both femininity and masculinity (Bem, 1974). Because the BSRI was created over thirty years ago, Cheryl Holt conducted a study in 1998 to test the current validity of the BSRI. She used the same method that Bem used to test the descriptors, surveying the public, and found that all but two of the 60 descriptors were still valid in measuring masculinity and femininity (Holt, 1998).

The BSRI contains 60 descriptors; 20 are feminine, 20 are masculine, and 20 are neutral filler items. The participants rate themselves on those characteristics using a seven-point scale, ranging from never or almost never true to almost always or always true. Based on their scores, participants are categorized as near feminine, feminine, androgynous, near masculine, or masculine.

Procedure

The study was a 2x2 mixed design with perceptions (of self and scientists) and gender being the independent variables. The experimenter described the general procedure to all the participants in the room and handed out the BSRI to each. Participants were told to rate themselves on one sheet and to rate the typical scientist on the other sheet. The BSRI tests were handed out in two different orders; some participants received the self rating sheet first and then the scientist rating sheet; others received the reverse order, scientist and then self. The study was set up to measure the effects of perceptions (self and scientist) and gender on the Bem Sex Role Inventory scores.

Results

The data are scores on the Bem Sex Role Inventory. The design is mixed, with perceptions of self and scientist being the within factor and gender being the between factor. Scores were analyzed using a mixed design ANOVA. The results showed a significant main effect for the ratings of self and scientist, $F(1, 37) = 18.69$, $p = 0.000$. Scientists were rated with significantly more masculine traits than were the ratings of self. (M for self rating = -0.74, $SD = 3.15$; M for scientist rating = -3.32, $SD = 2.60$). There was also a significant interaction between the ratings given to self and scientist and the gender of the participant, $F(1, 37) = 5.30$, $p = 0.027$. Newman-Keuls tests showed that men rated scientists significantly more masculine than they rated themselves. Women also rated scientists significantly more masculine than they rated themselves. Moreover, men rated themselves significantly more masculine than women rated themselves. Therefore, women gave

more extreme ratings for self and scientist than men did. There was no significant difference in the way men and women rated scientists. (M for self ratings by men = -1.77, SD = 3.09; M for self ratings by women = 0.46, SD = 2.83; M for scientist ratings by men = -3.03, SD = 2.65; M for scientist ratings by women = -3.67, SD = 2.57). The interaction is illustrated in Figure 1.

Discussion

A significant difference was found for the ratings of self and scientist on the Bem Sex Role Inventory. The results show that participants perceived scientists to have more masculine traits than they perceived themselves to have. A significant interaction effect was also found. The difference between the scientist and self masculinity ratings were greater for women than for men. For women, the difference was 4.13, whereas the difference between the average ratings of scientist and self for men was only 1.26. Women saw themselves as having an average rating of 0.46, which is androgynous, and saw a typical scientist as having an average rating of -3.67, which is strongly masculine. The difference between self and scientist for men was not so large. Men gave themselves an average rating of -1.77, which is in the near masculine category, and they gave a typical scientist an average rating of -3.03, which is strongly masculine. Men rate themselves as much closer to the way they rate scientists than women do. No overall gender main effect was found. That is, the overall ratings on the BSRI did not differ depending on whether the participant was a man or woman.

Previous research reviewed in the introduction to this paper clearly indicates that children perceive scientists to be men and/or masculine, and that these perceptions are influenced by parents and teachers. The college students in our study continue to exhibit these stereotypical views of scientists. What is most interesting to us is the contrast between how college students describe themselves and how they describe scientists. Even for men, the difference in how they see themselves and scientists is significant. For women, this difference is not only significant, but pronounced. Women see a much greater gap between themselves and scientists than men do. Undoubtedly it is more difficult for women to imagine themselves to be scientists than it is for men. It is not surprising that, historically, more men than women go into science, thus perpetuating the view that scientists are men.

Our study did however have its limitations. For instance, the sample that we used was non-random and small in size. Also, each participant came from a university setting and might have a different view about science and scientists than a sample of same-age people who are not in college. One might expect that college students would have had more experience with science and scientists and be less susceptible to stereotypes. One the other hand, our sample came from a liberal arts university where Bachelor of Arts degrees are more numerous than Bachelor of Science degrees. Perhaps students choose our university in part because it emphasize science less than other universities do.

Our research and previous research deals with stereotypes that people apply to scientists. Two interesting areas remain. One is to investigate the perceptions of psychologists—perhaps variously described as scientists, clinicians, and professors. Another is to direct attention at how taking a science class from either a male or female professor who exhibits stereotypical feminine characteristics could change one's views. For a future study, we want to do just that. We believe that although people do have preconceived notions about science, exposure to "feminine" science would be a simple way for people to be able to generate alternate perceptions based on experience and to see that people with many different characteristics can "do' science. Keeping this in mind, we would generate a pre- post-test study that would evaluate students' perceptions of scientists before taking a science class from a professor who displays feminine characteristics. After taking the class, we would then test student perceptions and compare results. We believe that students who "do" science themselves and see both men and women beside them will begin to see less difference between themselves and scientists and be more motivated to pursue a career in science.

References

Bem, S. L. (1974). The measurement of psychological androgyny. *Journal of Consulting and Clinical Psychology, 42*, 155-62.

Fung, Y. (2002). A comparative study of primary and secondary students' images of scientists. *Research in Science & Technological Education, 20(2).* Retrieved September 29, 2005 from PsychINFO at http://web11.epnet.com/search.asp.

Holt, C. (1998). Assessing the current validity of the Bem Sex-Role Inventory. *Sex Roles, 39(11-12)*, 929-941. Retrieved September 1, 2006 from PsychINFO at http://web11.epnet.com/search.asp.

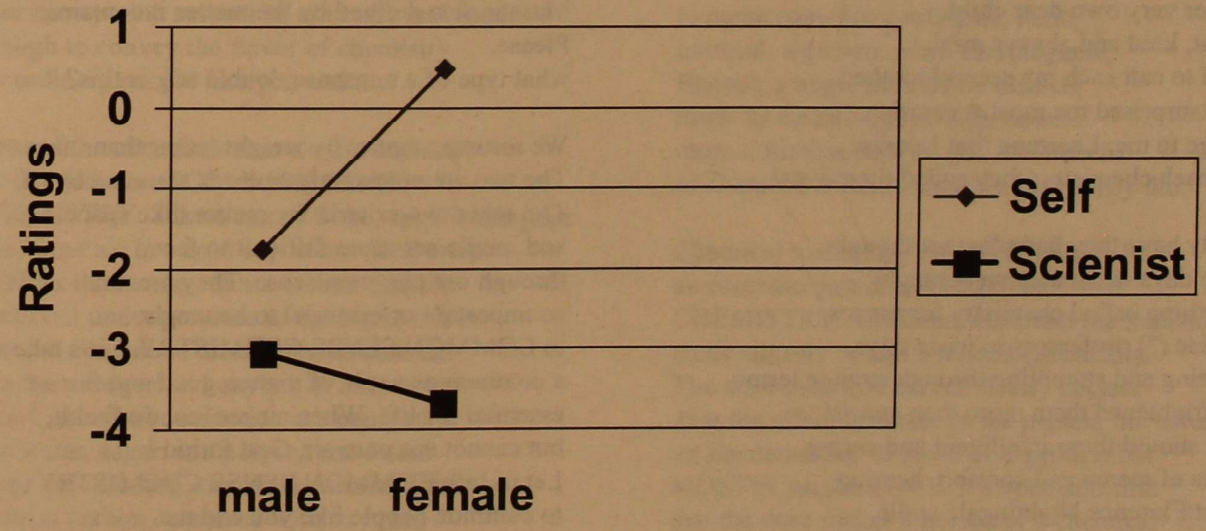
Palmer, D. (1997). Investigating students' private perceptions of scientists and their work. *Research in Science & Technological Education, 15(2),* 173-183. Retrieved September 28, 2005 from PsychINFO at http://web11.epnet.com/search.asp.

Papanastasiou, C., & Papanastasiou, E. (2004). Major influences on attitudes toward science. *Educational Research and Evaluation, 10(3),* 239-257. Retrieved September 28, 2005 from PsychINFO at http://web11.epnet.com/search.asp.

Tenenbaum, H., & Leaper, C. (2003). Parent-child conversations about science: The socialization of gender inequities. *Developmental Psychology, 39(1),* 34-47. Retrieved September 29, 2005 from PsychINFO at http://web11.epnet.com/search.asp.

Webb, A., & Speer, J. R. (1986) Prototype of a profession: Psychology's public image. *Professional Psychology: Research and Practice, 17(1),* 5-9. Retrieved September 18, 2005 from PsychINFO at http://web11.epnet.com/search.asp.

Figure 1: Interaction between Self and Scientist Ratings and Gender



COMMON SENSE CHEMISTRY

Basudeb DasSarma
429 25th Street Dunbar, WV 25064
Retired Professor of Chemistry.
West Virginia State College Institute, WV 25112
Tel: 304-768-1143 Email: Bdassarma@hotmail.com

Study of CHEMICALS is CHEMISTRY,
that can solve a lot of mystery
of society, our lives, nature and
everything around us – air, water, land,
appliances, cars, computers and toys,
and is the basis of our aches and joys.
Yet it emanates from COMMON SENSE
-simple, yet opens up secrets immense.

Believe it or not in two thousand and four
I was hospitalized, though never before,
for one hundred days in three hospitals.
Everybody thought, came and made calls
assuming this was the end of my life
after over eighty years of being alive.

The doctors and staff did their job,
but the nurses were angels from above,
who treated me the way only a mother can
of a sick, smelly, pathetic old man.
like her very own dear child,
patient, kind and always mild.
I used to call each my second mother.
What surprised me most was rather
strange to me. Learning that I devise
and teach chemistry; they rolled their eyes!

Hardly have they had pleasant thought
of the days when they were taught
something called chemistry for nurses
by wise (?) professors in those days,
sweating and stumbling through strange terms,
that frightened them more than germs!
Why should these intelligent and caring
givers of mercy and comfort, bearing
sweet Florence Nightingale smile,
so detest chemistry all the while?

Study of chemicals is called Chemistry
that has a very long interesting history
from Alchemists who dreamt of making gold,
discover Elixir of life, defy death and behold
the joys and bounties of life and living.

We partly achieved these early deserved things.
The gold we can make by transmutation
is however too expensive to make and own.
Wonder drugs have prolonged life a lot.
We have made new elements that were not
present on earth. Let us consider how easy
are the basics of COMMON SENSE CHEMISTRY.

In the absence of a better word, it is enough
to call everything in and around us as STUFF.
All stuff can be roughly divided into
MATTER and ENERGY. But these two
are really not so different, as we can see
one can be converted quite readily
to the other. Matter is the common name
for chemicals that often became
substance in common talks. Matter was
traditionally defined as stuff with mass,
stuff that takes up a definite space,
and a moment of inertia they possess.
Mass is also defined by the matter it contains.
Please,
what type of a nonsense double talk is this?

We measure matter by weight rather than mass.
The two are not exactly same as assumed by us.
The other two criteria for matter, like space
and inertia are more difficult to face
through our common sense. They are not
so important or essential to be taught
in COMMON SENSE CHEMISTRY. Let's take air,
a common example of matter, good and fair
essential for life. When air moves, we feel it,
but cannot see pure air, God forbid !
Let us talk COMMON SENSE CHEMISTRY
to common people like you and me.

We may better understand the stuff called energy
like light, heat, sound and electricity.
Matter and energy, interconvertible and actually
are different facets of the same stuff. Really
it is difficult to think of energy without matter
like that in our body. We shall see later

that energy is involved in every change
any matter undergoes, and hence
we shall concentrate on matter in common sense
chemistry for chemicals in life and lab.
Presence of and changes in chemicals have
normally been realized through
the senses of sight, sound, smell, taste and touch
too.

Modern technology did vastly extend
the probe through our senses and send
intelligence and imagination of human race
through the maze of knowledge at rapid pace.
Observation, detection, preservation, extension,
explanation and retrieval through computation
aided by silicon technology- all are
boons from chemistry square and fair.

Element that comes from word 'elementary'
is the simplest form of any and every
pure substance or matter. The smallest unit
of an element that can take part and refit
in a chemical reaction, is called an ATOM,
meaning matter's indivisible ultimate form.

For elements, there are ninety natural ones,
but COMMON SENSE CHEMISTRY can be done
with only a few like fifteen or so that constitute
over ninety-nine percent of our body and food,
enough to convey the flavor of chemistry
for ordinary people like you and me.

Most of the chemicals that we use and see
are the domain of COMMON SENSE
CHEMISTRY. Take a can of Coke, open it and pour
into some ice in a cold cup at fast food store.
Bubbles that come out are carbon dioxide
dissolved under pressure in the liquid inside.
The can is made of aluminum, an element
like the nitrogen and oxygen in air present
over Coke. Carbon dioxide is a compound
like water, sugar, and polystyrene found
in the cup. Carbon dioxide is formed when
element carbon combines with oxygen,
dihydrogen oxide, with common name water,
from hydrogen and oxygen. Styrofoam a polymer,
CxHy, finds multiple usages here and there.
Binary compounds are formulated as AmXn,
where A, X are elemental symbols, x,y, m, n,
the number of respective combining atoms,
as in CO_2, H_2O and CxHy for compound forms.

The elements are presented in a few columns
and rows in the Periodic Table that comes
from Mendeleef, a Russian pioneer,
an enormous benefit to chemists forever.

Each element is designated by a name,
and a one- or two-letter symbol, each to claim
an Atomic Number equal to the number
of PROTONS in its central nuclear chamber
that may contain NEUTRONS, similar to proton
but neutral. The nucleus, surrounded by electrons,
negatively charged, extremely lighter than a proton
and neutron. The number of protons at the center
equals the number of electrons that are
around it, forming a neutral atom like our
solar system to some extent. We need not go far
from this simple model in most of the affair
of common sense simple chemistry
for ordinary folks like you and me.

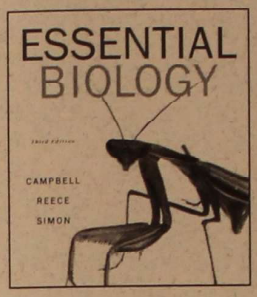
Chemical reactions result from the loss, gain or
exchange of electrons between two or more
atoms, while the protons remain the same
in our COMMON SENSE CHEMISTRY game.

The word "chemical" as a noun
to most people brings a frown
to mean something artificial, toxic,
harmful, a poison, often carcinogenic.
Bhopal, a tragic man-made disaster
made us conscious and help bolster
more critical review of risk and benefit
of chemical industry than we previously did.

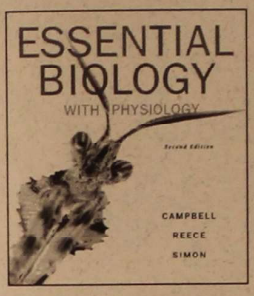
Chemical toxicology should definitely be
an essential part of COMMON SENSE
CHEMISTRY. All chemicals affect the human body
made entirely of and operated chemically.
The dose-response curves clearly declare
that the effect depends on the person, the nature
of the chemical, its route of exposure, but
MOSTLY on the DOSE. It's open and shut
that the dose makes a poison of a chemical
which at a low dose is harmless or beneficial
like Botulin, the deadliest toxin known by man
used in wrinkle-removing Botox shots on women.

New Titles from Benjamin Cummings

Introductory Biology



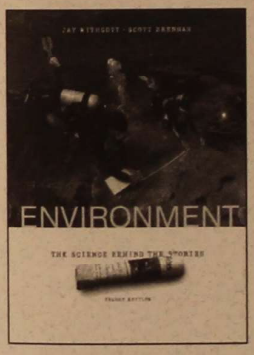
***Essential Biology,* Third Edition**
by Neil A. Campbell, Jane B. Reece, and Eric J. Simon
©2007 • 544 pages • Paper w/CD-ROM
0-8053-6842-6



***Essential Biology with Physiology,* Second Edition**
by Neil A. Campbell, Jane B. Reece, and Eric J. Simon
©2007 • 752 pages • Paper w/CD-ROM
0-8053-6841-8

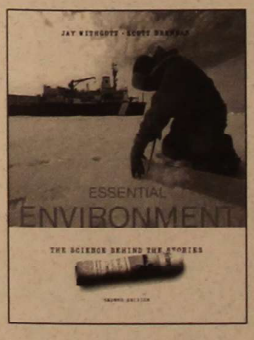
Hailed for clear writing, scientific accuracy, and real-world examples, these brief non-majors biology texts focus on the core concepts students need to make scientifically informed decisions throughout their lives.

Environmental Science



***Environment: The Science behind the Stories,* Second Edition**
by Jay Withgott and Scott Brennan
©2007 • 672 pages • Cloth
0-8053-8203-8

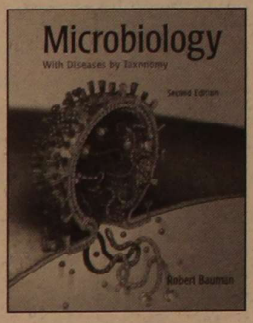
The second edition retains all the popular features of the landmark first edition—including its integrated central case study approach, and focus on current data and critical thinking.



***Essential Environment: The Science behind the Stories,* Second Edition**
by Jay Withgott and Scott Brennan
©2007 • 414 pages • Paper
0-8053-0640-4

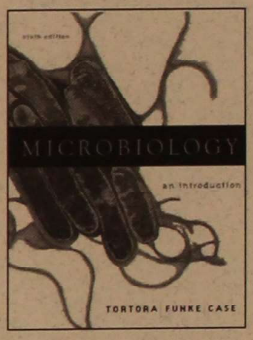
This brief 15-chapter text retains all the popular features of the landmark first edition—including its integrated central case study approach, and focus on the scientific process, current data and critical thinking.

Introduction to Microbiology (Mixed Majors)



***Microbiology with Diseases by Taxonomy,* Second Edition**
by Robert Bauman
©2007 • 896 pages • Cloth w/CD-ROM
0-8053-7678-X

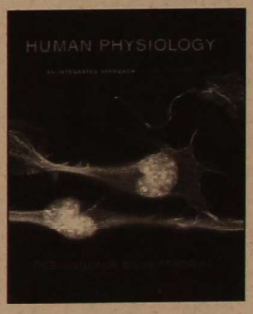
This new edition is filled with lively vignettes, fascinating research findings, engaging illustrations, and memorable, student-friendly explanations.



***Microbiology: An Introduction,* Ninth Edition**
by Gerard J. Tortora, Berdell R. Funke, and Christine L. Case
©2007 • 928 pages • Cloth w/CD-ROM
0-8053-4790-9

This text is trusted, reliable, and thoroughly up-to-date. Students and instructors praise its careful balance of microbiology concepts and applications.

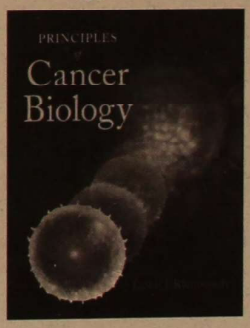
Human Physiology



***Human Physiology: An Integrated Approach,* Fourth Edition**
by Dee Unglaub Silverthorn
©2007 • 912 pages • Cloth w/CD-ROM
0-8053-6851-5

A human physiology text designed and written for today's students.

Biology of Cancer



Principles of Cancer Biology
by Lewis J. Kleinsmith
©2006 • 352 pages • Paper
0-8053-4003-3

This engaging book focuses on providing students with a "big picture" view of cancer, focusing on key concepts for both students and a general audience.

Visit our online catalog at www.aw-bc.com for more information and to request examination copies.

PEARSON
Benjamin Cummings
www.aw-bc.com