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Editorial policy is located on the inside front cover. *Information for contributors* is located on the inside back cover. Manuscripts and inquiries about publication and information about advertising should be sent to the editor: Kenneth J. Curry, University of Southern Mississippi, Post Office Box 5018, Hattiesburg, MS 39406-5018.

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Editorial

Election results! Congratulations to **Susan Nodurft** who will assume responsibilities as president-elect of the Mississippi Academy of Sciences this summer and to **Charles Swann** who will assume responsibilities as one of the three Academy directors.

Call for papers—the deadline is moved back from last year! We have come to that time of the year when you should start thinking about assembling your research or other creative efforts to present at our annual meeting. This issue contains the first of two calls for abstracts. The second and final call will appear in the October issue (but don't wait that long). Why two calls? This year we have rolled back the abstract deadline from 1 October to 1 November (actually the second since the first is a Sunday). That allows us to make the traditional July call and place a second call in our October issue. This will mean more scrambling for us to meet the printer and mailing deadlines to get the abstract issue to you on time, but it does give you an extra month over last year to organize what you have been doing. We hope this will help you and encourage submission of abstracts from those of you that had difficulty meeting the 1 October deadline. In addition, we have decided to accept late abstracts ***if*** there is room in the divisional program for which they are submitted and publish them in the April issue of the Journal. Previously late abstracts were not published.

The Mississippi Junior Academy of Sciences. You will notice that we have published again this year papers from high school students that won our Junior Academy paper competition. The Academy would like your help in promoting the annual Junior Academy competition. Contact Joan Messer, who heads our Junior Academy, for deadlines and other information about the competition. Winners receive a generous scholarship to most of the state universities and their paper is published in this journal after receiving peer review. You can reach Joan at joanmesser@aol.com or 601-477-4067.—Ken Curry

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Executive Officer's Column

We have just returned from a walk-through of our convention facility in Tupelo and were quite pleased. The staff of the Ramada Inn there seems most anxious to make sure that we have an excellent Annual Meeting in 1999. This facility is more dispersed than our typical meeting site but the meeting rooms themselves are of excellent quality. We look forward to seeing you there. Now, if the people working on Highway 45 will only cooperate and finish the four-laning!!

I'd like to call your attention to a fascinating report that has recently been published. It is from the Boyer Commission on Educating Undergraduates in the Research University and is entitled "Reinventing Undergraduate Education: A Blueprint for America's Research Universities." The Mississippi Academy of Sciences serves as a forum for researchers in the state and is also vitally interested in education issues. This report looks at the realities and possibilities of undergraduate education in research universities. While this state has only two such recognized institutions (according to Carnegie Foundation classification), the concepts in the report can be readily extrapolated to most colleges and universities.

As in any groundbreaking report, the recommendations will be controversial but well worth examining. Key among them is the concept that students learn best through inquiry rather than simple exposure to knowledge. Research is the ideal method of inquiry in a modern university. The report talks of making universities student-centered. While this is no doubt exceedingly difficult in large institutions, students are a major reason, if not the major reason, for the existence of most colleges and universities. As research attains a momentum of its own, we sometimes leave the students behind. This report urges us to bring them along with us. Its recommendations are actually rather radical and perhaps unrealistic when examined in detail. However, it is important to appreciate the overall concept of student importance and the linking of research with students. It is also very useful to examine the examples of what some schools are doing in an effort to improve their teaching efforts.

This report is available at the following WEB site:

http://notes.cc.sunysb.edu/Pres/boyer.nsf

For those of you who actually want to hold paper in your hands, simply go to this site and request a free hard copy. The Commission that produced this work has many significant members including the President of the National Academy of Sciences, the former Chancellor of Berkeley, and a Nobel Prize winner in physics.—John Boyle

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Frequency Averaging for Mismatch Mitigation

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Matched-field processing is a parameter estimation technique for localizing an acoustic source in a waveguide. The signal is observed at an array of hydrophones in the presence of spatially correlated noise (that also propagates in the same ocean environment as the signal). Incoherent broadband averaging is a technique used with matched-field processing that capitalizes on the broadband nature of the target acoustic source to acquire additional gain against ambient noise and to decrease the background variance common to matched-field sonar output. Incoherent broadband averaging has been applied earlier [J. Acoust. Soc. Am. 91, 1447–1455 (1992)] for improving signal-to-noise gain, and [J. Acoust. Soc. Am. 88, Suppl. 1, S27 (1990)] for source localization in the presence of random phase errors in a Pekeris environment. In this paper, this method is applied using a more general environmental model and realistic sound speed mismatch errors. Both the Minimum Variance Distortionless Response and the Sector Focused methods are used to compare the localization stability. The results indicate that while incoherent frequency averaging can improve performance for moderate mismatch, it can also degrade performance for more severe mismatch errors. More specifically, while performance improved as bandwidth increased for slight mismatch, performance degraded as bandwidth increased for severe mismatch. Also, while Sector Focusing performed better than Minimum Variance Distortionless Response under the high mismatch conditions used in this test, it did no better than MVDR in performance versus bandwidth trials.

Matched-field processing (MFP) (Bucker, 1976; Klemm, 1981; Porter et al., 1987; Fizell, 1987) is a promising technique for localization of a submerged acoustic source in a wave guide (e.g., shallow water). It is accomplished by correlating the complex pressure field predicted at the elements of a hydrophone array with the complex pressures obtained by Fourier transforming the measured acoustic signals. The predictions are possible only if sufficient environmental data is available and if the source location is assumed. The normalized correlations are plotted against assumed source range and depth to produce what is called an ambiguity surface. The matched-field ambiguity surface typically contains, in addition to the signal peak, several false peaks or sidelobes which make detection and localization of an acoustic source ambiguous. The sidelobes represent the essential non-uniqueness of this type of solution to this particular inverse problem. Sidelobe contamination is more severe in range independent environments and for low signal-to-noise ratios. Recent studies have shown that the sidelobe problem is not so severe when MFP is applied to complex range-dependent environments (Zala and Ozard, 1989; Perkins et al., 1989).

The correlation between predicted and measured fields can be calculated with one of several available estimators, such as the replica correlator, the maximum likelihood (ML) or the Minimum Variance Distortionless Response (MVDR) estimator developed by Capon (Capon, 1969; Hinich, 1973). Though MVDR is known to achieve good noise and sidelobe rejection, it becomes unstable in the presence of severe mismatch as occurs, for example, when the sound speed profiles used in the calculations do not closely match the profiles actually present in the water when the sound is propagated through it. It has been shown by Frichter, et al. (1990) that Sector Focusing (SF) is more stable and accurate against random phase errors

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in source localization. Smith et al. (1993) used a "sliding window" implementation of sector focusing (denoted SWSF herein) to demonstrate the superiority of SWSF over MVDR against sound speed mismatch.

Ambiguity surfaces produced by MFP suffer from sidelobe contamination due to the primary source and competing discrete noise sources (e.g., surface ships). Noise sidelobes can occur at ranges and depths within the search region even though the noise source itself may be far removed from the region. The incoherent broadband averaging technique demonstrated in the literature (Smith et al., 1992, 1990; Akundi, 1992) enhances the capability of already existing processing techniques by suppressing the noise and signal sidelobes through incoherent averaging of several MFP ambiguity surfaces produced at different processing frequencies. The rationale behind this technique is that the locations of the noise and signal sidelobes on the ambiguity surface are generally frequency dependent whereas the location of the signal peak is generally independent of the frequency. Incoherent frequency averaging should therefore enhance the signal peak and suppress the sidelobes. In this paper, we used both MVDR and SWSF in conjunction with incoherent frequency averaging to compare the stability of the localization performance of MVDR and SWSF under mismatched conditions.

As a preparation to the simulation studies to be presented later in this paper, we introduce the environmental and noise models that are used in this study. A detailed discussion of incoherent broadband averaging of the ambiguity surfaces was done earlier (Smith et al., 1992) by one of the authors, and a detailed discussion of the philosophy and implementation of SWSF was also done earlier (Smith et al., 1993) by one of the authors. The reader may refer to those articles for details.

Environmental Model—The transmission of sound to any great distance in shallow water occurs primarily through normal mode propagation. This is due to the fact that significant reflection from the surface and sediment bounds the wave propagation in the vertical, thus creating a cylindrically symmetric waveguide. This symmetry is rarely exact in an actual shallow water environment. But the range independent, cylindrically symmetric case possesses most of the salient features that are essential in a study such as this one.

The calculations for the acoustic pressure field were performed using the SACLANTCEN Normal-mode Acoustic Propagation (SNAP) model (Jensen and Ferla, 1979). This model was chosen because it enables acoustic conditions in shallow water ocean environments to be treated realistically while maintaining reasonable computational requirements. The environment consists of a half space divided into three layers: a depth variable water column sound speed with constant density and volume attenuation; a depth variable sediment sound speed with constant density and attenuation; and a constant speed, density and attenuation in the bottom.

Noise Model—Of major importance in MFP is the manner in which processing algorithms deal with various components of the ambient noise field presented to the array. The noise field can be subdivided roughly into two categories—ocean noise and system noise. System noise can be further subdivided into two components which are either uncorrelated or correlated at the hydrophone array. Ocean noise can be roughly subdivided into near and farfield components. Both these noise components are correlated at the hydrophone array. In the following simulations, two noise components have been incorporated into the model: a white noise component to simulate uncorrelated noise components and a (correlated) farfield noise component. The white noise component is modeled by adding a multiple of the identity matrix to the data covariance matrix. The farfield correlated noise component is obtained using a RANDI type model (Hamson and Wagstaff, 1983) and is also added to the data covariance matrix.

In this work we have used the RANDI noise model to calculate noise-only data covariance matrices by assuming several discrete point sources randomly distributed over range from 10 km to 100 km, at a depth of 7 m. The signal source is placed at a range of 5 km. This is meant to simulate a shipping channel behind the signal source. A vector of complex pressures at the array is calculated as a coherent sum of the contributions from the noise sources. A rank-one matrix is formed as the outer product of this vector of complex pressures with itself. Several such realizations are averaged to obtain the final (full rank) simulated noise-only covariance matrix. Each realization is prepared with noise sources displaced from their positions in the previous realization in a way that simulates ship speeds between 0 knots and 14 knots. The time between realizations is simulated to be 1 s. The rank-one matrix from the signal source is added to the noise-only covariance matrix after being appropriately weighted to achieve the desired signal-

to-noise ratio. White noise is added as the appropriate multiple of the identity matrix.

Two quantities are used to define the levels of the two types of noise: the signal-to-noise ratio (SNR), and the modal-to-white noise ratio (MWR), where

MWR = 10 log [power in modal component/power in white component]. (1)

In the present work, all the ambiguity surfaces were produced with an MWR of 10 dB and an SNR of -10 dB.

Configuration of the Simulated Experiment—The experiment simulated here is performed in a range independent, shallow water waveguide of 100 m depth and 1 g/cc density overlaying a sediment layer of 500 m thickness, 2 g/cc density and an acoustic attenuation of 0.0425 dB/λ. These are underlain by an isospeed, semi-infinite basement with properties matching the base of the sediment layer so that reflections at the sediment-basement interface may be neglected. Shear wave propagation was not incorporated into the problem. A vertical line array of 20 evenly spaced hydrophones spanning the water column is suspended in the waveguide and extends from 3 m to 98 m depth. The hydrophones therefore have a spacing of 5 m which is adequate for proper sampling of the first 20 modes since the sampled mode functions are approximately sinusoids with wavelengths equal to twice the water depth divided by the mode number. Thus, adequate spatial sampling occurs when the hydrophone spacing is equal to the distance between nodes for the highest order mode sampled. The source is placed at a depth of 75 m and a range of 5 km from the array. The search region extends from 2 m to 98 m depth, and from 3 km to 7 km in range.

Sector focusing requires the choice of a sector size, placement and dimensionality for its implementation. The sliding window implementation used here and in some previous studies (Smith et al., 1993) places the sector so that the current search point is contained within it. Hence, the sector slides along with the search point. When the search point is near a boundary of the search space, it is not centered in the sector; otherwise, the search point is centered in the sector. The sector size and dimensionality (number of eigenvectors used) are usually chosen by trial and error. Here the sector size was chosen to be 200 m in range and 20 m in depth with a dimensionality of 7. This gives a performance midway between MVDR and standard linear replica correlator. That is, its performance should be more stable against mismatch than MVDR (but not as stable as linear replica correlator) with a significantly higher resolving ability than linear replica correlator (but with not as much resolving ability as MVDR). Its sidelobe suppression characteristics should be midway between linear replica correlator and MVDR. While these characteristics are not being quantified in this work, they are commensurate with what is observed on the ambiguity surfaces generated, and they are mentioned in order to give the reader some intuition for expected sector focused performance here.

Simulations—The parameters used to represent the mid-latitude summer environment assumed for the simulations of water column sound speed in this work are essentially identical to those used by Feuillade et al. (1989). In the present work, we are only dealing with the variations in the water column sound speed profile since it is well known that this type of mismatch error has the greatest impact on matched field localization. Also, it presents the greatest problem because it is subject to dynamics which vary over minutes (solitons) to hours (fronts) to days (eddies) as compared to sediment dynamics which vary over months to years. In principle, the sediment could be adequately sampled so that successful matched field localization could occur in that region for several months before the sediment sampling had to be repeated. But the water sound speed profile would have to be sampled at all points in the region from moment to moment, and this is clearly impossible even in principle. Thus, the matched field community is most concerned with the type of mismatch that is being addressed in this paper.

Figure 1 shows the generic sound speed profile for a mid-latitude environment and a typical sediment for a shallow water area. Shown in Fig. 1 are all the water column sound speed profiles used in the experiment. However, at the scale of Fig. 1, it is difficult to see the various profiles.

Figure 2 shows the water column profiles on a scale which allows their differences to be seen. The line marked with 0% shows the un-mismatched profile used to simulate the data. The other curves show the mismatched profiles used to generate the mismatched replicas for matched field processing. They are denoted by -10%, -20% and -30%, respectively, indicating the percent of a depth variable, historical standard deviation that was subtracted from the original profile to generate the mismatched profiles

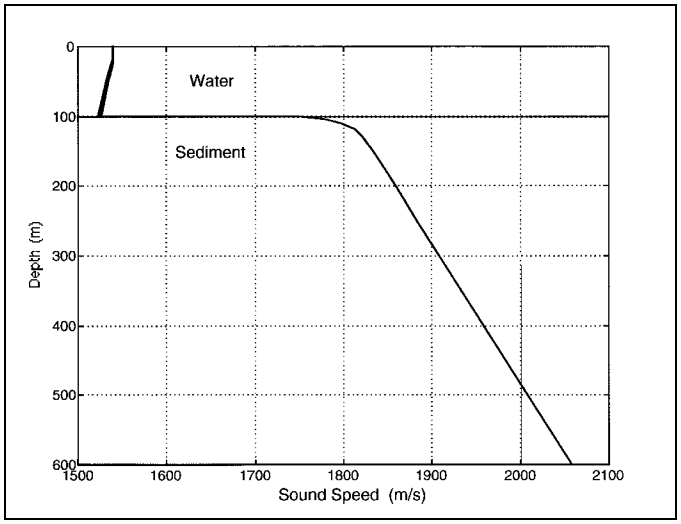

Figure 1. Simulation environment showing water column sound speed profiles and sediment sound speed profile.

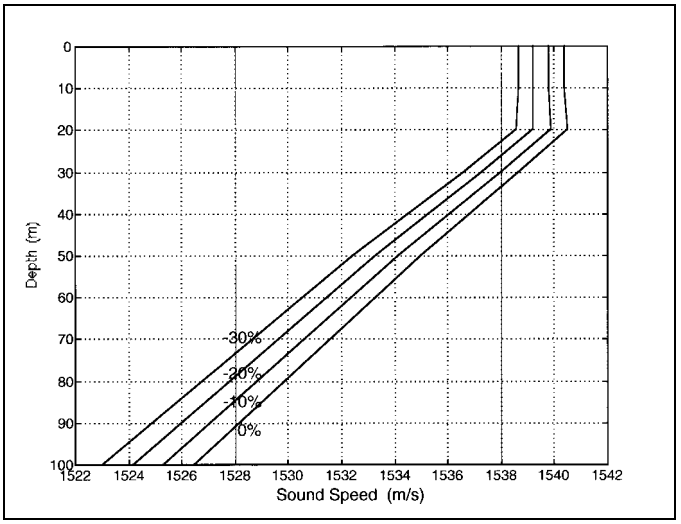

Figure 2. Water column sound speed profiles used in the simulations showing % mismatch.

(Feuillade et al., 1989). Careful examination of Fig. 2 shows that the deviation of the sound speed profile is not uniform and gradually increases with depth. We see that by using this method, the profile gradient was varied together with the offset, so that mismatch was simulated which was realistic from the point of view of matched field processing.

In this study we investigated the effect of frequency averaging on source localization with water column sound speed mismatches of -10%, -20% and -30% using the MVDR and SWSF estimators. Sixteen ambiguity surfaces were created in the frequency range of 150 Hz to 300 Hz (1 octave) with frequency values spaced 10 Hz apart. A sample component MVDR surface prepared at 150 Hz is shown in Fig. 3 for the -10% mismatch case, while the average over the 16 MVDR surfaces (1 octave) is shown in Fig. 4.

It is clear from Figs. 3 and 4 that all of the surfaces are dominated by surface noise. However, at this slight mismatch (-10%) the highest submerged peak on all the component surfaces as well as the averaged surfaces is the source peak. All averaging adds to this case is a cleaner surface with fewer false submerged targets. This is true for SWSF as well as for MVDR. There is a trend toward less clutter in the component surfaces as the frequency is increased, but this is an artifact caused by processing only 10 modes for each of the frequencies—a practice which was followed throughout the entire paper for computational efficacy.

The apparent reduction of clutter with increasing frequency is caused by the fact that there are more modes for higher frequencies than for lower frequencies, but the clutter information is distributed uniformly (a simplifying assumption) over all the modes. Thus, the clutter information per mode

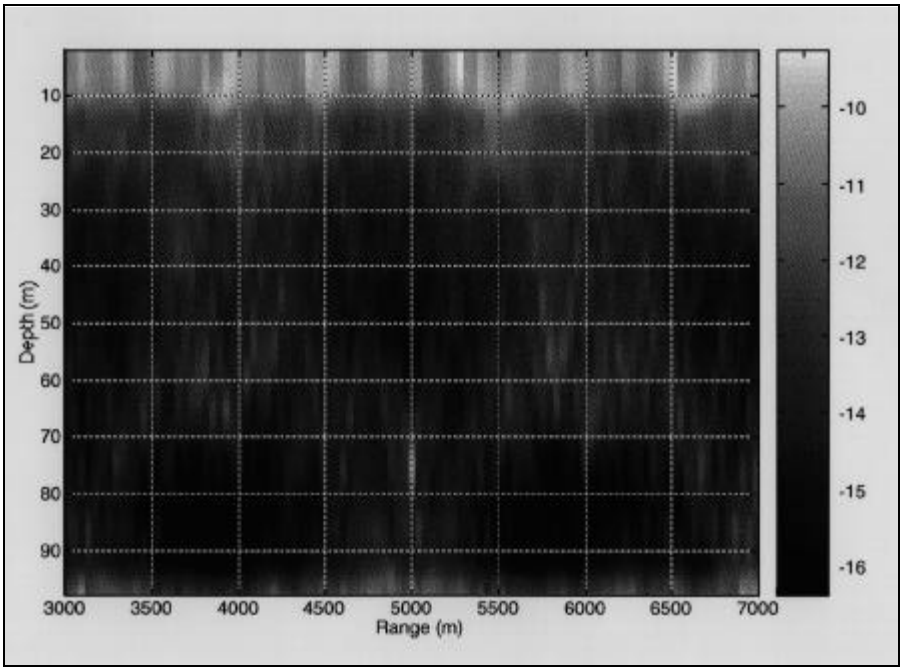

Figure 3. Sample component MVDR ambiguity surface (150 Hz) used for frequency averaging for the -10% mismatch case.

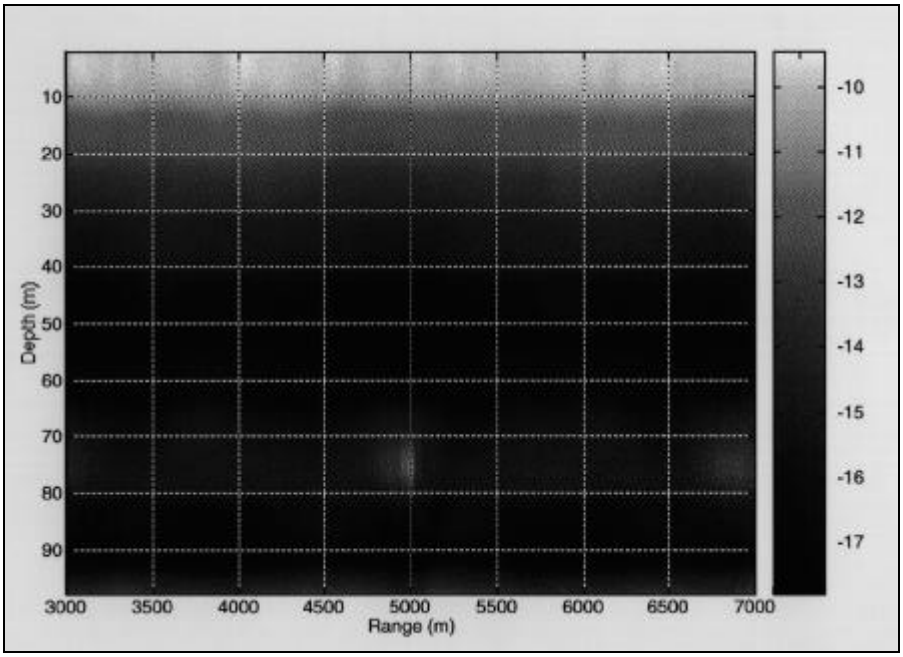

Figure 4. MVDR surface for the -10% mismatch case averaged over 1 octave.

decreases with increasing frequency. so there is less clutter information in 10 modes at higher frequency than there is in 10 modes at lower frequency.

Sector focused processing is done in a reduced dimension subspace of the vector space spanned by the eigenvectors of the data covariance matrix (Smith et al., 1993). This gives rise to the additional structure seen in Fig. 5 as compared to Fig. 4 since fewer eigenvectors are available to SWSF to null out sidelobes. Other than that there is very little difference between the averaged MVDR and SWSF surfaces (Figs. 4 and 5).

As mismatch is increased to -20%, both MVDR and SWSF suffer signal peak degradation. This is caused by the frequency dependence of the mismatch induced range localization error addressed by Shang and Wang (1991) which spreads the peak in range so that averaging is not as effective as it would be without this complication.

At -30% mismatch, comparison of the MVDR component surface for 150 Hz (Fig. 6) and the SWSF component surface for 150 Hz (Fig. 8) shows that the component surfaces localize the target at the wrong location with considerable clutter. The clutter problem at all frequencies seems to be worse for SWSF (Fig. 8) than for MVDR (Fig. 6). The averaged surfaces (Figs. 7 and 9) show smoothing of the background (i.e., clutter reduction), but (even ignoring surface noise peaks) MVDR incorrectly localizes on a sidelobe at 6680 m range while SWSF incorrectly localizes on a bottom position at 5120 m range. At this maximum mismatch (considered in this paper) it is clear that the spreading caused by the mismatch induced localization error has completely smeared the target signal response so that we have gained little by frequency averaging.

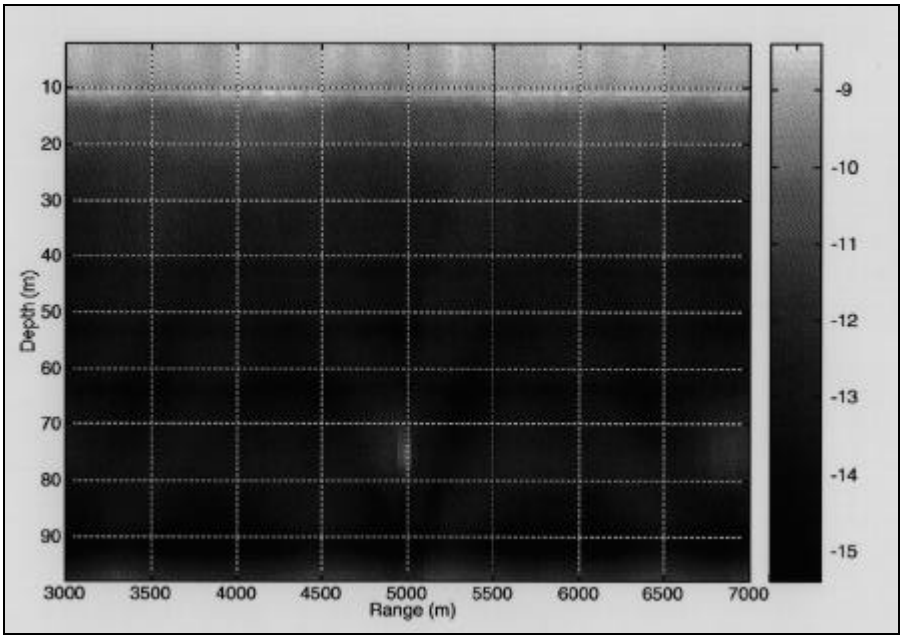

Figure 5. SWSF surface for the -10% mismatch case averaged over 1 octave.

To quantify the performance of frequency averaging for the two estimators used in this study, a measure of peak height relative to background roughness was used. It is called Peak-to-Background Resolution (PBR), and is defined as

PBR = 10 log [(Peak Height - Surface Average) / Surface Standard Deviation], (2)

where the surface average and standard deviation are calculated with a small patch containing the signal peak removed. This measure has been

used in several previous publications (Smith et al., 1990, 1992, 1993; Akundi et al., 1992) and (in lieu of a complete "Receiver Operating Characteristics" study) is the relevant performance measure here since the goal of frequency averaging is to smooth the background while enhancing the target peak. In this study, only the lower two-thirds of the ambiguity surfaces were included in the calculations of the PBRs. Hence, the effect of the surface noise on the PBR is minimized.

Figure 10 shows this performance measure plotted as a function of bandwidth for the three mismatch cases (-10%, -20% and -30%) and the two estimators considered. The bandwidth was increased by starting near the center frequency (225 Hz) and adding frequencies on alternate sides of center until all component frequencies from 150 Hz to 300 Hz were included. The specific order of inclusion was [220, 230, 210, 240, 200, 250, 190, 260, 180, 270, 170, 280, 160, 290, 150, 300 Hz]. These 16 component frequencies comprised the full octave of averaging used in this study.

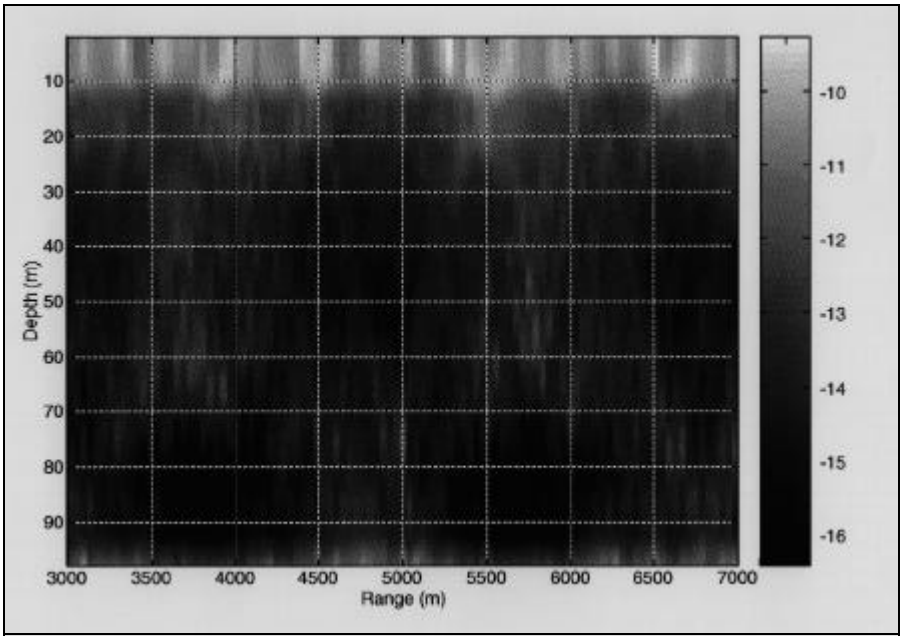

Figure 6. Sample component MVDR ambiguity surface (150 Hz) used for frequency averaging for the -30% mismatch case.

The most striking feature of Fig. 10 is that performance increases with increasing bandwidth only for the least severe mismatch case (-10%)! For the more severe mismatch cases, increasing bandwidth lowers performance. This is, of course, a direct result of the frequency dependence of the mismatch induced range localization error mentioned earlier.

Another very noticeable feature of Fig. 10 is the smoothness of the MVDR performance curves relative to those of SWSF. The reason for the variance in the SWSF performance plots (also noticeable to a much lesser degree in the MVDR performance plots) is the greater variation in (ambiguity surface) peak height across frequency with SWSF than with MVDR. For MVDR that variation was 1.5 dB, whereas for SWSF it was 2.9 dB in the -10% mismatch case. Had the performance versus bandwidth been calculated by including frequencies in increasing (or decreasing) order, the variation in either plot would not have been apparent.

It can also be seen from Fig. 10 that for the -30% mismatch case, SWSF did produce a significantly higher value of PBR than did MVDR. In fact, the SWSF PBR is higher for the -30% case than it is for the -20% case. These anomalies are not important

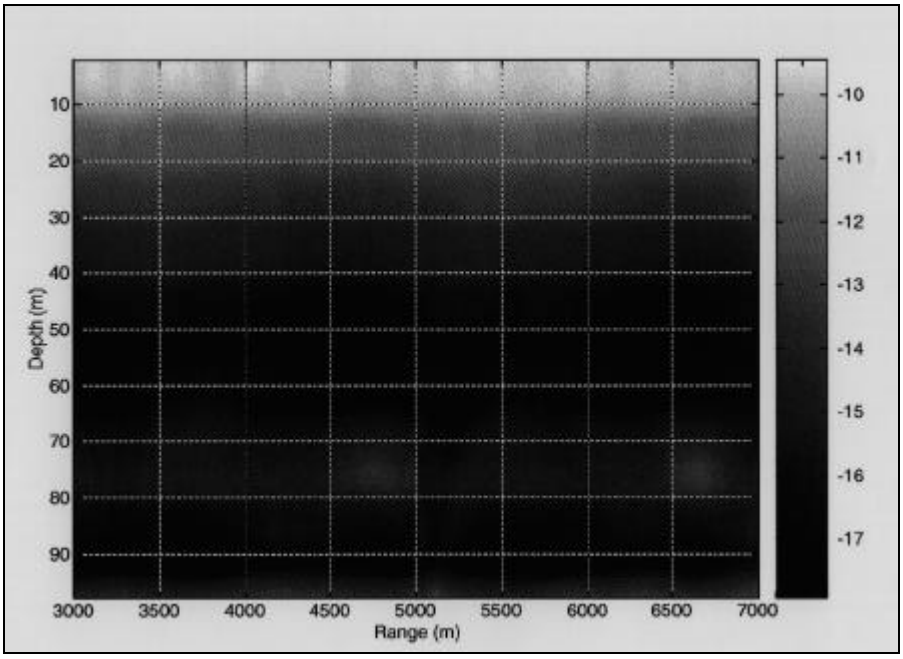

Figure 7. MVDR surface for the -30% mismatch case averaged over 1 octave.

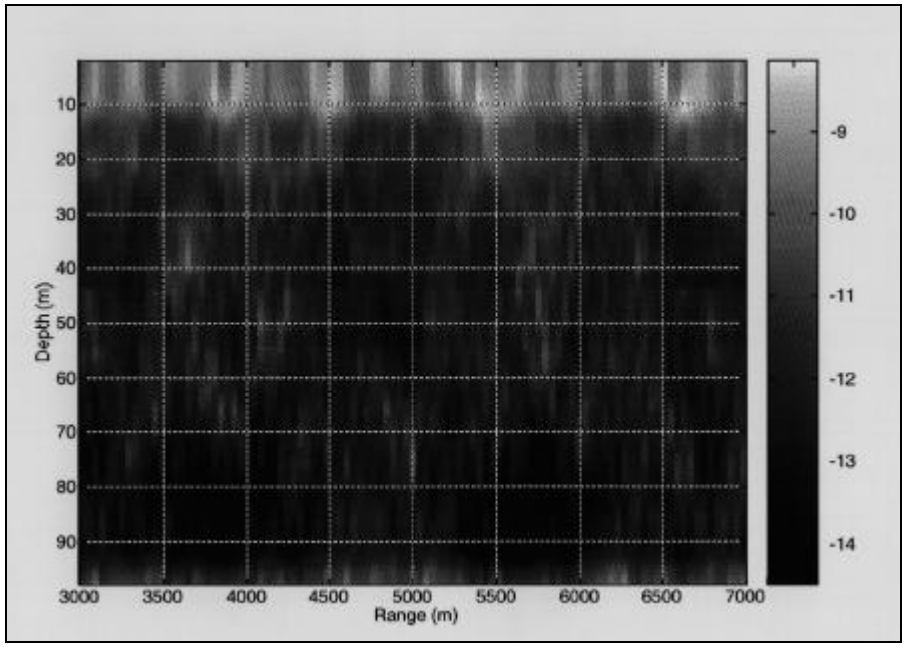

Figure 8. Sample component SWSF ambiguity surface (150 Hz) used for frequency averaging for the -30% mismatch case.

since such small changes in PBR are being considered here (0.5 dB for SWSF). That SWSF out-performed MVDR for a given case is a hollow point given that the performance for this case failed to increase with bandwidth.

CONCLUSION

The performance of the incoherently frequency averaged MVDR and SWSF matched field estimators was compared at low signal-to-noise ratio in a realistic, mismatched shallow water environment. The startling conclusion of this paper is that increasing bandwidth will not necessarily improve performance!

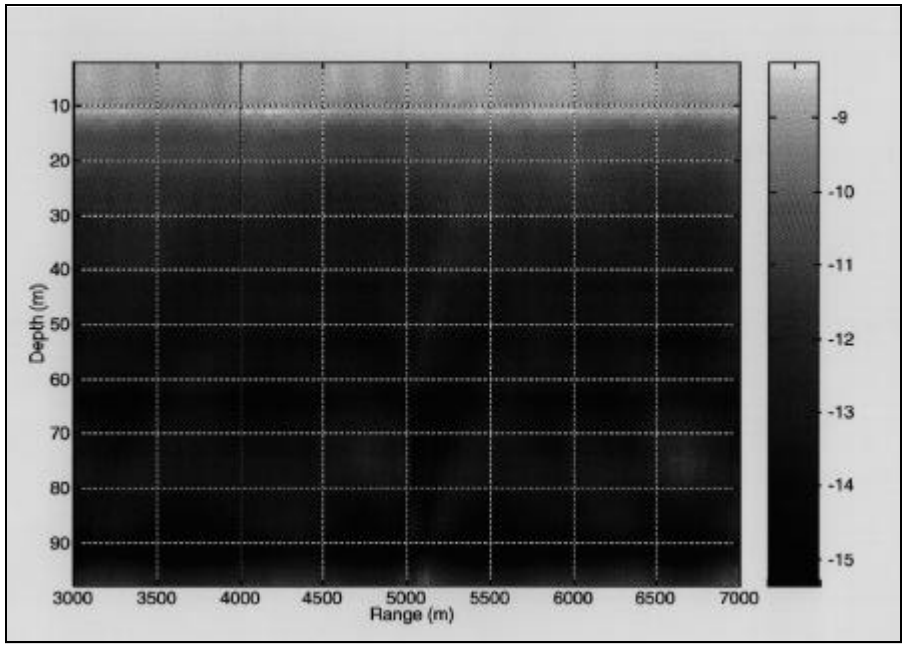

Figure 9. SWSF surface for the -30% mismatch case averaged over 1 octave.

Performance did improve as bandwidth was increased when the mismatch was slight, but performance degraded for both estimators as bandwidth increased for severe mismatch. This is, of course, a direct result of the frequency dependence of the mismatch induced range localization error.

The hope was that sector focusing would produce better incoherent frequency averaging than MVDR. This was to be demonstrated by comparing the slopes of the MVDR versus SWSF performance curves (PBR vs. bandwidth). Disqualifying the higher mismatch cases for obvious reasons, the comparison must be made only for the -10% mismatch case. The slope of the MVDR performance curve shown in Fig. 10 is 0.00763 dB/Hz, while for SWSF it is 0.00452 dB/Hz. Thus, sector focusing averages no better than MVDR.

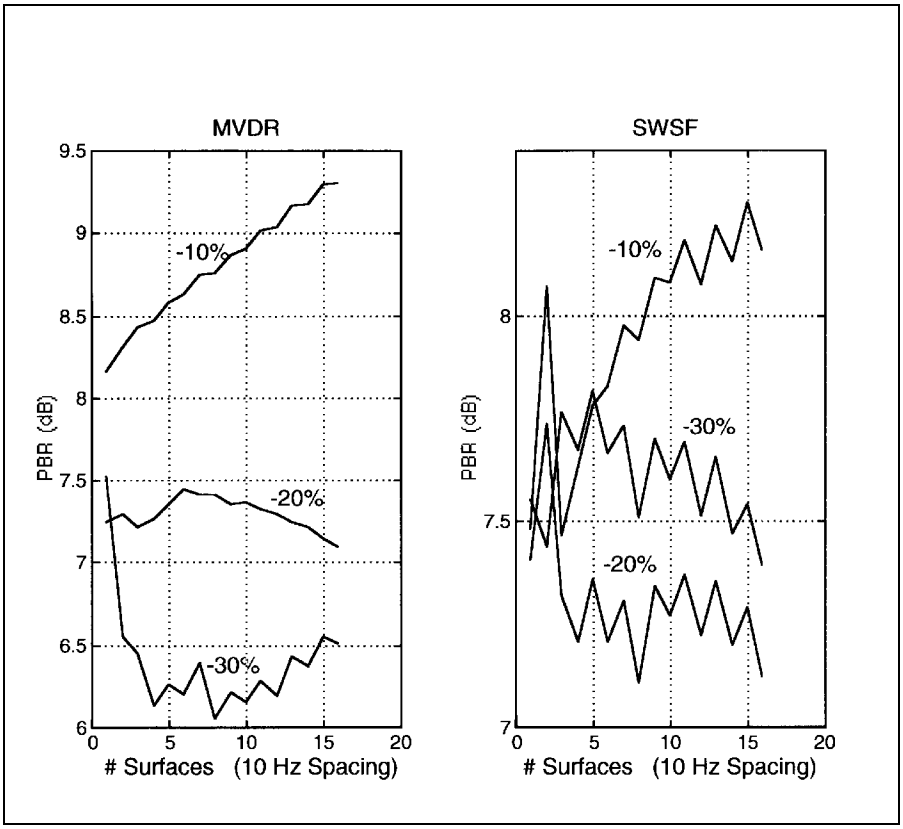

Figure 10. Performance measure (PBR) versus bandwidth for the three mismatch cases and two estimators considered in this study.

ACKNOWLEDGMENTS

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Work Under Way on New Museum of Natural Science

In March 1995 the Mississippi Legislature appropriated $14 million in funding for the new Natural Science Museum building in LeFleur's Bluff State Park and, in doing so, set off an extensive planning effort to assure that the funds were used to the best possible advantage.

During the past two-years the Museum staff and the architectural firm, Barlow, Eddy & Jenkins, have researched the latest innovations in Museum architecture and have analyzed our services to design the best building for the needs of our audience. The new Museum will provide greater access to existing educational programs, as well as new interpretive opportunities. Special emphasis will be placed on resource management, wetlands restoration, and protection of threatened, rare, and endangered species.

The Museum's existing theme, "Mississippi's Web of Life," has provided a dynamic interpretive framework and will remain the focus of the new facility's exhibits programs. The theme will be enhanced through access to a variety of interpretive exhibits and nature trails designed to prompt first-hand exploration of the natural world.

By providing both indoor and outdoor educational settings, the new Museum facility will enable students, teachers, and other visitors to view environmental, conservation, and other scientific studies in the context of everyday life.

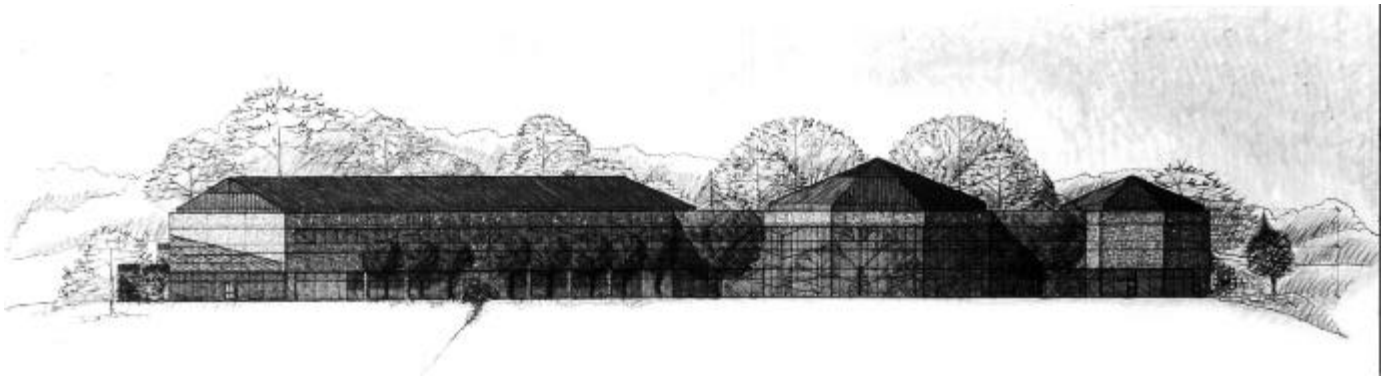
Exhibits will be designed to please the eye with a variety of different views of the state's unique life forms, past and present, and to educate visitors on the importance of relationships that bind living things to one another. The goal of the new exhibits plan is to spark interest in and provide understanding of mankind's critical role in determining the status and future prospects of life in Mississippi and the world at large.

The exhibit design criteria includes providing freedom of movement and a variety of sensory experiences for visitors, as well as a sense of continuity between indoor learning spaces and outdoor experiences.

Construction of the new Mississippi Museum of Natural Science in Le Fleur's Bluff is on schedule, with the building projected to be finished next spring and exhibit and aquarium installation to be complete by fall of 1999.

The Potomac Group, museum consultants from McLean, Virginia, have estimated that the new facility will attract at least 200,000 visitors per year.

State bond money will cover all construction costs, extensive landscaping, the aquarium system, including a large greenhouse-terrarium with lots of room for fish, turtles, alligators, and native plants.



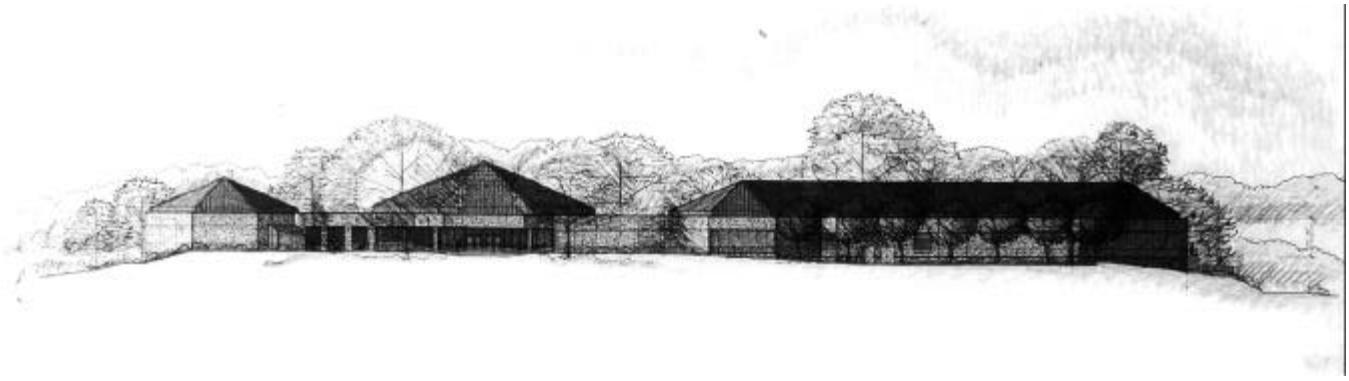
EAST ELEVATION

When completed the new facility will provide:

- ★ An aquarium system with 6 times the amount of water in our present system on Jefferson Street, featuring marine and freshwater fishes
- ★ A 200 seat auditorium
- ★ Two classrooms for school groups and adult programming
- ★ Preschool room specially designed for our youngest visitor
- ★ A 2,500 sq. ft. library
- ★ A Gift Shop
- ★ An exhibit hall for temporary and traveling exhibits
- ★ Laboratories and Collection ranges to accommodate our present biological archives in a safe environment, with separate work space and collection ranges for Paleontology collections
- ★ Larger, more realistic habitat exhibits--representing the diverse ecosystems of Mississippi, from the Northeast Hills to the Delta Bottomlands to the Barrier Islands
- ★ More hands-on exhibits, including microscopes, and a bird watching area
- ★ More than 2½ miles of nature trails that connect the Museum site to Mayes Lake
- ★ 300 beautiful acres adjacent to the Pearl River, including cypress swamps, sandy creek bottoms, and steep wooded bluffs

Two million dollars from the private sector is needed to cover the cost of additional habitat dioramas, native wildlife exhibits, and fossil displays.

Opportunities exist for individuals and corporations to become a part of the Museum of Natural Science through memberships and designated giving. Any size donation is certainly appreciated and can help with the new exhibits. Various levels of membership are available.



WEST ELEVATION

Novel Biotechnological Applications of the Chaperone Like Activity of Protein B23

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Recombinant proteins often accumulate within the cell as insoluble protein aggregates or inclusion bodies. Also, expressed proteins become denatured during purification which is a major problem in biotechnology. The purpose of this paper was to find a chaperone protein and employ it as a refolding machine. If this chaperone proves to be useful, it may help solve these current problems in the biotechnological industry. Molecular chaperones are an abundant group of proteins that facilitate the post translational folding, renaturation and transport of nascent polypeptides *in vivo*, and they also help prevent damage from heat and oxidation. Nucleolar protein B23 is an abundant, multifunctional phosphoprotein, a ribosome assembly factor and a putative molecular chaperone which could serve as a refolding system. The goal was to prove that B23 is able to function as a chaperone protein and can protect other proteins, to check the viability of *Escherichia coli* at elevated temperatures in an expression system overproducing B23, and to develop a novel refolding chromatography. It was found that *E. coli* expressing B23 had a drastically higher survival rate at elevated temperatures than *E. coli* without B23 expression. Denatured liver alcohol dehydrogenase (LADH) model enzyme was used to analyze chaperone like effects of protein B23, and it was found that the protein protected and prevented aggregation of LADH at elevated temperatures. A novel refolding chromatography, where B23 was immobilized on the affinity column, was developed to refold apparently irreversibly denatured LADH enzyme.

Abbreviations: LADH liver alcohol dehydrogenase; HIV-1 Human Immunodeficiency Virus-1; NAD^+ nicotinamide adenine dinucleotide; GdmHCl guanidine-hydrochloride; DTT dithiothreitol; SMCC succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate; IPTG isopropyl-1-thio-β-d-galactopyranoside; BSA bovine serum albumin.

Researchers in laboratories and biotechnological industries alike are often confronted with protein misfolding and protein aggregation upon overexpression of genes in host cells such as *Escherichia coli* (Guise et al., 1996). But why doesn't this problem occur in naturally living cells? These cells come with a folding machinery provided by Mother Nature in the form of molecular chaperones. Molecular chaperones prevent inappropriate liaisons between their charges and tempters that would lead them astray (King et al., 1996; Buchner, 1996). One class of chaperone molecules, called chaperonins, performs the important task of shepherding newly made proteins away from each other and helping them fold into the correct shape (Fenton, Horwich, 1997). Although the functions of the numerous molecular chaperones are not fully understood, their major roles are: promoting the proper folding of newly synthesized proteins, maintaining the folding pattern of existing proteins, recognizing and correcting the conformations of misfolded proteins, and preventing the misfolding of proteins under stress conditions. The activities of some chaperones are carried out in specific locations within the cell; i.e., in the mitochondria, endoplasmic

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reticulum, cytosol or nucleus, although a few chaperones are found in multiple compartments (Hendrick and Hartl, 1993).

A suborganelle of the nucleus, the nucleolus is the site of RNA synthesis, processing and ribosome subunit assembly (Alberts et al., 1994). This multistep process includes transcription of preribosomal RNA and attaching ribosomal RNA to ribosomal proteins which are synthesized extranuclearly. These factors simply assist ribosome synthesis and do not become a part of the ribosomes. The assembly process requires numerous nonribosomal proteins which fall into several classes. Among these classes is a group of nucleolar nonribosomal proteins which contain highly acidic and basic segments such as nucleolin, protein B23/No38 and Nopp 140 (Olson, 1990). Recent findings (Szebeni and Olson, 1998) have shown that nucleolar protein B23 prevents protein aggregation and has chaperone-like activity *in vitro*. It is believed that the natural chaperone substrates of B23 are ribosome proteins (Szebeni and Olson, 1998).

B23 is relatively small and highly acidic (molecular weight/pI = 38,000/5.1) and the level of sequence conservation among species is very high. Protein B23 is expressed in at least two isoforms (Chan et al., 1985, 1986). The two proteins, designated B23.1 and B23.2, contain 292 and 257 amino acids, respectively. In their C-terminal sequences, there is a unique 37 residue extension in B23.1 and a 2 residue extension in B23.2 (Chang et al., 1989). Protein B23 forms a specific complex with the Human Immunodeficiency Virus-1 (HIV-1) Rev protein (Fankhauser et al., 1991; Umekawa et al., 1993) which is also localized in the nucleoli of HIV-infected cells.

It is a known fact that the overexpression of foreign proteins with concomitant overexpression of chaperone proteins in *E. coli* enhances the level of certain recombinant proteins in a native folded structure (Goloubinoff et al., 1989; Escher and Szalay, 1993; Amrein et al., 1995). This gives hope to the idea that B23 as an overexpressed foreign chaperone may also be capable of renaturing recombinant proteins, and in the future it can be used as a special tool for the more effective production of other overexpressed foreign proteins by the biotechnological industry.

Numerous experiments have shown that chaperone proteins *in vitro* are able to refold damaged or unfolded proteins. From these observations arises the question of how this *in vitro* stationary folding can be transformed to a continuous process. The second half of this report focuses on the development of a refolding chromatography where the chaperone protein is immobilized on the matrix and could in the future be employed by the biotechnological industry for the production of continuously refolded proteins.

MATERIALS AND METHODS

Recombinant Protein B23.1 and B23.2—Proteins used in these studies were produced in *E. coli* and purified essentially as previously described (Umekawa et al., 1993) with a slight modification as reported earlier (Szebeni, 1996).

Thermal Aggregation Measurement of Alcohol Dehydrogenase—Lyophilized horse liver alcohol dehydrogenase was obtained from Fluka and used for standard aggregation assay. The aggregation of LADH upon heat denaturation was monitored by measuring the apparent absorption due to light scattering at 360 nm at 48°C for up to 80 min in teflon-stoppered, semi-micro, black walled, and had a 10 mm pathlight. In each experiment the total volume in the cuvette was 400 µl. The turbidity was recorded automatically every 60 seconds. Proteins and buffers were mixed in the cuvette and kept on ice.

Enzyme Activity Measurements of Alcohol Dehydrogenase—Enzyme activity of LADH was assayed in 50 mm sodium phosphate buffer (pH 8.0) containing 0.2 mm NAD^+ and 1 mm ethanol in a final volume of 1 ml. The rate of reduction of NAD^+ was monitored spectrophotometrically at 340 nm according to the method of Lee et al. (1995).

Denaturation and refolding of LADH–LADH was denatured for 15 min in 6 M guanidine-HCl, 0.1 M phosphate buffer (pH 7.6), 0.5 mm DTT and then separated from metal ions on a Sephadex G25 column equilibrated in 0.1 M phosphate buffer (pH 7.6), 6 M guanidine-HCl (GdmHCl), 5 mm EDTA as described by Jaenicke and Rudolph (1990). The denatured protein fraction was used for refolding essays after 85-fold dilution with 0.1 M phosphate buffer (pH 7.5), 0.5 mm DTT, 3 µM $ZnCl_2$ at room temperature. Aliquots from the diluted mixture were used to measure the enzyme activity.

Fluorescence Experiments—Fluorescence spectra were recorded on a Aminco A2 fluorometer at 20°C. The slit width for both excitation and emission corresponded to 4 nm, and the scan rate was 240 nm/min.

Protein Linking to BioR-Rad Affi Gel 10—Affi-

Gel 10 gel is an amino terminal crosslinked agarose gel with a 6-atom, hydrophilic arm. B23 was immobilized to Bio-Rad Affi-Gel 10 as described by company literature and as reported earlier (Szebeni, 1997). The succinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate (SMCC) bifunctional linker first reacted with the amino group of the matrix. In the second step, the sulfhydryl (-SH) group of the SMCC reacted with the B23's -SH.

***Bacterial Culturing*—**Bacteria were cultured in LB medium and the number of living cells was determined by plating aliquots on Luria plates and counting colonies (Sambrook et al., 1989).

RESULTS

***1) Is Eukaryotic Protein B23 Able to Function as a Chaperone Protein in* E. coli?—**It is well known that overexpressed chaperone proteins in *E. coli* can raise the stability of other proteins and can protect the bacteria from the damaging effects of elevated temperatures, therefore increasing the bacteria's thermal resistance. To determine if eukaryotic chaperone-like protein B23 is able to function in a prokaryotic cell, *E. coli* was utilized for a bacteria death rate measurement. Two cultures were exposed to elevated temperatures, the control being *E. coli* cells and the second culture was the *E. coli* cells overexpressing B23 (BL21de3). Bacteria were grown and induced with isopropyl-1-thio-β-d-galactopyranoside (IPTG). Two 50 ml cultures were placed in the incubator at 37°C. After 30 min one set of tubes was placed at 50°C. Samples were removed at 2 hour intervals, and the numbers of surviving colony-forming units/ml were determined by the standard spread plate method on Luria plates. No ampicillin was used in the experiment. Sixty-four percent of the bacteria overproducing B23 survived the 50°C incubation, while the survival rate of the nonproducing B23 bacteria was less than 5% (Figure 1).

To determine if the overexpressed B23 contributed to the high survival rate of the test group, only one sample of B23 expressing bacteria was induced with

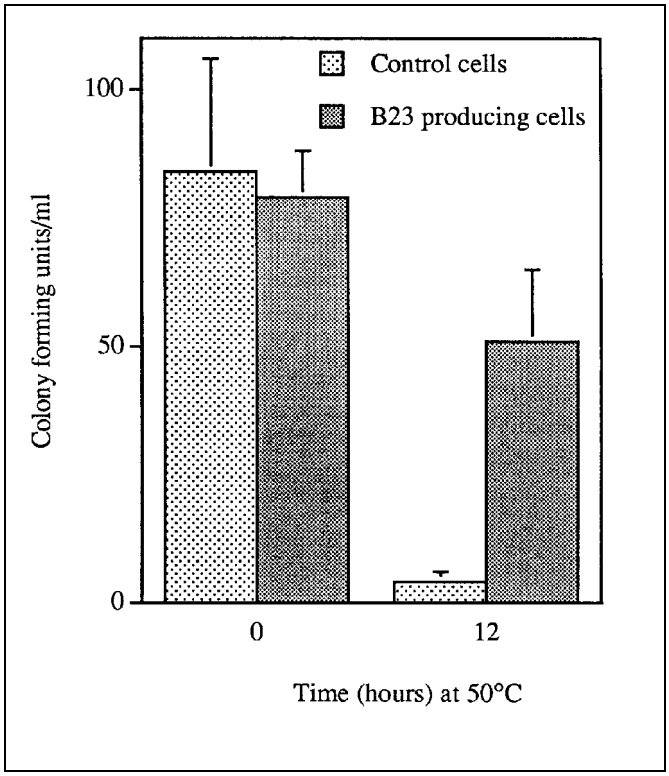

Figure 1. Survival rate of *Escherichia coli*. The cells producing B23 had a higher survival rate after incubation of 50°C than the cells that did not produce the chaperone. The data are shown as the mean ± SE from four determinations.

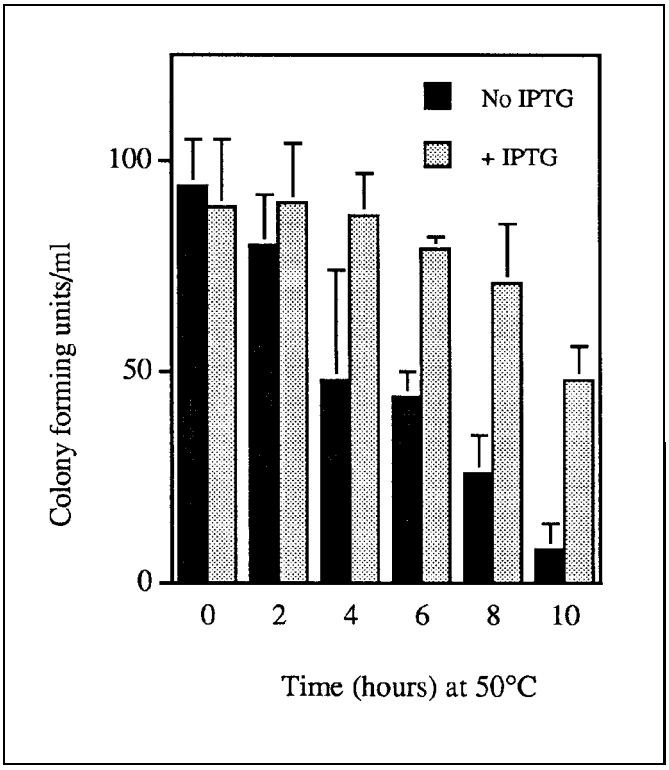

Figure 2. Protection of *Escherichia coli* against elevated temperature after induction of protein B23 expression. IPTG induced *E. coli* had a higher survival rate after incubation at 50°C than the uninduced cells. The data are shown as the mean ± SE from four determinations.

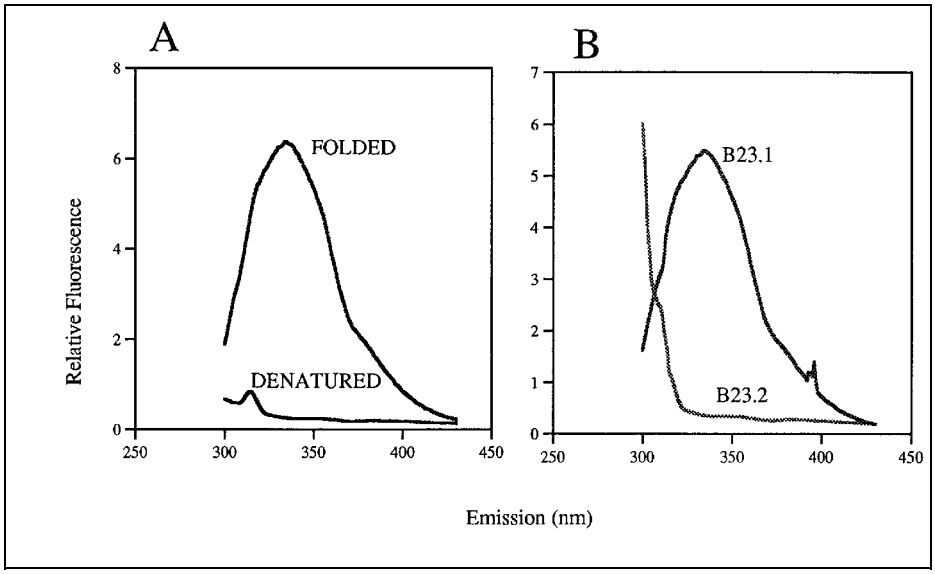

Figure 3. The intrinsic fluorescence emission spectrum of LADH and B23.1 and B23.2. Panel A: LADH was denatured in GdmHCl and the fluorescence emission spectrum was measured (excitation at 285 nm). Panel B: B23 isoforms were measured under the same conditions as LADH.

IPTG (Figure 2). Both groups were incubated at 50°C for 10 hours and samples were removed every 2 hours. The numbers of surviving colony-forming units/ml were determined and compared with the IPTG lacking control. It was found that the number of surviving bacteria in the control decreased more rapidly than in the IPTG induced group. It should be noted that the weak survival rate of the control group was due to some B23 production even without induction of IPTG. The B23 production in these cells was shown with western blotting. These experiments suggest that eukaryotic B23 found a function for itself in a prokaryotic cell.

2) Developing Refolding Chromatography

Which Chaperone Substrate is a Good Model for Refolding Chromatography?—To show that protein B23 isoforms possess chaperone-like activities and to find a suitable model enzyme for the refolding chromatography, liver alcohol dehydrogenase was used as it has previously been shown to serve as a substrate for chaperones (Szebeni and Olson, 1998).

Conditions of assay temperature and concentration of LADH were chosen to ensure that aggregation of the unfolded protein could be measured in the light scattering assay. The light scattering measurements showed that at a concentration of 100 µg/ml, LADH aggregated when the temperature was increased from 0 to 48°C, reaching maximum turbidity in about 40 min. However, the aggregation was largely prevented when a 1:1 molar ratio of protein B23 was added to the incubation medium containing LADH. Both isoforms showed the same kind of chaperone effect on LADH thermal aggregation, suggesting that the unique C-terminal end of protein B23.1 does not contribute to its chaperone-like activity. In control experiments the presence of excess bovine serum albumin (BSA) in place of protein B23 did not prevent the increases in aggregation. These data suggest that both isoforms are suitable for refolding chromatography and LADH is a convenient model protein for testing refolding chromatography.

Is the Structure of LADH Protected by Protein B23?—The thermal aggregation of LADH at 48°C is relatively a simple assay, but it is crude and does not mirror the subtle structural changes of the protein. To follow the more delicate structural changes of the protein, the intrinsic fluorescence of the protein was measured. The fluorescence of proteins originates from phenylalanine, tyrosine and tryptophan residues. LADH has two tryptophan residues. For the measurement of intrinsic fluorescence of LADH, 285 nm excitation and 335 nm emission was selected which is the selective wavelength for tryptophan residues (described in Materials and Methods). To observe the chaperone-like activity of B23 toward LADH, B23.2 isoform was used because this isoform has no tryptophan residues but it shows the same kind of chaperone activity. Figure 3 shows the difference between the spectrum of LADH and the two B23 isoforms. LADH was denatured with 6 M GdmHCl in 100 mm phosphate buffer for 15 min and then diluted (85-fold) with phosphate buffer. The changes in the emission spectrum of the denatured LADH were measured in the presence and absence of protein B23. Both samples were diluted the same way, and the results are shown in Figure 4. The slow return to the native state (spontaneous refolding) was observed in the emission spectrum and it was significantly faster when a 1:1 molar ratio of protein B23.2 was added to the denatured enzyme. These data further suggest that

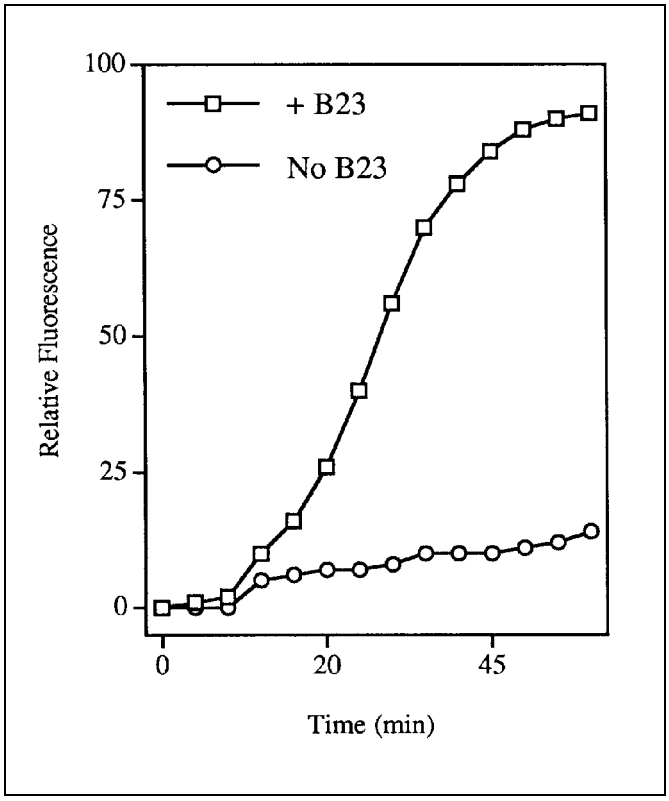

Figure 4. Reconstitution of intrinsic fluorescence of LADH in the presence and absence of protein B23. LADH was denatured with 6 M GdmHCl and diluted 85-fold with phosphate buffer. The changes in the emission spectrum of the denatured LADH were measured in the presence and absence of protein B23 (285 nm excitation and 335 nm emission).

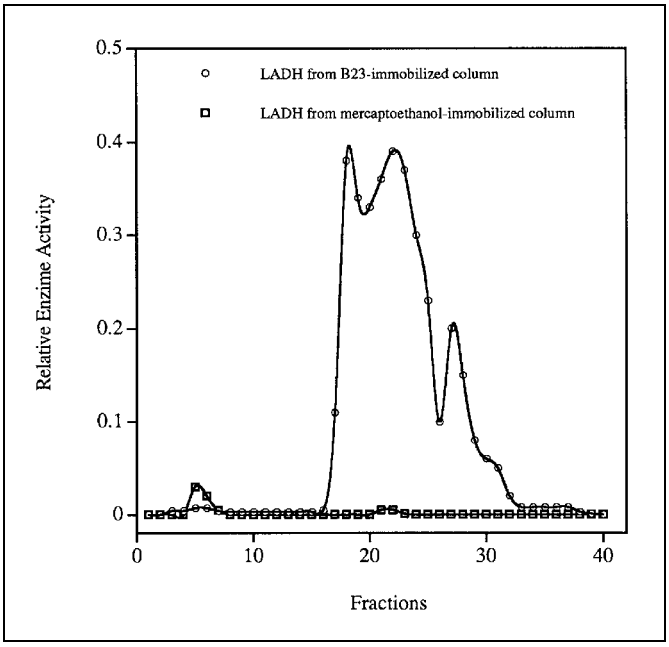

Figure 5. Refolding of LADH in the presence of immobilized protein B23.1. GdmHCl denatured LADH was loaded onto and eluted from a column featuring either immobilized protein B23.1 or immobilized mercaptoethanol. The enzyme activity of the fractions was measured.

B23 is able to act as a chaperone protein not only protecting LADH from thermal aggregation, but also helping in its refolding.

Protein B23 Protects the Catalytic Activity of Liver Alcohol Dehydrogenase—Enzymatic activity is a more sensitive measure of the native state of a protein than the aggregation assay. LADH is a NAD^+ dependent dehydrogenase and the catalytic activity of the enzyme may be assayed by measuring the change in absorbance at 340 nm that accompanies the interconversion of NAD^+ and NADH coenzyme. LADH was denatured in 6 M GdmCl and diluted (85-fold) as aforementioned.

The returning activity of the denatured enzyme was measured in the presence and absence of protein B23 at a molar ratio of 1:1 (B23 : LADH). This assay was based on the findings of Szebeni and Olson (1998). The enzyme was fully unfolded and de-activated after GdmCl denaturation, and in the absence of B23, the activity came back very slowly. When B23 was present, 74% of the enzyme's activity was detected after only 60 minutes. In the control experiments it was found that the addition of B23 to native solution of LADH did not influence the specific activity of the enzyme. The dramatic effect of B23 on the refolding of de-activated LADH is an important indication that B23 is a chaperone protein.

Immobilization of Protein B23 to Matrix—The idea behind the refolding chromatography was to immobilize a protein that showed chaperone-like activities *in vitro*, and employ it as a continuous "refolding machine." For this purpose protein B23 was linked to BioRad Affi-Gel 10 with the help of Sulfo SMCC as reported earlier (Szebeni, 1996) and as described in Materials and Methods. LADH was denatured in 6 M GdmHCl and diluted as already mentioned. One ml of this diluted LADH was mixed gently with 1 ml immobilized B23-Affi-Gel mixture for one hour at room temperature. Affi-Gel 10 linked with β-mercaptoethanol was used as control. After one hour of incubation, the mixture was centrifuged and in the (200 µl) supernatant LADH activity was measured. The B23-Affi-Gel sample showed 12 times greater catalytic activity than the control. The protein

concentration in the supernatant was determined by Bio-Rad protein assay. There was no significant difference detected, therefore the increased enzyme activity is the result of the refolding.

***Refolding Chromatography*—**In the aforementioned experiment the suitability of immobilized B23 for column chromatography was indicated. Five ml LADH enzyme was denatured by GdmHCl and diluted as described above. Then it was added to the column (16 x 30 mm) and was eluted with 50 mm phosphate buffer, pH 7.4, at slow pump speed (100 µl/min) at room temperature. Fractions of 100 µl were collected and the activity of the enzyme was measured (Figure 5). The fractions showed high LADH activity after 3.5 ml elution volume. From the control Affi-Gel 10 column, with immobilized β-mercaptoethanol, no active LADH was eluted.

DISCUSSION

The results presented here support that the overexpression of foreign chaperone proteins in *E. coli* can be an important tool in biotechnology and genetic engineering; eukaryotic chaperones can help bring about the correct folding of eukaryotic proteins in a prokaryotic cell. It was further shown that in an *in vitro* environment a continuous "protein folding machine" can be developed and, after further improvement, could be used for large scale recombinant protein purification. It is possible that not all of the B23 molecule plays a part in refolding. If this is so, the immobilization of the chaperone active sequence of B23 would be a possible step ahead. This could increase the life expectancy of the column. Interestingly, the covalently linked protein B23 to CNBr activated Sepharose 4B did not show any signs of chaperone activity. This suggests that for B23 to function as a chaperone the protein needs moving space. This observation will be useful in the future development of refolding chromatography methodology.

ACKNOWLEDGMENTS

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The Agricultural Potential of Estuarine Waters

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Because of the current strain on the world's fresh water supply, a study was designed to investigate whether estuarine waters could be used for limited periods of time as an alternate water source in the irrigation of rice, the staple food for over half of the world's population. Two test groups, each containing five replicate chambers of *Oryza sativa* var. Cypress, were used to test the effects of salinity on rice during two different stages of the life cycle. The first test group was exposed during the late vegetative (earlier) stage and the second group was exposed during the reproductive (later) stage. Chlorophyll concentrations were measured after the plants were exposed to varying salinities for three weeks. Plants in both groups were then grown to maturity in order to determine the effects of various salinities on yield production and root length. Root lengths were previously found to be a sensitive indicator of salt stress. Exposure to the highest salinity (8 ppt) during the vegetative stage caused only a slight loss of chlorophyll. However, exposure to 8 ppt during the reproductive stage resulted in a significant loss of chlorophyll. Grain production and root lengths were more affected by saline exposures during the reproductive stage. Growth in rice exposed during the vegetative stage was much more stable. Results of this study indicate that estuarine waters with salinities no higher than 8 ppt. can be safely used for rice cultivation during the vegetative stage for periods up to three weeks. This data may be of most significance during droughts when estuarine waters could be appropriately substituted for fresh water.

Irrigation, population, and industry have put extreme demands upon Earth's supply of fresh water, demands which will continue to escalate as the world's population continues to grow. As these trends progress, the necessity of water conservation also increases. Water conservation becomes especially important in times of severe water shortage. Rice, the world's most important agricultural crop, requires extraordinary amounts of water for growth, and is, therefore, of utmost concern when discussing agricultural water conservation. In Asia, for example, where over half of Earth's population resides, rice is the staple food (Williams, 1996), and a severe water shortage there would be devastating to the many people completely dependent on rice. Because of possibilities such as this, a study was designed with the purpose of discovering a method for reducing the consumption of fresh water in agriculture. This study specifically investigated the use of estuarine waters as an alternate for or a supplement to fresh water for limited periods of time in the cultivation of rice.

An estuary is a semi-enclosed coastal body of water which is freely connected to the open sea and at least one fresh water source, usually a river or stream. As such, in an estuary, sea water is measurably and continually diluted with fresh water. Because of this lower salinity, as well as a high level of organic material, a great diversity of plant life flourishes in estuaries that could not survive in full strength sea water (Weber et al., 1992). These facts led the researchers to hypothesize that estuarine waters may serve as an alternative or supplemental water source in the irrigation of rice.

Rice was chosen as the test plant for this study

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primarily because of its adaptability and its importance to millions of people world wide. It provides 25 to 80 percent of the calories in the daily diet of 2.7 billion Asians, more than half of the world's population. In 1993, rice harvested around the world amounted to 520 million metric tons, and nearly all of this went towards human consumption (White, 1994). Rice is, in short, the most important food crop in the world today, and a poor harvest could be devastating to millions of people. Fortunately, rice is also one of nature's most adaptable plants, an important trait when considering the possibility of using estuarine waters as a supplement or a substitute for fresh water in the cultivation of rice (White, 1994).

This study has been developed over three years. Preliminary experimentation showed that *Zizaniopsis milacea*, Southern Wild Rice, could germinate in salinities of up to 3 parts per thousand (ppt) which first established in this study that rice did have some tolerance to saline waters. There was, however, an observable drop in root length and shoot height of rice seedlings grown in saline solutions as low as 3 ppt. Preliminary investigations also demonstrated that, of the parameters measured (root length, shoot length, dry biomass, and wet biomass), root length was the most sensitive indicator of a salt stress in young rice.

The main experimental emphasis following preliminary investigations was the measurement of the effects of saline waters on later stages of the rice life cycle. It was hypothesized that mature rice could withstand higher salinities and longer periods of exposure than the juvenile rice could in the preliminary investigations. This hypothesis was tested by observing and measuring the effects of sustained salinity on the two later life cycle stages of *Oryza sativa* var. Cypress rice.

There are four distinct stages in the life cycle of the rice plant. The first stage is germination. The second is the vegetative stage which lasts about 55 days and ends when the panicles first form. The third is the reproductive stage which usually lasts about 35 days and ends at flowering. The fourth and final stage is the ripening stage which lasts about 30 days and ends with harvesting (Vergara, 1970). Many texts, however, divide the life cycle of the rice plant into only two stages, vegetative and reproductive (Miller, 1994). These "two" stages became the basis for the objectives of this study which were [1] to investigate and [2] to compare the salinity tolerance of late-vegetative rice and reproductive rice.

MATERIALS AND METHODS

***Design*—**Wooden frames were built in order to hang lights directly over the rice. Two Sylvania Gro-Lux lights and two 100 watt fluorescent lights were hung approximately six inches above each group of plants. The lights were moved up as the rice grew. In addition, a 1000 watt Metal-Halide lamp was suspended in the center of the growth area. Ten growth chambers were constructed from Rubber-Made tubs fitted with drainage apparatuses. Each chamber had a layer of gravel under a layer of soil about 25 centimeters deep to prevent sediment loss. Each chamber contained 30 plants grown from seeds planted an inch beneath the top of the soil. The ten chambers, were divided into two groups of five which were designated Block 1 and Block 2. Block 1 contained plants tested during the late vegetative stage. Block 2 contained plants tested during the reproductive stage. One chamber in each block served as a control. The other four test chambers, each received a salinity treatment of 1, 2, 4, or 8 parts per thousand. The saline solutions were made using commercial ocean salts (Forty Fathoms Sea Salts) which contain many of the elements found ionized in estuarine waters. The salinity treatments lasted three weeks.

After 50 days of growth, the plants in Block 1 received their salinity treatment. Normally, at day 50, rice is approaching the reproductive stage which indicates that there are only a few days remaining in the average vegetative stage. However, because of an initial problem with light intensities, it was felt that the growth stages might have been slightly delayed. This assumption proved correct when the rice began panicle formation, which signifies the beginning of the reproductive stage, about 30 days late (Vergara, 1970). After three weeks of treatment, the plants in Block 1 were flushed with fresh water, and for the duration of the study, these plants were grown in fresh water. Salinity treatments on the rice plants in the five chambers of Block 2 started at day 100. After three weeks of treatment, the soil in these five chambers was flushed with fresh water. At this time, all of the chambers in Blocks 1 and 2 were drained in preparation for harvesting. The rice was harvested after 141 days of growth.

Rice requires large amounts of nitrogen for growth. In this study, 18.7 g of nitrogen rich (20% N) rose

fertilizer was added to each chamber. This translated into 120 pounds of nitrogen per acre, the amount of nitrogen required under field conditions (Miller, 1994). All of the required nitrogen was worked into the soil before the rice was planted. However, because of the large amount of drainage that was required for the salinity treatments, an additional nitrogen application of 4.7 g of fertilizer per chamber (30 lbs/acre) was administered on day 50. During experimentation, there was a problem with algae growth. To combat this, all of the algae was removed from the water manually, and 0.5 g of $CuSO_4$ in solution was distributed to each chamber.

Test Conditions and Routine Monitoring—During this study several parameters were measured on a routine basis. Temperature was measured twice daily. The low was measured around 7:30 AM before the lights came on. The average low temperature was approximately 24° C. The high was measured around 3:30 PM after the lights had been on for 8 hours. The high temperature was usually close to 30°C although it was much warmer on several occasions. The lights were on for 14 hours daily. The pH of the water was measured daily and then three times a week when it became apparent that pH was remaining fairly constant. The water remained slightly alkaline throughout experimentation. A soil analysis, performed by Mississippi State University before the rice was planted, indicated that the soil was slightly acidic (pH of 6.9). An analysis performed after the test found that the soil had become basic (pH of 7.8). Also, as expected, the Na concentration in soils exposed to saline waters was higher than in unexposed soils. The salinity of the test water was measured every other day during salinity treatments. After the first week of both treatments, the saline waters were drained, and freshly prepared saline waters were introduced. This was done because the salinity dropped slightly during that first week, mostly due to dilution with leftover fresh water from the initial draining of the growth chambers.

Test Response Measurements—Immediately after each three week salinity treatment was completed a chlorophyll analysis was carried out. Approximately eight inches of leaf was cut from fifteen plants in each chamber. Leaves were then clipped into small pieces and two representative samples weighing approximately 0.5 g were taken from each group of plant material. Samples were homogenized using a Pro-Scientific 250 homogenizer and placed in 90% acetone to extract the chlorophylls. The samples were then centrifuged and the solution was decanted off. Adsorption readings were measured on a Perkin-Elmer UV Visible Spectrophotometer at 663, 645, and 630 nm with chlorophylls a and b quantified using SCOR/UNESCO equations and concentrations converted to mg/g wet weight of plant material (Strickland and Parsons, 1968).

Grain production of rice was also measured and compared. Grain harvest began with the cutting of the new rice from the plants while it was still on the panicle. The rice was removed from the panicle by placing it inside a tire inner tube and beating it on the ground. Then the rice from each chamber was weighed collectively on a Sartorius analytical balance.

Maximum root length of individual plants was determined by collecting 15 plants from each chamber. The longest root on each plant was measured. Mean root length was calculated for each salinity level tested during the vegetative and reproductive stages.

Statistical Treatment—One way analysis of variance (ANOVA) was applied to the mean values of chlorophylls *a* and *b* and mean root lengths of plants exposed during the vegetative and reproductive stages. A least significant difference multiple range test was then applied to identify groups of means within each of the data sets that could not be distinguished statistically at the 95% confidence level [$p<0.05$] (Statgraphics Plus, 1995).

RESULTS AND DISCUSSION

Salinity Effects on Root Length—Although the root lengths of germinating and young seedlings are very sensitive to salt stress, ANOVA results indicated that root length was not significantly affected by the test salinities used during the more mature vegetative stage of rice development (Figure 1). Mean root length at the highest salinity during the reproductive stage increased significantly as compared to the control with no significant differences seen at lower salinities (Figure 1). Although the controls in Block 1 (vegetative stage) and Block 2 (reproductive stage) were slightly different, data comparisons were made only between plants in the same block. Hence, plants exposed during the vegetative stage were only compared to the control in Block 1. This allowed a more accurate depiction of the effects of salinity in the event that growth conditions were slightly different between Blocks 1 and 2.

Salinity Effects on Biomass of Grain Yield—Rice yields from plants tested during the vegetative stage

remained constant with increasing salinities up to 4 ppt as compared to the control but dropped at 8 ppt (Figure 2). Rice yields from plants tested during the reproductive stage were moderately elevated as compared to the control with peak production occurring at 2 ppt (Figure 2).

Chlorophyll Responses of Plants Exposed to Saline Conditions—There were no noticeable changes in plant coloration at any salinity level during the vegetative or reproductive stages. In rice exposed during the vegetative stage, concentrations of both chlorophylls *a* and *b* increased slightly as compared to the control in salinity levels of up to 4 ppt. At 8 ppt, the concentration of chlorophyll *a* dropped slightly below that of the control and the concentration of chlorophyll *b* dropped back to that of the control. Neither concentrations were statistically different from the control at 8 ppt (Table 1). In rice exposed during the reproductive stage, concentrations of chlorophylls *a* and *b* exhibited an overall downward trend as salinities increased. In fact, levels of chlorophyll *a* dropped by 43% when plants were exposed to 8 ppt. Chlorophyll *b* decreased by 44% at 8 ppt. Both chlorophyll concentrations at 8 ppt were statistically different from the control.

It is clear from these data that saline waters with lower salinities (1, 2, and 4 ppt) exert minimal effects on *Oryza sativa* var. Cypress. The higher salinities (mainly 8 ppt) had a noticeable effect on the rice in this study although plants grown in 8 ppt were still quite capable of producing rice. In fact, there was no noticeable drop in yield production in rice exposed during the reproductive stage and only a slight drop in yields from rice exposed during the vegetative stage. Chlorophyll concentrations, which are sensitive indicators of salt stress even when visible symptoms are not apparent (Velageti et al., 1990), showed that rice exposed to salinities during the vegetative stage was more capable of withstanding the salt stress than rice exposed during the reproductive stage. Root lengths and yield biomasses supported the chlorophyll data by exhibiting a stronger salinity effect when the plant was exposed during the reproductive stage. All growth measurements were much more stable in rice exposed during the vegetative stage.

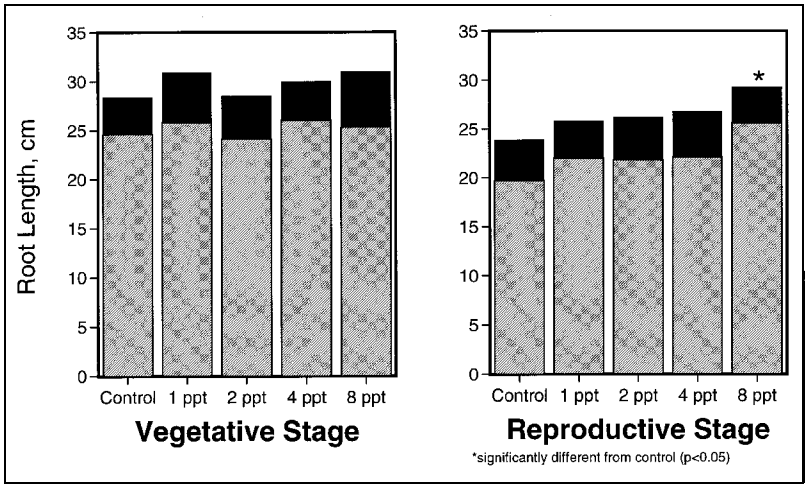

Figure 1. Effects of salinity exposure at two growth stages on root length of *Oryza sativa* var. Cypress rice. (Note: solid black bars represent standard deviation.)

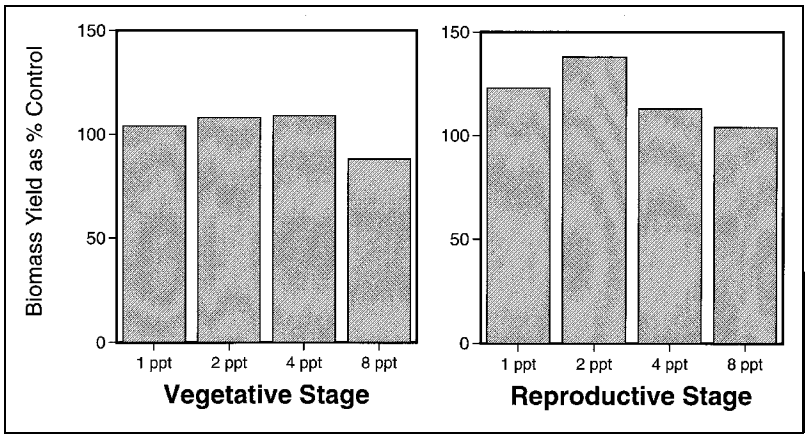

Figure 2. Effects of salinity exposure at two growth stages on yield production of *Oryza sativa* var. Cypress rice.

An interesting trend noticed in this study was that as salinity levels increased, growth responses tended to increase as well. This phenomenon is not uncommon and has been noticed particularly in pesticide studies where low levels of stress actually produced positive growth results (Lytle and Lytle, 1996).

CONCLUSIONS

The researchers feel that there is potential in using estuarine waters to safely irrigate rice during periods of water shortage. Based on this study, the length of time using estuarine waters should not exceed three weeks at a maximum salinity of 8 ppt although further research may prove a higher tolerance of saline exposure. While plants exposed during the reproductive stage were still capable of producing rice, the optimum time for irrigating with estuarine waters

is during the vegetative stage. This means that the rice farmer should wait a substantial amount of time after germination to begin using estuarine waters and should discontinue irrigation of rice with estuarine waters after flowering. During germination and grain production rice simply cannot handle the stress of saline exposure.

Applicable uses of this data may not occur under normal circumstances (although there are places, such as lower Louisiana, where salt is entering the ground water). However, during times of drought or other such periods of water shortage where locally grown rice is the staple food, the use of estuarine waters to irrigate rice would be possible and beneficial as long as the criteria mentioned above are adhered too. This would definitely reduce the amounts of fresh water being used in rice cultivation which would leave more fresh water for humans, who do not have the capability, as does rice, of surviving on saline waters.

Table 1. Effects of salinity exposure at two growth stages on concentrations of chlorophyll pigments of *Oryza sativa* var. Cypress rice.

	Vegetative Stage		Reproductive Stage	
Treatment	Chlorophyll *a* mg/g	Chlorophyll *b* mg/g	Chlorophyll *a* mg/g	Chlorophyll *b* mg/g
Control	3.61±0.02[a]	1.08±0.01[a,b]	3.46±0.33[a]	1.01±0.10[a]
1 ppt	3.78±0.04[b]	1.14±0.01[b,c]	3.04±0.01[b]	0.879±0.003[b]
2 ppt	3.94±0.01[c]	1.19±0.01[c]	3.45±0.06[a]	1.01±0.01[a]
4 ppt	4.32±0.08[d]	1.33±0.04[d]	2.96±0.03[b]	0.851±0.002[b]
8 ppt	3.51±0.06[a]	1.08±0.02[a]	1.96±0.03[c]	0.567±0.004[c]

Values are means of two replicates ± standard deviation; values not sharing letter in column are statistically different ($p<0.05$).

ACKNOWLEDGMENTS

The researchers gratefully acknowledge the tireless help of Dr. Julia Lytle, Dr. Tom Lytle, and the Environmental Chemistry Department of the Gulf Coast Research Laboratory in Ocean Springs, Mississippi. The researchers further acknowledge the valuable assistance of Dr. Richard T. Dunand of the Louisiana State University Rice Research Center, Dr. Ronald L. Sass of Rice University, and Dr. Ted Miller of the Mississippi State University Agricultural Experiment Station. The researchers finally thank the Ocean Springs High School Science Department and especially Mr. Joe Mason for four years of encouragement and support.

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Chemistry and Chemical Engineering Division Report

Thirty-three (33) presentations of papers were presented along with 16 posters. The topics ranged in scope from biochemistry to the chemistry of concrete.

For comparison, the current chair reviewed old programs of the nineties and late eighties and found the following numbers of presentations and posters.

Year	Papers Presented	Papers as Posters	Total
1986	36	0	36
1987	28	0	28
1988	56	3	59
1989	42	2	46
1990	17	6	23
1991	30	3	33
1992	49	7	56
1993	39	0	39
1994	58	4	62
1995	49	19	68
1996	29	13	42
1997	28	18	41
1998	33	16	49

If a trend can be observed, it is probably the increase in papers as posters. From this writer's perspective this is possibly due to more undergraduate papers being presented in this manner and not as talks. In the papers this year only three of the talks were from undergraduate institutions while half of the posters were so identified. Such a trend will possibly please those in charge of arrangements. Fewer meeting rooms will be needed. However, this writer finds this trend disturbing. He would prefer to see more undergraduate and graduate students getting the experience of speaking and responding to questions from an audience of mostly strangers. This develops much more poise than speaking one-on-one in a poster presentation.

Several years ago, the division sponsored a symposium on the teaching of physical chemistry. Perhaps it is time for the division to sponsor another educational symposium. Some may feel that this should be done in the Science Education Division. However, this writer visited one session of that division and found it to be, with a few exceptions, primarily papers on secondary and elementary education with little on teaching the real content of college level chemistry.

This year's business meeting had reasonably good attendance. For next year, Dr. Edward Valente of Mississippi College was elected chair and Dr. Ken S. Lee of Jackson State University was elected vice-chair. Also at the business meeting an extended discussion took place concerning the abstract submission date of October 1. Over the years the date has apparently crept up from November 15, to November 1, and now October 1. It was expressed that most undergraduates do not have sufficient work completed by this date to see the final direction of their investigations and prepare abstracts. The division is sensitive to the difficulties that the editors face in preparing the program but would appreciate any relief in a later submission date. This expression was unanimous from the division. Some members of the division also reported difficulties in trying to make E-mail submissions of abstracts.—R.A. Berry

First call for abstracts for the 1999 Annual Meeting of the Mississippi Academy of Sciences

The Mississippi Academy of Sciences
will hold its **1999** annual meeting
Thursday and Friday, **February 25 and 26**
at the Ramada Inn in
Tupelo, Mississippi

Abstracts are due no later than 2 November 1998. A form for abstract submission is included in this issue.

MISSISSIPPI ACADEMY OF SCIENCES ABSTRACT FORM/MEMBERSHIP FORM

ABSTRACT INFORMATION

Abstract title _____

Name of presenting author(s) _____

Telephone _____ Email _____

Name of Academy member _____

(One author must be a current member of the MAS; 1999 membership dues must be paid.)

Check the division in which you are presenting

___ Agriculture and Plant Science	___ Health Sciences	___ Psychology and Behav. Neuroscience
___ Cellular, Molecular and Dev. Biology	___ Math., Computer Sci. and Statistics	___ Science Education
___ Chemistry and Chemical Engineering	___ Marine and Atmospheric Sciences	___ Social Sciences
___ Geology and Geography	___ Physics and Engineering	___ Zoology and Entomology

Type of presentation

___ Poster presentation ___ Workshop
___ Lecture presentation ___ Invited symposium

If the presenting author for this paper is also presenting in another division, please list the other division: _____

Audio-visual equipment needs

___ 2" x 2" slide projector
___ Overhead projector

Other audio-visual equipment including computers and computer projection equipment must be provided by the speaker.

MEMBERSHIP INFORMATION

New ___ Renewal ___

Mr. Ms Dr. _____

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Telephone _____ Email address _____

PLEASE INDICATE DIVISION WITH WHICH YOU WISH TO BE AFFILIATED _____

Regular member $25	Student member $5	Life member $ 250
Educational $150	Corporate Patron $1000	Corporate Donor $500

CHECKLIST

The following MUST be DONE:

___ ★ Enclose copy of abstract (even if abstract has been submitted electronically)
___ ★ Complete and enclose abstract form /membership form(this form)
___ ★ Enclose the following payments (make check payable to Mississippi Academy of Sciences):
  ___ $25 per abstract
  ___ $25 regular membership fee OR $5 student membership fee (1999 membership must be paid for abstract to be accepted)
___ ★ You must supply a check #_____ or P.O. #_____ (credit cards are not accepted)

In addition you MAY preregister at this time:

___ Enclose the following payments:
  ___ $12 regular member (Preregistration before Feb. 1, 1999)
  ___ $5 student member (Preregistration before Feb. 1, 1999)

NOTE: Late abstracts will be accepted with a $10 late fee and only if there is room in the appropriate division. They will be published in the April issue of the MAS JOURNAL.

MISSISSIPPI ACADEMY OF SCIENCES—ABSTRACT INSTRUCTIONS
PLEASE READ ALL INSTRUCTIONS BEFORE YOU SUBMIT YOUR ABSTRACT

Your paper may be presented orally or as a poster. Oral presentations are generally 15 minutes. The speaker should limit the presentation to 10–12 minutes to allow time for discussion. Instructions for poster presentations are given on the reverse side of this sheet.

Enclose a personal check, money order, institutional check, or purchase order for $25 publication charge for each abstract to be published, payable to the Mississippi Academy of Sciences. The publication charge will be refunded if the abstract is not accepted.

At least one author must be a member of the Academy at the time the paper/poster is presented. Payment for membership of one author must accompany the abstract.

Attendance and participation at all sessions requires payment of registration. Complete pre-registration information will be sent in January.

Note that two separate fees are associated with submitting a paper for presentation at the annual meeting of the Mississippi Academy of Sciences. An abstract fee is assessed to defray the cost of publishing abstracts and a membership fee is assessed to defray the costs of running the Academy. Preregistration payment ($12 regular; $5 student) may accompany the abstract, or you may elect to pay this fee in January or pay full registration costs at the meeting.

Abstracts may be submitted typed or printed on clean white paper. Abstracts received in this form will be scanned into a computer. Leave ample margins and use a sanserif type font to help minimize errors in scanning.

Abstracts may be submitted as a WordPerfect (Windows or DOS), ASCII, ANSI, or .RTF file on a PC readable diskette. Formatting should be minimal. This abstract submission form and the appropriate fees should be sent by US mail even if a diskette is used for the abstract.

Abstracts may be submitted by e-mail or entered directly through the MAS website. The URL is http://www.msstate.edu/Org/MAS/ MAS.HTML. This abstract submission form and the appropriate fees should be sent by US mail even if the abstract has been submitted electronically.

Submit your abstract and appropriate fees to the Abstracts' Editor, John Boyle, TO BE RECEIVED NO LATER THAN NOVEMBER 2, 1997.

Dr. John Boyle
Mississippi State University
Dept. of Biochemistry
P.O. Drawer 9650
Mississippi State, MS 39762

FORMAT FOR ABSTRACT

Your abstract should be informative, containing: (a) a sentence statement of the study's specific objectives, unless this is given in the title; (b) brief statement of methods, if pertinent; (c) summary of the results obtained; (d) statement of the conclusions. It is not satisfactory to state, "The results will be discussed."

Your abstract, including a concise, descriptive title, author(s), location where work was done, text and acknowledgment, may not exceed 250 words.

The title should be all capital letters. Use significant words descriptive of subject content.

Authors' names start a new line.

The institution where your research was done should include city, state, and zip code. Do not include institutional subdivisions such as department.

The abstract should be one paragraph, single spaced, starting with a 3-space indentation.

Use standard abbreviations for common units of measure. Other words to be abbreviated, such as chemical names, should be spelled out in full for the first use, followed by the abbreviation in parenthesis. Do not abbreviate in the abstract title.

Special symbols not on your printer or typewriter must be in black ink.
Use italics for scientific names of organisms.
Begin authors' names on a new line. Place an asterisk (*) after the presenter(s), if there are multiple authors.
Use superscripts for institutional affiliations where necessary to avoid ambiguity.
Refer to these examples as guides.

EXAMPLES OF TITLES AND AUTHORS:

[single author, no ambiguity about designated speaker or affiliation]
AN EXPERIMENTAL MODEL FOR CHEMOTHERAPY ON DORMANT TUBERCULOUS INFECTION WITH PARTICULAR REFERENCE TO RIFAMPICIN
Joe E. Jones, Mississippi State University, Mississippi State, MS 39762
Abstract body starts here . . .

[two authors, one designated speaker, different affiliations, but no ambiguity]
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Abstract body starts here . . .

[two authors, both designated as speakers, different affiliations, but no ambiguity]
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Joe E. Jones[1], Ralph A. Smith[1]*, and Alice D. Doe[2], [1]Mississippi State University, Mississippi State, MS 39762 and [2]University of Mississippi Medical Center, Jackson, MS 39216
Abstract body starts here . . .

GUIDELINES FOR POSTER PRESENTATIONS

The Academy provides poster backboards. Each backboard is 34" high by 5' wide. Mount the poster on the board assigned to you by your Division Chairperson. Please do not draw, write, or use adhesive material on the boards. You must provide your own thumb tacks.
Lettering for your poster title should be at least 1" high and follow the format for your abstract. Lettering for your poster text should be at least 3/8" high.
Posters should be on display during the entire day during which their divisional poster session is scheduled. They must be removed at the end of that day.
Authors must be present with their poster to discuss their work at the time indicated in the program.