APPENDIX I

PROTEIN RESIDUE ANALYSIS

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I. INTRODUCTION

Subsistence studies have been a major avenue of archaeological research for the past 40 years because they can focus attention on such issues as prehistoric nutrition and demography, occupational seasonality, and methods of resource procurement, among other topics. The ability to identify the components of prehistoric diet is thus a primary tool in understanding patterns of human behavior.

The tidal lowlands of the Mid-Atlantic coastal plain contain biotic communities characterized by wide species breadth and high productivity. Locally abundant animals include numerous types of fish, shellfish, and waterfowl, plus rodents, turtles, and deer. The expansion of human populations in the coastal region has been credited in part to the diversity and richness of these available prey species (Custer 1989). As human population densities increased during the Late Archaic and Early Woodland periods, it is reasonable to assume that groups exploited greater numbers of species in an increasing variety of habitats. Changing patterns of faunal diversity and richness within human diet should therefore be apparent in the archaeological record (Byrd 1997; Reitz 1982). Unfortunately, much of the coastal plain, and Delaware in particular, labors under a burden of poor faunal preservation because of a combination of weak soil development and strongly acidic soils. The record of prehistoric subsistence in the region can therefore be read, in general, only indirectly, through functional analyses of artifacts and features, and by inferences gained from soil chemistry, pollen cores, or phytolith samples.

The detection of relict protein residues lifted off stone tools is, in contrast, a more direct approach to the identification of prehistoric faunal exploitation. The possibility of long-term survivability of organic proteins in archaeological contexts and the ability to index these proteins by taxa may provide an avenue toward understanding ancient subsistence exploitation when skeletal elements are absent. Beyond the mere identification of prey species, however, is the opportunity to map out the behavioral landscape of these resources, and in turn to clarify the scheduling and logistical planning used in the organization of subsistence strategies.

II. PROTEIN DETECTION AND IDENTIFICATION

The search for residues of proteinaceous materials on prehistoric tools evolved from forensic techniques developed initially from research on immunology and later expanded for law enforcement applications (Newman et al. 1998; Nutall 1901, 1904). G.H.F. Nutall was an early researcher of human blood groups and devised a method of detecting and identifying suspect blood. Subsequent discoveries revealed that all organic fluids and tissues were similarly detectable and potentially identifiable (Bjorklund 1952). The ability to detect and speciate blood and tissue at a crime scene or on criminal evidence was an important development in the repertoire of forensic medicine and continues to be an important tool today. Building on this research, Thomas Loy sought to demonstrate the possibility of detecting relict proteins on stone tools and identifying their species of origin (Loy 1983). Using lithic samples from western Canada, Loy reported positive results by means of a technique utilizing comparative crystal morphology to assess hemoglobin protein molecules. The crystalline forms of hemoglobin, being genetically determined, are in theory effective diagnostic markers for species of origin (Hyland et al. 1990:106). Loy reasoned that it would be a fairly straightforward matter to characterize and compare crystal shapes and arrive at a determination of species identification.

Because of the newness of the crystallography method, Loy's results went unchallenged for several years, but by the early 1990s at least three serious problems had surfaced in Loy's methodology to dampen enthusiasm for it. First, experimental research appeared to indicate that hemoglobin molecules were adversely affected by environmental exposure in the long term, such that proteins were sufficiently degraded to be incapable of crystallization (Smith and Wilson 1992:238). Second was the concern of sample

contamination voiced by Custer et al. (1988), Hyland et al. (1990:106), and Smith and Wilson (1992:238). They suggested that the introduction of environmental agents, such as salt crystals, would prove difficult to discriminate from protein crystals, thus obscuring a definitive identification. The third problem with crystallographic protein analysis was whether crystal structure could be unambiguously characterized by species. Remington (1994) and Smith and Wilson (1992) both concluded that this could not be achieved because of the fairly limited number of ways in which proteins can crystallize, making the identification process quite difficult. In addition, they both questioned whether optical analysis could be sufficiently rigorous to distinguish closely matched crystal forms. Loy has responded to some of these concerns (Loy 1994; Loy and Dixon 1998), but the consensus on the use of comparative crystal morphology as a tool for protein analysis has been that it is overly subjective, difficult to replicate, and inaccurate (Cattaneo et al. 1993; Downs and Lowenstein 1995; Marlar et al. 1995; Smith and Wilson 1992).

Crystallographic analysis is essentially a physical method of determining protein origins, relying on optical scans of crystal form to establish a phylogenic order of protein residues. Another method of residue detection is the Hemastix chemstrip, employed by Loy (1983) and others (Custer et al. 1988), which relies on a chemical reaction to indicate the presence/absence of blood. Hemastix is a color-responsive test that is highly sensitive to blood hemoglobin and found use as a quick and inexpensive means of screening specimens. Those exhibiting no reactions to the presence of hemoglobin could be eliminated from further testing, while positive reactions could be confirmed by other means, providing a good test of Hemastix accuracy. The Hemastix method, however, suffers from its reliance on hemoglobin as its target protein because of hemoglobin's limited survivability through time in exposed environments (Margaret Newman, personal communication 1998; Smith and Wilson 1992). When used as a screening device at several sites in Delaware, Hemastix yielded extremely low rates of positive reactions. Of 680 tools tested at the Leipsic Site, no positive reactions were recorded (Custer et al. 1996:112); at the Snapp Site, three positive reactions were obtained from 967 tests (Custer and Silber 1995:169-170). Further testing that may have confirmed these results was considered unwarranted because of the scarcity of positive reactions. It is suggested that the poor survival rate of hemoglobin may have been responsible for these discouraging results.

III. IMMUNOLOGICAL TESTING

The developmental path of protein residue studies took an important step forward in the late 1980s with the adoption of immunological methodologies that take advantage of the type of antigen-antibody reactions that are the basis of an organism's ability to resist infectious disease. Antibody molecules are proteins capable of chemically recognizing the surface structures of specific foreign proteins (antigens) and that bind with them, creating an immunity to the disease-causing agents (Loy and Dixon 1998; Malar et al. 1995). The presence or absence of antigen-antibody reactions and the specificity of each reaction are the foundation of an immunological approach to residue analysis. Antigens, as unknown residues, are paired with antibodies of known species-specific antisera to elicit potential reactions (Downs and Lowenstein 1995:13). There are multiple advantages to this kind of approach. It is easily reproducible, evaluates results using objective criteria, relies on well-established forensic techniques, and is sensitive to minute quantities of residue. Depending on the specific technique used, microgram (10^{-6}) to picogram (10^{-12}) concentrations are detectable (Marlar et al. 1995:28-29).

Several techniques utilizing immunological assays have been developed, each with specific strengths and weaknesses. All rely on the binding properties of antigen to antibody to reveal potential associations between residues and known antisera, but differ in the way these reactions are validated and measured. In the method called cross-over immuno-electrophoresis (CIEP), closely paired antigen and antisera will form a visible precipitate if there is a positive reaction between them. CIEP is sensitive to microgram concentrations of antigenic material (Marlar et al. 1995:29). One drawback of this procedure is that the strength of a positive

reaction is difficult to quantify and relies on an operator's visual inspection of the precipitate. Other methods overcome this problem with double-antibody techniques, the use of a second antibody as an immunoreactant label to detect and measure the signal strength of a reaction (Eisele et al. 1995:37-38): a radioactive tracer is linked to the second antibody by radio immunoassay (RIA) (Downs and Lowenstein 1995:14); a color-sensitive enzyme is used in enzyme-linked immunosorbent assay (ELISA) (Hyland et al. 1990); colloidal gold as the agent for gold immunoassay (GIA) is responsive to specific molecular weights of suspect proteins (Petraglia et al. 1998:540). Besides producing quantifiable results, these methods are between one to three orders of magnitude more sensitive than CIEP, with GIA reported to have the ability to detect picogram concentrations (Eisele et al. 1995:38, Marlar et al. 1995:28). Some of the disadvantages of the double-antibody techniques are potentially dangerous radioactivity (in the case of RIA) (Hyland et al. 1990:106), the fact that immunoglobulin target proteins have been experimentally shown to exhibit high susceptibility to environmental degradation (ELISA) (Cattaneo et al. 1993:40), and the concern that extreme sensitivity may induce false positive results through bacterial contamination (GIA) (Newman et al. 1996:679).

IV. CRITICISM OF CIEP

The focus of criticism regarding CIEP analysis essentially turns on one theoretical and one methodological issue. It is known that proteins degrade with time and exposure, but it remains unclear how far proteins can degrade and still be detected and identified by taxa (Cattaneo et al. 1993; Eisele et al. 1995; Fullagar et al. 1996; Loy 1994; Loy and Dixon 1998; Newman et al. 1996). Loy and Hardy (1992), at one end of the spectrum, claimed they were able to detect biologically viable immunoglobulin proteins on tools from a 90,000-year-old Neanderthal cave site in Israel. It is conceivable that the protection offered by the rock overhang of the cave slowed the normal progression of microbial decay of the proteins. The semi-arid conditions of the eastern Mediterranean may also be a contributing factor to the continued biological activity of some proteins. Other researchers, however, reject this time span as impossibly long. In separate experimental studies, Cattaneo et al. (1993) and Eisele et al. (1995) both noted the near total degradation of immunoglobulin proteins within months after burial of test bifaces in outdoor soils. It appears unlikely given these results that immunoglobulin proteins could survive for the lengths of time claimed by Loy and Hardy. The Cattaneo and Eisele experiments do indicate, however, that different proteins exhibit differential survivability. Albumin appears to have greater resistance to microbial decay than the immunoglobulins (α, β, and γ). This is significant for the current study because albumin is the target protein for this immunological testing program.

Of methodological concern is the relative cross-reactivity of the different immunological testing procedures. This relates directly to the specificity of the supposed taxonomic identifications, and can vary from species level to family or even order. Obviously, as the identifiable taxonomic units become more inclusive, or phylogenically broad, the data lose value as specific behaviors and habitats of individual species get lumped with increasingly divergent groups of animals. For example, a positive reaction to protein residue identifiable only to the mammalian order Carnivora, could include such disparate animals as the red fox, striped hyaena, harbor seal, and giant panda, among many others. Striking off non-indigenous species from the list of possible prey would eliminate having to explain how striped hyaena, harbor seal, or giant panda residues had made their way onto Eastern Woodland bifaces, but we are still left with a dizzying variety of native species with vastly different habitats and behaviors that could be eligible suspects for the detected proteins.

The problem of cross-reactivity has been linked with the performance of all immunological testing techniques, particularly for CIEP with regard to oversensitivity and accuracy (Cattaneo et al. 1993; Eisele et al. 1995; Fullagar et al. 1996). The claim of oversensitivity is a real concern for evaluating CIEP's reliability. If correct, this situation might be expected to produce a slew of false positive results that would be impossible to separate from true positive reactions. In response to this concern, the CIEP laboratory testing procedure

engaged for the Puncheon Run project was modified to increase specificity by diluting the solution of every positive reaction to 1:10 or 1:20 strength and retesting each positive sample. Only positive results from this final test were then reported.

V. REGIONAL CIEP STUDIES

Several studies utilizing the CIEP approach have been undertaken in the Mid-Atlantic region. At the Indian Creek V Site (18PR94) in Prince Georges County, Maryland, a two-step procedure used chemstrips as an initial screening device followed by CIEP testing (LeeDecker et al. 1991). Of 41 artifacts testing positive to the chemstrip procedure, 29 yielded positive results to protein residue analysis, a success rate of 64 percent. Eleven animal residues were identified at the family level, including, in order of frequency, Cervidae (deer), Bovinae (bison), Muridae (mouse), Tetraonidae (quail), Salmonidae (trout), Hominidae (human), Canidae (dog), Leporidae (rabbit), Caviidae (guinea pig), and Ursidae (bear). Also detected was a residue identified as nonspecific bracken fern (Polypodiaceae).

More equivocal results were obtained from the Two Guys Site (7S-F-68) in Sussex County, Delaware. In that study, 11 of 50 artifacts returned positive results for protein residue, a success rate of 22 percent, yet only one of seven artifacts determined positive by chemstrip yielded a positive CIEP result (LeeDecker et al. 1996). The low correlation between chemstrip and CIEP results suggests either that the chemstrip technique is not a reliable predictor of protein residues at the family level, or that CIEP itself lacks a consistent capacity to detect protein residues. Residues identified at the family level from Two Guys included Cervidae, Canidae, Caviidae, Leporidae, Bovinae, Tetraonidae, and Ursidae.

At four sites in the northern Virginia Piedmont, Petraglia et al. (1996) achieved a 20 percent success rate using CIEP on 100 artifacts. Seven residues at the family level were detected, including Anatidae (duck), Cervidae, Leporidae, Canidae, Felidae (cat), Ursidae, and Hominidae. A positive response to "rat/guinea pig" antiserum apparently indicates sensitivity at the order level (Rodentia). Significantly, by combining protein residue analysis with a study of microwear damage, Petraglia et al. were able to demonstrate a relationship between remnant residues and functional tool use. Microwear patterns identified as hide scraping were detected on two tools yielding cervid residue (1996:131).

Research along Kettle Creek, a tributary of the West Branch of the Susquehanna River in Pennsylvania, yielded positive CIEP results from the Kettle Creek West (36CN165) and Kettle Creek East (36CN199) sites (Petraglia, Knepper, and Rissetto 1998). This study produced a positive identification rate of 24 percent on 25 submitted artifacts. Identified residues included Cervidae, Canidae, and Leporidae. The authors noted that despite site placement on a high-order stream, there were no positive reactions to the Salmonidae antiserum, a family-level protein that is common to trout, salmon, char, and whitefish (1998:31). They concluded that the absence of reactions to fish may result from excessively small concentrations of antigens, sampling bias, or occupant preference for non-Salmonids. This test for fish is somewhat limited, however, because brook trout (*Salvelinus fontinalis*) is the only species indigenous to the inland river systems from the family Salmonidae (Cooper 1983).

VI. RESEARCH STRATEGY

In the present study, a contextual approach was used to refine the selection process of possible food sources for protein residue testing because of the diversity of native fauna in the Delaware coastal plain. Extensive wetlands intermixed with riverine and estuarine environments suggest that site occupants had access to the abundant aquatic resources surrounding them. Site catchment analysis, based on distance, abundance, ease of capture, nutritional value, and the processing time of resources, clearly orders fish and shellfish as high

ranking food sources. Given their high rank in any likely human diet within this ecozone, it seemed appropriate to search for fish residues on stone implements. This would accomplish two main goals. First, targeting locally available animals would satisfy Stuart Feidel's requirement that residue analysis be undertaken within a context of possible prey species rather than from the commercial availability of antiserum, and would have the effect of eliminating interpretations that were ". . .without obvious and demonstrable faunal associations" (Fiedel 1996:145). Second, by selecting fish with known behaviors and ranges, insights into issues relating to settlement seasonality, technology, and subsistence organization may be gained. A similar research strategy based on the ethology of deer was undertaken by John Cavallo (1991, 1994) and has proven useful in delineating patterns of prehistoric sites by moving beyond a simplistic view of cultural geography.

Little is known of the exact nature of fish populations in the Delaware Bay and its drainages during prehistory. Population dynamics and the diversity of species have been greatly influenced by the high level of industrial fishing during the last century, by the extent of industrial and residential pollutants, and by the intentional and unintentional introduction of non-indigenous species to the region. Although modern trawl data are not viewed as a particularly good fit to the characteristics of the early bay, the identities of native versus introduced fish species are fairly well established in the literature (Cooper 1983; Lee et al. 1976; Raasch and Altemus 1991; United States Geological Survey 1999).

Specific fish species were selected for capture and antiserum development based on several criteria. Abundance was viewed as an essential property of any sampled fauna because of the increased likelihood that a clustered and abundant food source would have been included in prehistoric diets. Fish that school or spawn in great number are more efficiently exploited than non-aggregated species (Butzer 1982:226; Reitz and Quitmyer 1988:105). Bay anchovy (*Anchoa mitchilli*), Atlantic croaker (*Micropogonias undulatus*), weakfish (*Cynoscion regalis*), and striped bass (*Morone saxatilus*) are among the most abundant aquatic resources in Delaware Bay, a factor used in the selection of these species.

Fish exhibiting diverse behaviors and habitats were chosen to gauge the variability of hunter-gatherer adaptations to spatial and seasonal factors. For example, American eel *(Anguilla rostrata*) leave the tidal creeks in large numbers during the fall to spawn seaward, a behavior known as catadromy. In contrast are anadromous, or upstream spawners, which move into the Delaware River and tidal streams during the spring and early summer. Sturgeon, alewife, gizzard shad, and striped bass are anadromous species included in this study.

Technological considerations play a role in human adaptational responses to animal behaviors, particularly as they relate to the size of potential prey species. The susceptibility to fishing gear is in large measure a factor of the size of the fish. Gill nets, for instance, are highly selective for catch size based on mesh width. Small fish swim through the net untouched, and very large species can back away from the net. Bay anchovy are the most abundant species in Delaware Bay but are small, necessitating the use of fine-mesh netting or basket technology as fishing implements. Thus the choice of gear will be determined by the kind of fish being targeted (Greenspan 1998:973). With this in mind, test specimens of variable sizes were selected to assess the range of possible fishing technologies practiced by the inhabitants of the Puncheon Run Site. Habitat also plays a role in selection of technology. Species that make their way up tidal creeks may have been most efficiently exploited by constructing weirs or tidal traps. Some large, open-water species, such as sturgeon and mature striped bass, are strong and capable of damaging nets. Gaffing, or spearing in combination with nets, is the most efficient means of catching large fish.

In all, 10 fish species were represented in the study (Table I-1). In addition to searching for aquatic resources, five terrestrial species were tested, consisting of deer, bear, rabbit, guinea pig, and turkey. Guinea pig (*Cavia porcellus*) is a non-indigenous mammal, native to South America. The suspected specificity of the antiserum

for guinea pig is at the order level, making it responsive to all members of Rodentia, including field mice, voles, squirrels, and woodchuck. Sub-order specificity would reduce cross-reactivity solely to the porcupine among North American species.

Table I-1: Antisera Specificity and Origin

Antiserum	Level of Specificity	Origin
fish		
Alewife *Alosa psuedoharengus*	not specific	Delaware Bay
American eel *Anguilla rostrata*	Order	Hudson River
Atlantic croaker *Micropogon undulatus*	Order	Delaware Bay
Atlantic sturgeon *Acipenser oxyrhynchus*	not specific	Delaware Bay
Bay anchovy *Anchoa mitchilli*	Species	Delaware Bay
Catfish Ictaluridae	Family	commercial
Gizzard shad *Dorosoma cepedianum*	Species	Hudson River
Striped bass *Morone saxatilus*	Order	Delaware Bay
Trout Salmonidae	Family	commercial
Weakfish *Cynoscion regalis*	Species	Delaware Bay
animals		
Bear Ursidae	Family	commercial
Guinea pig *Cavia porcellus*	Order	commercial
Rabbit Leporidae	Family	commercial
White-tailed deer *Odocoileus virginianus*	Family	commercial
Wild turkey *Meleagris gallopavo*	Family	commercial

Seventy-three stone tools were selected for protein residue analysis, 15 from Locus 1, one from Locus 2, and 57 from Locus 3. The majority of submitted specimens were found in the Metate and Feature 30 blocks, two large activity zones within Locus 3. The Metate block excavations centered around a very large grinding slab, or *metate*, in close association with three fire-cracked rock (FCR) clusters and a lithic assemblage of some 8,000 artifacts. Thirty-four bifaces, one scraper, and one grinding stone (*mano*) were submitted for

analysis from this zone. The second activity zone contained a cluster of three very large pit features and a total lithic assemblage of about 1,700. Nine bifaces and two scrapers were submitted for analysis from this zone. An additional eight specimens were tested from general locations within Locus 3. Thirteen bifaces were submitted for protein testing from the Buried Plowzone area and two bifaces from the Silo Pit area in Locus 1.

All submitted artifacts were recovered from subsoil or feature contexts. Artifacts from plowzone contexts were rejected for analysis because of possible contaminants from agricultural fertilizers. Contaminants introduced into the soil matrix surrounding artifacts have been noted as a likely source of false positive results presented in previous studies (Custer et al. 1988; Hyland et al. 1990; Marlar et al. 1995). With regard to this concern, soil samples from several locations within each activity zone were collected and forwarded along with the artifacts to test for possible contamination.

The legitimacy of the protein residue program rested on the development of a means to evaluate the accuracy and reliability of the program CIEP methodology. It is important to be able to understand with what level of confidence the results can be accepted in order to interpret them in a meaningful way. Assumptions about aquatic resource location and behavior proved useful in modeling subsistence strategies, but without some confirmation of the efficacy of the protein program, any conclusions concerning fish exploitation and consumption using residue data would be strictly conjectural. To resolve this question, a series of control tests were made during Stage 2 of the residue study to measure the ability of the prepared antisera to detect and classify correctly the taxonomic identities of modern fish proteins. Live samples of menhaden (*Brevoortia tyrannus*), American eel (*Anguilla rostrata*), striped bass (*Morone saxatilis*), and weakfish (*Cynoscion regalis*) were collected from Hudson River populations and butchered with replicant bifaces. Each replicant tool was then placed into a Petri dish, enclosed within a Ziploc bag, and refrigerated for one month. Prior to submittal for analysis, each replicant specimen was removed from its container and gently swabbed with sterile cotton gauze soaked in distilled water to remove adhering scales and tissue. The point of this exercise was to obligate CIEP to discriminate modern, non-degraded proteins of similar species from those supposedly detected among the archaeological samples. If CIEP was not able to fulfill this task, then the validity of the methodology would be weakened and its utility placed in doubt.

VII. RESULTS

The results of the Puncheon Run study can be separated into three categories: first, the status of antisera development; second, the tally of archaeological specimens tested against the antisera; and third, the result of the butchering exercise on the replicated tools.

A. ANTISERA DEVELOPMENT

Atlantic sturgeon and alewife antisera exhibited non-specific reactions; in other words, they cross-reacted with all other blood samples, and were thus eliminated from artifact testing. The antisera of white perch, striped bass, and Atlantic croaker were able to discriminate reactions only to the level of their common order, the Perciformes. The Perciformes are composed of several major families of fish native to Delaware waters, including the perch, bass, darter, drum, and sunfish (Jackson 1998; Raasch and Altemus 1991). A fourth subject specimen, American eel, likewise was cross-reactive to its order, the Anguilliformes, but this order is phylogenically very narrow. The only consequential member of the order native to the Delaware Bay is the American eel, so we can regard this antiserum with high confidence as specie-specific within the geographic parameters of this study. The antisera of bay anchovy, gizzard shad, and weakfish (sea trout) are species-specific, reflecting no cross-reactivity to other species.

B. ARCHAEOLOGICAL SPECIMENS

Of 73 archaeological artifacts submitted for testing, 18 produced positive reactions to subject antisera, an identification rate of 24.7 percent (Table I-2). This rate compares favorably with the 15.2 percent rate achieved recently at the Bugas-Holding Site in Wyoming using the same CIEP procedures (Shanks et al. 1999:1188). Four bifaces from Locus 1 tested positive for the presence of guinea pig, gizzard shad, deer (2), and American eel antigens (see Table I-2). One specimen (97/58/38) yielded positive results for guinea pig and gizzard shad. Depending on the specificity of the antiserum, guinea pig might indicate the presence of one of several native families within the order Rodentia. The three soil samples submitted for control purposes returned negative.

Table I-2: Positive Results of Protein Residue Analysis from Loci 1 and 3

Sample No.	Area/Provenience	Tool Class	Raw Material	Positive Antiserum	Associated Feature
Locus 1					
97/58/38	BPZ/ unit 195/c-3	biface	jasper	Guinea pig Gizzard shad	-
98/2/79	BPZ/ unit 266/c-3	point	jasper	Deer	-
98/2/356	BPZ/ unit 360-b-2	point	chert	American eel	10
98/2/1144	Silo/ feature 64/c-7	point	chert	Deer	64
Locus 3					
97/55/139	block 6/b-3	biface	quartz	American eel	-
97/55/535	block 6/b-2	biface	quartzite	Deer	4
98/2/241	feature 33/a-2	point	jasper	American eel Bay anchovy Deer	33
98/2/260	feature 35/b-2	biface	jasper	American eel Deer	35
98/2/300	unit 352/b-2	point	jasper	Bay anchovy Catfish	-
98/2/219	Metate/ unit 333/ b-3	point	jasper	Deer	-
98/2/510	Metate/ unit 374/ b-3	point	slatey chert	Striped bass	36
98/2/529	Metate/ unit 397/ b-2	biface	quartz	American eel	96
98/2/803	Metate/ unit 424/ b-3	drill	chert	Deer	-
98/2/974	Metate/ unit 396/ b-3	biface	quartz	Atlantic croaker	96
98/2/1171.1	Metate/ unit 456/ b-3	biface	quartz	American eel	97
98/2/1352	F30/ unit 472/ b-2	point	jasper	Gizzard shad	37
98/2/1379	F30/ unit 473/ a-3	uniface	chert	American eel	37
98/2/1381	F30/ unit 488/ b-3	point	chert	Gizzard shad	37

In Locus 3, six bifaces from the Metate block were positive for striped bass (1), Atlantic croaker (1), American eel (2), and deer (2) (see Table I-2). Eleven soil controls were submitted to test for contamination. Three of the soil samples reacted positively, to guinea pig, gizzard shad, and deer, respectively. These

reactions may indicate either modern or ancient animal activity, or false positive reactions resulting from contaminants in the soil.

At the Feature 30 activity zone, approximately 60 meters to the southeast of the Metate block, three tools recovered from the periphery of Feature 37 were positive; two bifaces reacted to gizzard shad, and one scraper to American eel (see Table I-2). Soil controls from Feature 37 fill were negative.

Two positive reactions were obtained from bifaces recovered from the Block 6 excavations, located about 8 meters east of the Metate block. The identified species were American eel and deer. Other positive results from Locus 3 bifaces include Bay anchovy and catfish (Unit 352), American eel and deer (Feature 35), and American eel, Bay anchovy, and deer (Feature 33). The jasper projectile point from Feature 33 was associated with charcoal radiocarbon dated to 2,480±40 BP (Beta-136091).

C. REPLICATED SPECIMENS

In addition to the archaeological assemblage, several replicated bifaces and one flake fashioned from local cobbles were submitted for residue testing. The cobbles were obtained on site from a point-bar in the lower reach of the Puncheon Run. Two of the replicant bifaces (Catalog Nos. 98/2/9913 and 98/2/9915) were included as unutilized specimens to test the claim that the CIEP technique often returns false positive results. To the initial surprise of the researchers, both of these tools reacted positively to deer/elk antiserum, which seemed to justify the criticisms leveled at the procedure. Upon reflection, however, a simpler explanation was adopted. After each cobble had been split freehand by hammerstone, they were bifacially thinned with an elk billet, and finally pressure flaked using deer antler. Antler contains an abundance of proteinaceous material, which could have been easily transferred to crevices and joints in the bifaces in the form of fragments or dust during percussion and pressure flaking. By attempting to replicate accurately the process of prehistoric flintknapping, it was assumed that unutilized modern bifaces would react negatively to protein antisera. Through unanticipated means, CIEP confirmed its ability to detect and identify correctly protein residues of the anti-deer serum.

The broader test of CIEP's ability to detect and identify the antigens of known butchered species yielded highly equivocal results (Table I-3). Only Specimen No. 98/2/9914 yielded the correct butchered fish (weakfish) plus the anticipated deer residue from billet use during manufacture. A second replicant specimen (No. 98/2/9903) was correctly identified as having butchered American eel, but it also returned a false positive identification for catfish. Specimen Nos. 98/2/9911 (menhaden) and 98/2/9916 (American eel) were misidentified as catfish, and No. 98/2/9909 (weakfish) was misidentified as turkey. Specimen Nos. 98/2/9906 and 98/2/9908 (striped bass) and No. 98/2/9913 (menhaden) yielded negative results.

Table I-3: Results of Protein Residue Analysis from Replicant Tools

Sample No.	Tool Class	Raw Material	Butchered Species	CIEP Result
98/2/9903	point	jasper	American eel	American eel Catfish
98/2/9906	biface	chert	Striped bass	-
98/2/9908	point	chert	Striped bass	-
98/2/9909	biface	quartzite	Weakfish	Turkey
98/2/9911	biface	jasper	Menhaden	Catfish
98/2/9913**	biface	chert	Menhaden	-
98/2/9914	point	chert	Weakfish	Weakfish Deer

Table I-3 (continued)

Sample No.	Tool Class	Raw Material	Butchered Species	CIEP Result
98/2/9916	flake	quartzite	American eel	Catfish
98/2/9913*	biface	chert	none (antler use)	Deer
98/2/9915	biface	jasper	none (antler use)	Deer

*Submitted for testing Jan. 28, 1999
**Resubmitted Oct. 19, 1999

VIII. DISCUSSION

The lack of consistent identification of butchered species among the replicated bifaces is a serious obstacle in the validation of the CIEP testing procedure. The apparent cross-reactivity of some of the antisera (most notably catfish and turkey) and the absence of reactivity of others create a problem of reliability that calls into question the results obtained from the archaeological sample. The experimental sample achieved a success rate of 40 percent, very close to the 37 percent rate of Leach and Mauldin's experimental tests (1995:1021), yet far below the 100 percent rate recently reported by Shanks et al. (1999:1186). The poor results may, in part, be accounted for by the protocols used for the experimental butchering. By placing the replicant tools in Ziploc bags within a refrigerator, the tools were inadvertently introduced into an environment conducive to the growth of microbes. Microbial attack is known to be responsible for protein decay (Eisele et al. 1995:45). Upon removal of the study tools from the sealed bags, white cotton-like mold was seen on both menhaden-treated specimens and one of the striped bass. Mold may have promoted protein decay on those tools, as well as others, although the extent of the possible degradation is not known.

Taken at face value, the results of the study suggest a clear preference for aquatic food resources over terrestrial foods. Of the seven positive reactions to protein residue analysis, six were to fish antisera (see Table I-2). Freshwater species (trout and catfish) were not detected. These results are in accord with the model of subsistence strategies elicited from the site catchment and landscape analyses, which indicated that migratory marine fish and shellfish would be highly ranked resources given their abundance, predictability, and clustered behaviors. In contrast, exploitation of terrestrial mammals was probably undertaken on an opportunistic basis because of the higher costs incurred in hunting dispersed prey. It must be emphasized, however, that these results do not readily translate into caloric contributions to human diet by marine and terrestrial resources, and thus an actual reckoning of the relative importance of each food group is not available. Nonetheless, it seems reasonable that fishing would have provided a more steady intake of food compared with hunting even if greater meat-weight was procured during each *successful* hunting foray (Jones 1978:36). This distinction between successful and unsuccessful is important because of the greater probability of consistent fishing success over hunting, and thus a more constant dependability of diverse aquatic resources to fulfill food needs. Table I-4 presents energy, protein, and fat values for the species identified by residue analysis.

An unresolved question emerged from the identification of deer antigens on three of the replicant tools: to what degree do positive reactions of archaeological specimens to deer antiserum reflect deer butchering or a lithic manufacturing process that utilized deer or elk antler? White-tailed deer are consistently ranked among the most important prehistoric food resources in the Eastern Woodlands (Cavallo 1991:111; Purdue 1986:66), so positive protein reactions to deer are not at all surprising. It should be apparent, however, that *a priori* assumptions about deer hunting are a potential bias with regard to interpretations about the *meanings* of protein residue results.

Table I-4: Nutrient Data for Positively Identified Residue Species

Species	Energy (Kcal/100g)	Protein (G/100g)	Fat (G/100g)
American eel[1] (*Anguilla rostrata*)	184	18.4	11.7
Anchovy[1] (Engraulidae)	131	20.4	4.9
Atlantic croaker[1] (*Micropogon undulatus*)	104	17.8	3.2
Catfish[2] (Ictuluridae)	95	16.4	2.8
Gizzard shad[3] (*Dorosoma cepedianum*)	66	33.6	4.6
Striped bass[1] (*Morone saxatilus*)	97	17.7	2.3
White-tailed deer[1] (*Odocoileus virginianus*)	120	23.0	2.4

Sources: 1-USDA (1998); 2-USDA (2000); 3-Watt and Merrill (1963)

An emphasis on subsistence fishing, with its relatively high manufacturing and maintenance costs, carries with it implications regarding complex social organization and sedentism. Recent calculations of time expenditures on net manufacture indicate that, minimally, hundreds of hours of labor are required for the material collection, cording, and netting of a single 100-foot-long gill net (Lindström 1996:138-139). Costs rise exponentially as smaller mesh size is made. (Again, prey selection can be seen as an important consideration in choice of technology and allocation of labor inputs.) Such labor expenditures are possible only if community food-gathering is capable of providing enough resources to make up for the lost (or rather, delayed) labor of the net-makers (or trap-makers, weir-makers, etc.), an effort that implies that labor has been organized into increasingly efficient task groups. As increased efficiency in productive tasks is selected for its effect on increased output, we should see the adoption of task or craft specialization, since specialization is more efficient than a generalist mode of production (Pianka 1978:253). This kind of labor transformation is indicative of increased social complexity and has been tied to the effects of increased population pressure (Arnold 1992:63; Byrd 1997:52; Testart 1982).

With regard to sedentism, it has been noted that investments in stationary weirs and tidal traps are likely to encourage the stable attachment of a people to a place to ensure the protection and improvement of those resources (Byrd 1997). This sense of emotional attachment has been termed the *emergence of notions of ownership* (Riches 1995), and the exertion of territorial rights (Dyson-Hudson and Smith 1978).

The extent of a group's commitment to at least a semi-sedentary lifestyle can be assessed by indications of occupational seasonality. At both Locus 1 and Locus 3, evidence of spring visits is inferred from the presence of spring-spawning striped bass and gizzard shad residues, and autumn occupations from residues of catadromous, or fall-spawning, eel. Multi-seasonal site occupations are viewed as possible characteristics of a semi-sedentary lifestyle, reflecting a resource-rich environment capable of satisfying a broad range of subsistence requirements. In this type of highly productive setting, there may have been little incentive to engage in continuous residential mobility as a strategy to procure resources.

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