EVALUATION OF FRESHWATER MUSSELS (MEGALONAIAS GIGANTEA) AS A NEW PROTEIN SOURCE

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ABSTRACT

This study was undertaken to evaluate the potential of a commercially important species of freshwater mussel (FWM), Megalonaias gigantea, as a high protein food source, and to assess levels of potentially hazardous contaminants in these FWM collected at four sites in the Tennessee and Cumberland rivers.

The microbiological examination of FWM showed a total aerobic plate count and total coliform count of 10^6 cells/g and 10^3 cells/g, respectively. Surface river water (SRW) samples showed total aerobic plate counts and total coliform counts of 10^3 cells/ml and 10 cells/ml, respectively. Fecal streptococci were absent or present at very low levels in both the FWM and SRW samples.

The biological oxygen demand in the SRW indicated significant differences (0.01 level) among locations and ranged from 1.30 to 8.20 mg/l. The chemical oxygen demand indicated significant differences (0.05 level) among locations and ranged from 1.09 to 18.19 mg/l.

The proximate composition of freeze-dried flour made from FWM showed 4.09% moisture, 40.71% protein, 3.70% ether extract, 14.77% ash, and 36.74% nitrogen free extract. Seven short chain volatile fatty acids (VFA) identified in FWM flour, ranged from C2 through C7. Acetic and propionic acids predominated amounting to 47.29 and 40.30 mg/100 g of flour, respectively. A variety of long chain fatty acids were found in the extracted FWM flour lipids. The two dominant fatty acids were C16:0 and C16:1, which accounted for 32% of the total fatty acids present.

Eight pesticide residues were detected in composite flour locations. These were benzene hexachloride (BHC), heptachlor, heptachlor epoxide,

aldrin, dieldrin, pp'DDE, endosulfan-1 and methyl parathion. Only BHC and methyl parathion exceeded federal standards.

The FWM had a relatively high content of calcium, manganese and iron. SRW samples showed low concentrations of elements tested. The heavy elements, lead, cadmium and mercury, were found in low concentrations in the FWM and SRW samples.

Amino acid analysis showed that FWM contained at least 17 amino acids including the essential amino acids. Glutamic acid, aspartic acid, arginine, lysine, leucine, glycine, valine and alanine were the major acids present.

A sensory panel was asked to evaluate a soup made with FWM and record overall acceptability. The product was scored moderately acceptable. From all indications, FWM have the potential of being a new protein source.

CHAPTER I

INTRODUCTION

As a result of expanding world population and growing demands on an inefficient food supply, it is essential that new and improved sources of human food be developed. In the developing countries food production has not kept pace with population growth. This can be attributed to poor food distribution, land use and economics. Twelve and one-half percent of the world's population receive an inadequate amount and quality of available protein foods, and possibly one-half of the world's population is undernourished and suffers from some form of protein-calorie malnutrition (Beleau et al., 1975; Satterlee, 1975; Kahn, 1981; USDA, 1974; and Weldin et al., 1975).

An ideal dietary protein source is one which has an amino acid pattern that allows full use of its complement of amino acids. Animal proteins possess many characteristics of the hypothetical ideal protein. They are composed of and therefore supply all the essential amino acids in balanced amounts unlike plant protein sources (FAO, 1970b; and Whitaker and Tannenbaum, 1977).

With the demand for animal protein growing, it is becoming necessary to look for new or underutilized sources such as species of freshwater shellfish currently being commercially harvested for the shell. The flesh is discarded and considered an industrial waste product. If the flesh could be utilized as a food source, a new product would be available to the public. The freshwater mussel represented an

important food supplement in the diets of the American Indians and early settlers and has the potential of becoming an adjunct to the food supply today (Coker, 1919; Cook, 1946; Munson et al., 1971; and Parmalee and Klippel, 1974).

Shellfish such as freshwater mussels are usually underutilized because no efficient method for harvesting exists, no adequate processing method by which the initial quality of the product can be retained has yet been devised, and there is a lack of commercial markets. Another important factor is the acceptance of such an unfamiliar product by the consumer (Pirie, 1975; Sidwell and Ambrose, 1974; and Spinelli et al., 1977).

A literature search failed to reveal work on the nutritional potential of freshwater mussels. However, extensive work with marine shellfish has indicated that freshwater mussels could parallel clams as a food source. The bulk of the dry matter is protein. Depending on season and species, protein content varies from about 13 to 18% on a wet basis (Coker, 1919; and Parmalee and Klippel, 1974).

Because of their size and availability, the species Megalonaias gigantea was selected for this investigation. The objectives of the study were to assess levels of potentially hazardous contaminants in Megalonaias gigantea collected from four sites, and to evaluate the potential of a commercially important species of freshwater mussel as a high protein food source.

CHAPTER V

SUMMARY

As a result of an expanding world population, deficiencies in both the amount and quality of foods available are predicted in developing countries. Food production has not kept pace with population growth, causing severe protein-calorie malnutrition. Therefore, it is imperative that new sources of potential foodstuffs from currently unexploited resources be investigated.

An unexplored potential source of protein is available from the freshwater mussel (FWM), Megalonaias gigantea. Harvest of these freshwater shellfish by commercial shellers from the Tennessee River generates in excess of 909 metric tons of mussel flesh each year. Presently, the flesh is discarded. If the FWM could be shown to be safe and free from environmental contamination, a new and potentially nutritious food source could be made available to the public.

The FWM were collected at four locations, three on the Tennessee River (Athens, AL; New Johnsonville, TN, South; New Johnsonville, TN, North) and one on the Cumberland River (Rome, TN). Samples of mussels and surface river water (SRW) were collected from each location and shipped to the laboratory. A minimum of 12 mussels (1200 g of mussel flesh) and 1 SRW sample (700 ml) were collected at each location at four separate times during the 1980 fall season.

The microbiological examination of the FWM and SRW samples used three bacteriological analyses: total aerobic plate counts (TPC),

total coliform counts (TCC), and fecal streptococci counts (FSC). The counts on FWM were affected at the 0.01 level by location, time of harvest and the interaction, location x time of harvest. The time of harvest for TCC, however, had no significant effect at the 0.05 level.

The three bacteriological indices showed small variations in mean log values for FWM among the locations: TPC values (5.0 to 6.1 cells per g), TCC values (3.3 to 3.7 cells per g), and FSC values (0.0 to 0.1 cells per g). Small variations were observed in the mean log values among the locations for SRW. The three bacteriological indices were: TPC values (2.9 to 3.5 cells per ml), TCC values (0.7 to 1.6 cells per ml), and FSC (0.0 to 0.1 cells per ml). In comparison to market ground beef which was found to contain a TPC of 10^7 cells/ml (Westhoff and Feldstein, 1976), the microbiological quality of FWM is quite acceptable for human consumption.

The biological oxygen demand (BOD) of the SRW was affected at the 0.01 level by the four locations, time of harvest and the interaction, location x time of harvest. The chemical oxygen demand (COD) was affected at the 0.05 level by location but was not significantly affected by the time of harvest and the interaction, location x time of harvest. The mean BOD value for location ranged from 1.30 to 8.20 mg/l. The BOD of SRW from New Johnsonville (South) was highest at 8.20 mg/l and Athens, AL, had the lowest BOD with 1.30 mg/l. The mean COD value for location ranged from 1.09 to 18.19 mg/l. The COD of SRW from New Johnsonville (South) was highest, at 18.19 mg/l, and Rome, TN, had the lowest COD with 1.09 mg/l.

The FWM were freeze dried to form a dry FWM concentrate. This concentrate was broken down with a mortar and pestle to form a flour.

The proximate composition of the FWM flour and flesh varied at the 0.01 level due to the four locations, time of harvest and the interaction, location x time of harvest. The FWM flour on the average contained 4.09% moisture, 40.71% protein, 3.70% crude fat, 14.77% ash, and 36.74% nitrogen free extract. The FWM flesh contained 84.41% moisture, 6.29% protein, 0.65% crude fat, 2.20% ash and 5.83% nitrogen free extract. Variation between locations in the composition of the FWM flour and flesh could have resulted from seasonal varieties in the habitat, the age of mussel, food supply and sex of the mussel.

Seven short chain volatile fatty acids (VFA) were identified in freshwater mussel flour from the four locations. These acids were acetic, propionic, butyric, isovaleric, valeric, caproic and heptanoic acids. The concentrations of VFA from the four locations were different at the 0.01 level. Acetic and propionic acids were the predominant VFA in the flour which contained 47.29 and 40.30 mg/100g of flour, respectively.

Lipid content determined by the chloroform methanol extraction method in the FWM flour was affected by location at the 0.01 level. The percentage of lipid ranged from a low of 4.91 at Athens, AL, to a high of 9.38 at Rome, TN. The Rome, TN, location was significant by higher than the other three locations.

A wide variety of long chain fatty acids were detected in the extracted FWM flour lipids by gas liquid chromatography of methyl esters. In the FWM flour lipid, 7 fatty acids (C16:0, C16:1, C17:8, C17:0, C18:3, C18:1, C21:1) in the range of 3.70 to 24.88% accounted for 65% of the total fatty acid content. The two dominant fatty acids were C16:0 and C16:1, which accounted for 32% of the total fatty acid

content. The lipid of the FWM flour consisted of at least 50% unsaturated fatty acids.

Of the 47 possible pesticide residues that are detected with the procedure used, only 8 were found in the composite flour samples. These were benzene hexachloride (BHC), heptachlor, heptachlor epoxide, aldrin, dieldrin, pp'DDE, endosulfan-1 and methyl parathion. With two exceptions, the pesticide concentration (PPM) was below the "Action Levels" found in the Code of Federal Regulations. Polychlorinated biphenyl (PCB), identified as Arochlor 1254, was detected in the flour from three out of the four locations. PCB was below the maximum levels allowed by federal standards.

The metals measured in FWM flour and SRW were copper, chromium, manganese, magnesium, potassium, calcium, iron, sodium, zinc, aluminum, lead nickel, mercury and cadmium. The FWM had a relatively high content of iron, calcium, and manganese. SRW samples showed relatively low concentration of all elements tested.

The amino acid analysis showed that FWM contained at least 17 amino acids including the essential amino acids. Glutamic acid, aspartic acid, arginine, lysine, leucine, glycine valine and alanine were the major amino acids present.

The Hunter L color values of FWM flour were not significantly different between the four locations. Hunter "a" and "b" color values of the flour were affected (0.01 level) by location, time of harvest and the interaction, location x time of harvest.

A 62-member sensory panel evaluated three chowder soups made with freshwater mussels (Megalonaias gigantea), saltwater clams

(Mercenaria mercenaria) and a control consisting of all ingredients except the freshwater mussels or saltwater clams. An 8-point hedonic scale where 1 indicated "like extremely" and 8, "dislike extremely" was used to assess overall acceptability. The control soup had the highest score, while scores for saltwater clams and freshwater mussel chowders followed in succession. The scores ranged from 2.79 for the control chowder to 4.11 for the FWM chowder. The toughness of the muscular foot was considered with its high content of connective tissue as one factor in the scores of the freshwater mussels.

Gel electrophoresis showed the protein from FWM was a complex mixture of proteinaceous species which ranged from about 10,000 to 70,000 daltons in molecular weight. The source of the protein material and its treatment before analysis markedly affected the electrophoretic distribution of bands in the gels. Particularly, storage of FWM flour at room temperature decreased the number of bands that appeared.

From all indications, FWM could be a usable adjunct in the food supply, particularly as protein supplement in a dry flour form. While fish protein concentrate did not prove successful in the American markets, a large consumer market exists in Asian and African countries. Despite the lack of appeal to American tastes, freshwater mussels as a flour or even canned fresh, could have a place in the export trade American palates slowly become re-educated to the advantages of little-known food sources. Additional research is needed to develop processing techniques which take into account the inherent toughness of the mussel, as well as difficulties with shucking. New ideas are also necessary as to how this product should be utilized.

CHAPTER II

REVIEW OF LITERATURE

I. AVAILABILITY AND UTILIZATION OF FRESHWATER MUSSELS

North America, north of Mexico, has the world's largest concentration of freshwater mussel species. According to Burch (1975), the Unionacea of North America consists of 227 species, 46 genera, and 2 families (Margaritiferidae and Unionidae) within the Pelecypoda class and the Mollusca phylum.

Freshwater mussels are indigenous to lakes, streams, and rivers throughout this country, especially the Mississippi River, the Great Lakes, and their drainage tributaries. Surveys of unionid communities have been conducted routinely since the beginning of this century. The surveys were designed primarily to assess the freshwater mussel communities as a resource for commercial exploitation, for use in the pearl button and furniture industries, and most recently, as a source of shells for the cultured pearl industry. Also, information was gained on the diversity and relative abundance of mussels in a particular body of water and on the shell quality. Methods for surveying mussel biota and commercial harvesting include scuba diving, brailing, dredging, tonging, raking, and wading (Boeapple and Coker, 1912; Brice and Lewis, 1979; Coker, 1919; 1921; Huebner, 1980; Isom, 1969; Isom et al., 1973; Parmalee et al., 1980; Pennak, 1978; Schalie, 1938; and Stern and Felder, 1978).

Forty species of freshwater mussels have been collected for their shells. Seventeen species of primary importance in the manufacture of freshwater pearl button are in the following genera: Fusconaia, Megalonaias, Amblema, Quadrula, Actinonaias, Plagiola, Legumia, and Lampsilis. A sizeable industry was developed only to be displaced by World War II and the genesis of plastics which were used for button manufacture (Anon., 1981b; Coker, 1919; Parmalee, 1955; and Pennak, 1978).

The freshwater mussel was first taken in commercial numbers from rivers and streams in the latter part of the nineteenth century. Harvests from the late 1940's to the mid-1950's exceeded 91,100 metric tons per year. Today, approximately 909 metric tons of freshwater mussels are harvested from the Tennessee River by commercial shellers (Anon., 1981b; and Pennak, 1978).

The mussel is prepared for removal of the shell by steaming it in a cooker. After steaming, the flesh is removed. The animal bodies have been used for fish bait, fertilizers, and feed rations for swine and poultry. The wet tissues when dried can be ground to a fine meal and held indefinitely (Coker, 1919; Parmalee, 1955; and Pennak, 1978).

The toughness of the foot tissue can be overcome by grinding the wet meats through a sausage grinder, drying, and regrinding the dried mass in a mill. The resultant product is a coarse granular material (Coker, 1919). Coker (1919) suggested that the meats of the mussels may be used for human food if they were collected under sanitary conditions and prepared properly.

Analysis of the dried mussel meats indicate in general a high content of protein, glycogen, phosphoric acid, and lime (Coker, 1919). The average contents of dried mussel tissue were presented as follows: protein, 44.44%, moisture, 7.59%, ether extract, 2.84%, glycogen, 9.35%, carbohydrates, 13.02%, and ash, 22.76%. Coker (1919) found phosphoric acid (P_2O_5) to be 39.31%; lime ($Ca(OH)_2$), 34.71%; and silica (SiO_2), 15.86% in the ash. The size, weight, and species of freshwater mussel used in the analyses wree not presented.

In the early 1950's, workers in the cultured pearl industry in Japan found that small pellets from American thick-shelled bivalves formed excellent nuclei for cultured pearls. This discovery caused revival of the freshwater mussel industry for use in producing cultured pearls. Nuclei are produced by reducing the thick natural mother of pearl nacre of the inner shells to pellets of 7mm OD. These pellets are inserted along the inner margin of the shell in marine oysters. The pellets caused an irritation to the host oyster and consequently, the oyster secrets nacre around the pellet and initiates production of cultured pearls. A minimum of three years is required to produce a pearl of sufficient size for commercial marketing (Parmalee, 1966; and Pennak, 1978).

Pearl production in freshwater mussels can also be stimulated by placing a foreign object inside the bivalves. Objects such as pellets, pieces of wood, bone, metal, mud, or fine gravel have been used. Most pearls produced naturally or artificially are irregular in shape and variable in color.

The calcium carbonate component in the unionid shells is of reagent grade purity and the shell offers a source of high quality raw material for production of calcium carbonate (Nelson et al., 1966). Gerry (1980) used ground whole freshwater mussel shells which contained 34.2% calcium as a substitute for limestone in chicken rations. He concluded that the ground shells were an acceptable source of calcium for bone and egg shell formation.

Today, some naiad populations of freshwater mussels are threatened with extinction. Pollution, impoundments made by hydroelectric and other dams, stream channelization, introduction of foreign species with which they must compete, and overharvesting in many places all affected the maintenance of a viable population of these mollusks (Anon., 1981b; Burch, 1975; Coker, 1921; Fuller, 1974; and Pennak, 1978).

II. PRINCIPAL SOURCES OF CONTAMINATION OF FRESHWATER MUSSELS

Freshwater bivalves are known as filter feeders because they obtain food by using their gills to strain out and concentration zooplanton, phytoplankton, and other fine particulate matter, such as microorganisms from the water. Mollusks are often affected by adverse changes in the environment. Unlike freshwater fish and crustaceans, bivalves are sedentary organisms incapable of movement to avoid adverse environmental conditions (Churchill and Lewis, 1924; and Fuller, 1974).

Industrial discharges, uncontrolled runoff from agricultural lands, discharges of inadequately treated sewage, and adverse weather conditions such as flooding can affect the mollusk population by exposing them to large quantities of poor quality water. Water quality can

be evaluated by measuring the suspended and settleable solids, oxygen-demanding wastes, presence of coliform bacteria, and heavy metal and pesticide residues (Anon., 1977b).

High water quality is required for bivalve propagation and commercial harvesting. Filter feeding mollusks pump large quantities of water containing dissolved or suspended materials through their systems. The use of a depuration process with marine shellfish from moderately polluted water has been successful in Europe in reducing the microbiological contamination of shellfish beds and allowing year round commercial harvesting (Anon., 1977a; Anon., 1977b; Anon., 1979b; Fuller, 1974; Lutz and Incze, 1977; and Ritchie, 1977).

Environmental contaminants can adversely affect the flavor of the bivalves, making them inedible or unsafe for human consumption (Geyer, 1972). Also, marine mollusks can concentrate human pathogens such as typhoid, polio, hepatitis, and other microbial agents (Speck, 1976). However, there are no literature citations implicating freshwater mussels in the transmission of human pathogens to man.

Filter feeding mollusks accumulate trace metals such as mercury and copper from the sediment of the body of water or from the food chain. The concentration of trace metals in the body tissues generally reflects the actual concentration of the metals in the environment (Aoyuma et al., 1978; Cossa and Bourget, 1980; Davies and Pirie, 1980; Furr et al., 1981; Mahaffey et al., 1975; and Phillips, 1976a; 1976b; 1977). Foster and Bates (1978) indicated the usefulness of freshwater bivalves as a tool in monitoring industrial effluent

at the discharge site. This technique would allow a rapid, reliable, and inexpensive test for water quality.

Pesticides are transported to the aqueous environment either in solution or adhered to particles where the pesticides are deposited in sediment or taken up by the bivalves. The major chemical groups are chlorinated hydrocarbons such as DDT, organophosphorus compounds such as parathion, and carbamates such as carbaryl. There is also a variety of inorganic pesticides containing lead, arsenic, and mercury. Pesticide residues uptake may occur in mussel flesh directly through ingestion and absorption or indirectly through the food chain. Once the pesticides enter their environment their identities may undergo chemical and biological change (Grant, 1976; Hansen, 1980; Lippmann and Schlesinger, 1979).

Polychlorinated biphenyls (PCB) are sometimes found in the water environment and can become part of the human food supply. Substances such as PCBs, DDT and its metabolites, TDE and DDE, and heavy metals are nondegradable, persistent, and extremely toxic. PCBs and pesticides tend to concentrate in the adipose tissues of animals which occupy relative high position in the food chain (Langston, 1978; Lippmann and Schlesinger, 1979; and Munro and Charbonneau, 1978).

The public is becoming increasingly aware that thousands of carcinogenic (cancer-causing), teratogenic (birth-defect causing) and mutagenic (genetic-damaging) substances are being introduced into the water supply and environment (Doull et al., 1980; Dunn and Stich, 1976; and Munro and Charbonneau, 1978). The Food and Drug Administration (Anon., 1979a) and the Environmental Protection Agency (Anon., 1976b)

have established action levels for pesticides residues, trace metals and organic compounds such as PCB compounds in the environment to protect the consumer. Freshwater mussels have been used by state agencies in Michigan, Wisconsin, Minnesota, and Indiana to monitor pesticide chemicals in tributaries of the Great Lakes (Bedford and Zabik, 1973).

The quality of water of rivers, streams, and lakes depends to a large extent on the microbiological flora present. Microorganisms from soil, animals, and sewage may contribute to the flora. The kinds of bacteria found include the genera Pseudomonas, Chromobacterium, Acineobacter, Proteus, Micrococcus, Bacillus, Streptococcus (enterococci), Enterobacter, Escherichia, and Flavobacterium. Bacteria of the last three genera are probably contaminants rather than part of the natural flora (Frazier and Westhoff, 1979; and Harrigan and McCance, 1976).

Bacteria of the coliform group are considered the primary indicator of fecal contamination and water quality. The coliform group consists of a number of bacteria including the genera Klebsiella, Escherichia, Serratia, Erwinia, Enterobacter, and Proteus. All coliform bacteria are gram-negative rods and are associated with feces of warmblooded animals and/or with the soil. As an indicator of fecal contamination, fecal coliform bacteria have proven to be more valuable than the use of total coliform bacteria because fecal coliforms are restricted to the intestinal tract of warmblooded animals (Anon., 1976b; and Frazier and Westoff, 1979).

Because freshwater mussels are filter feeders, they may concentrate pathogenic bacteria and viruses from the waters which are affected by domestic pollution. The bacteria in the water establish themselves

on the muscle and membrane surfaces, gills and in the intestinal tract of mussels. Ruppert and Draughon (1982) isolated specific genera of bacteria from freshwater mussels. The predominant genus was Pseudomonas (Appendix A). In marine bivalves the genera Alcaligenes and Flavobacterium are predominant (Speck, 1976).

The freshwater clam species, Corbicula, which is a member of the super family Sphaeriacea, can maintain vast populations in harsh environments. In its native Asian range, Corbicula is used as food to support a commercial fishery. However, some California populations have also exploited the freshwater clams for local oriental markets. High counts of coliform bacteria have been recorded in Corbicula taken from polluted rivers on Taiwan. In addition, the consumption of raw Corbicula flesh has been implicated as a source of Eschinostoma (Trematoda) infections of humans (Sinclair, 1964).

III. STRUCTURES OF FRESHWATER MUSSELS

The general anatomy of freshwater mussels in the super family Unionacea is represented by Megalonaias gigantea in Figure 1. A large visceral mass extends from the dorsal midline. At the end of this mass is the "foot," which protrudes between the valves. The foot organ is responsible for the limited locomotion and the burrowing action of the mussel (Barnes, 1963; Burch, 1975; Pennak, 1978; and Weisz, 1968). Two pairs of large, double layer gill demibranches project into the mantle cavity, one pair on each side of the visceral mass. Cilia on the gills draw food-bearing water through the incurrent siphon. The water passes through the gills and exits via the excurrent siphon.

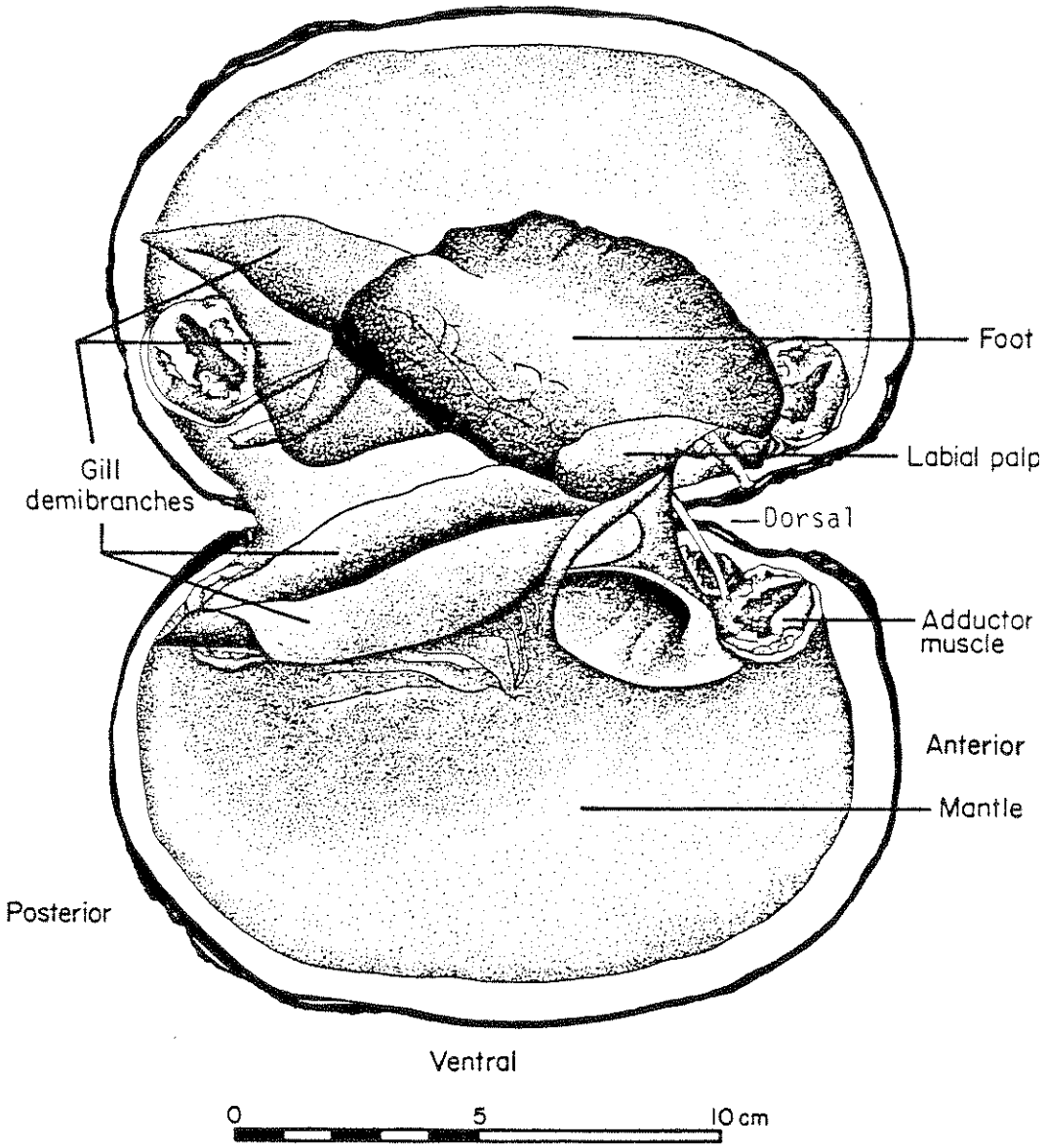

Figure 1. General anatomy of the freshwater mussel Megalonaias gigantea.

Food particles are strained out on the gill surfaces where they are caught up in a mucus secretion. Strands of the mucus are propelled by the cilia toward the labial palp which in turn propels food-containing mucus toward the mouth. The heart, digestive tract, excretory organs, and reproductive organs are located in the visceral mass. Two adductor muscles, one anterior and one posterior, are responsible for opening the closing the valves. Taxonomically, differences between the Unionacea (freshwater mussels) and Sphaeriacea (freshwater clams) are based on the reproductive features of these bivalves, marsupial characteristics of the gills, and the morphology of the shell (Barnes, 1963; Burch, 1975; Kraemer, 1979; Pennak, 1978; Sinclair and Ingram, 1961; Sinclair and Isom, 1963; and Weisz, 1968).

Megalonaias gigantea, like most other indigenous river mussels, is dioecious, and its zygotes develop within the female mussel's gill pouches before being expelled as immature glochidia. The glochidium larvae must parasitize a particular fish-host species before metamorphosizing into free-living adult river mussels. If the mussel is fortunate enough to drop from its host onto a suitable river bottom habitat, escape disease and predators, and find water of acceptable quality and a sufficient food supply, it may reach sexual maturity in 3 to 5 years. It may be harvested after 3 to 5 years when the shell has grown large enough to be commercially valuable (Anon., 1981b; Pennak, 1978; and Stern and Felder, 1978).

Artificial propagation of commercially important mussel species has been investigated by government and university researchers. The use of artificial propagation would preserve and maintain the remaining

natural beds of mussels, provide an assured supply of shells to meet market demands, and possibly permit selective breeding to improve growth rates of shell characteristics. Since natural reproduction requires a glochidial larvae-host relationship in the life cycle of the mussel, this could be eliminated by in vitro propagation in which an artificial host medium would be used. The key ingredient in this culture medium is fish blood serum. This could initiate an aquacultural industry. Aquaculture is defined as the culture or husbandry of aquatic animals or plants for commercial purposes or to increase natural stocks (Anon., 1981a; 1981b; Coker, 1921; Hurlburt, 1979; Poppensiek, 1972; and Ryther, 1981).

The general morphology of the freshwater mussel shell Megalonaisas gigantea is shown in Figure 2. The oldest part of a valve is the umbo, a bulge near the hinge from which growth proceeds in concentric rings. The two valves are basically mirror images of each other, held together at the dorsal margin by a tough elastic ligament and a series of projecting and interlocking hinge teeth (Figure 3). The internal surface of the shell is thickly coated with nacre, mother-of-pearl, which ranges in color from silver white through pink to dark purple. The overall shape of the shell and its degree of development of particular regions are used for identification purposes (Burch, 1975; and Pennak, 1978).

Recent work detailing the physiological functions of certain organs in freshwater mussels has been published (Dietz, 1979; Dietz and Findley, 1980; Hobden, 1970; Lee and Wilson, 1969; Mackie, 1978; and Pietrzak et al., 1973). Some of the research was concerned with measuring the enzyme activity of catalase and ATPase, shell structure

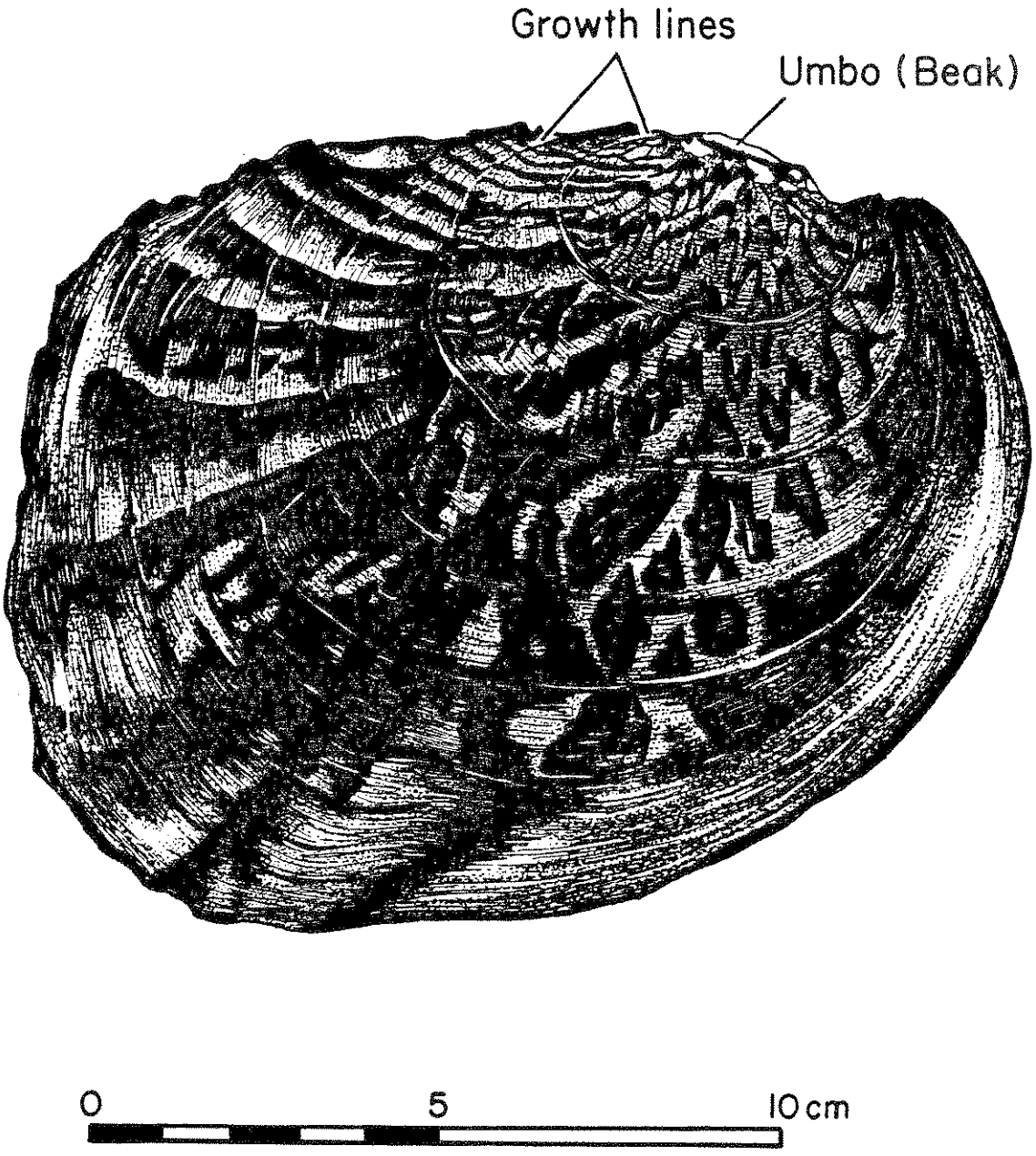

Figure 2. The external surface of the freshwater mussel shell (right shell) Megalonaias gigantea.

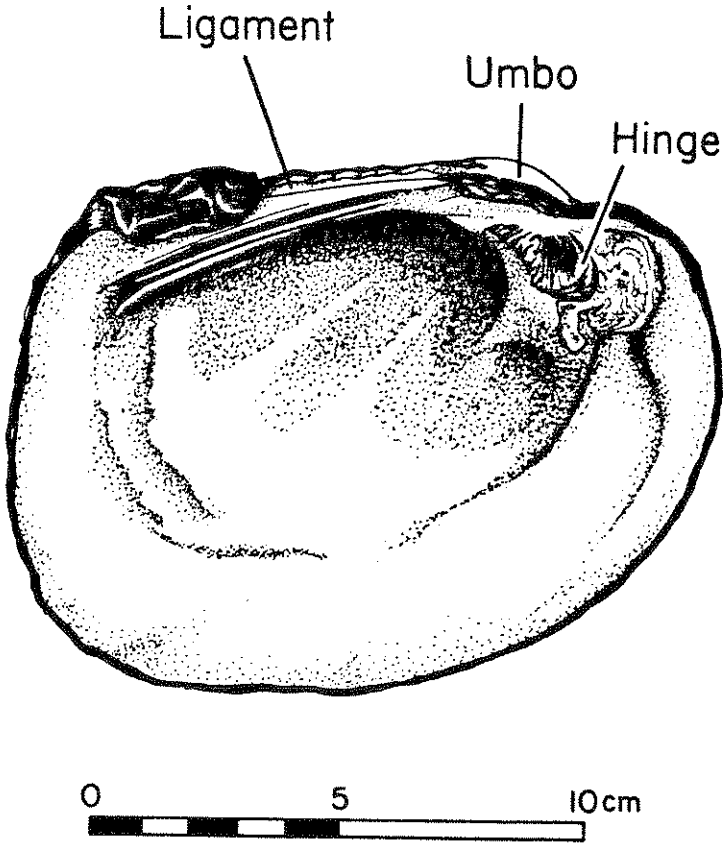

Figure 3. The general morphology of the freshwater mussel shell (left shell) Megalonaias gigantea.

and composition, translocation of sodium chloride in different species of freshwater mussels and the protein compounds of unionidextrapellial fluid.

IV. FRESHWATER MUSSELS AS A SOURCE OF PROTEIN

An unexplored potential source of protein is available from freshwater mussels. Parmalee and Klippel (1974) examined two species of Unionacea to determine the nutritional significance of mussels utilized by aboriginal peoples (Table 1). Analysis showed specimens to contain a high percentage of moisture but a relatively low percentage of protein (8 to 9%), carbohydrates, and fat and a low caloric value. According to Parmalee and Klippel (1974), archaeological sites ranging in time from the Early Archaic period through the Historic period showed that prehistoric cultural groups utilized freshwater mussels as a food source. Indians who once occupied eastern North America, especially the Mississippi River drainage region, exploited this food resource so extensively that archaeological sites of camps and villages revealed the presence of shell mounds (Matteson, 1953a; 1953b). Cook (1946) estimated the primitive diet and nutritional level of the inhabitants based on the size of shell mounds of San Francisco Bay excavation. Munson et al. (1971) recovered from excavation of a Middle Woodland Village in Illinois the shells of 27 species of freshwater mussels. They suspected the inhabitants used the mollusks as a food source. To a people subsisting primarily on plant food sources, the freshwater mussel would have supplemented the diet with protein and essential amino acids. Aboriginal man must have

Table 1. Proximate composition of two species of freshwater mussels (raw edible portions, 100 g)

	Pink Heel-Splitter[a] (*Proptera alata*)	Mucket[a] (*Actinonaias carinata*)
Moisture %	76.5	82.2
Protein (g)	9.5	7.8
Fat (g)	0.8	0.7
Carbohydrates (g)	7.8	4.5
Ash (g)	4.3	3.0
Fiber (g)	1.1	1.2
Calories (Kcal/g)	77.0	58.0
Sodium (mg)	23.0	7.0
Potassium (mg)	41.0	26.0
Iron (mg)	12.5	12.2
Calcium (mg)	370.0	320.0
Phosphorous (mg)	812.0	520.0
Riboflavin (mg)	0.3	0.2
Niacin (mg)	2.0	0.9
Ascorbic Acid (mg)	0.26	0.26

[a]Parmalee and Klippel (1974), p. 431.

initiated the first clambake as a way to steam open and cook the mussels (Parmalee and Klippel, 1974).

A literature search failed to reveal research on the food potential of freshwater mussels. However, extensive work with marine shellfish indicates that freshwater mussels could parallel marine bivalves as a food source.

The Need for New Protein Source in the Diet

As a result of an expanding world population, deficiencies are predicted in both the amount and quality of foods available (Finch, 1977). Today, except for North America and Australia, virtually all areas of the world are net importers of food (Beleau et al., 1975).

In his classic study published in 1778, Thomas Malthus predicted that the future world's population would overwhelm the earth's ability to produce food for everyone. The world's population is growing at the rate of 70 million people a year, with the developing countries accounting for 86% of this increase. Other factors affecting the supply of food are (1) the availability and use of land and other resources, (2) the technological advances for increasing yields through the use of fertilizers and improved seed varieties, (3) weather, and (4) the efficiency of food marketing and distribution systems. It is essential that new and improved sources of human food be developed (Hayes, 1981; Kahn, 1981; and USDA, 1974).

It has been established by the Food and Agriculture Organization (FAO) of the United Nations that possibly one-half of the world's population is undernourished and suffers from some form of protein-

calorie malnutrition (FAO, 1970b). The complex of diseases related to protein-calorie malnutrition, kwashiorikor (primary protein deficiency) and marasmus (severe undernourishment) is responsible for an infant mortality rate that is three to ten times higher and a death rate that is twenty to fifty times higher among one to four year old children in certain African countries than that of the industrialized nations of the world. Children below five years of age may account for 40% of the mortality. The low protein reserves of the body and poor nutrition status cannot sustain such children who often develop fevers from respiratory and gastrointestinal infections (Bogert et al., 1973; FAO, 1970b; and Loosli, 1974).

In the industrialized nations of the world, protein malnutrition is not a major public health problem because foods of animal origin are plentiful and usually account for about two-thirds of the protein intake. However, when protein-rich foods are unavailable to the poorer segments of a population, a higher incidence of infant mortality and general debility may occur (USDA, 1974).

In countries where protein intake is low and most of the protein is furnished by cereal grains or legumes, some of the essential amino acids such as lysine, tryptophan, methionine, and threonine are lacking in the diet. With the addition of animal protein, the biological value of a diet rich in cereal grains, beans, or other plant foods would be improved and contain the essential amino acids needed for body growth and development. The work of the United Nations (UN) health agencies, UN Children's Fund (UNICEF), the World Health Organization (WHO), the FAO, the UN Development Program (UNDP), and the UN Protein Advisory

Group (PAG) has developed programs to help alleviate the problems arising from nutritional deficiencies and food shortages and by formulating protein-rich supplements. These health agencies have assisted some countries in developing programs to educate the local population to use a wider variety of foods for improved nutrition and to raise the economic level to enable purchase of high quality protein foods. Protein-rich formulations which take advantage of locally available foods in the developing countries can supplement amino acids and conform to cultural food preferences. As a result, various protein mixtures, powders, and concentrates have been developed and recommended for alleviating protein malnutrition. Some of these protein sources are from single cell cultures, leaves, certain root vegetables, finfish, mollusks, crustaceans, and soybeans. Cost, availability, ease of preparation, keeping quality, and packaging are some of the criteria which determine the acceptability of protein supplement by the consumer in developing nations. Full utilization of all available resources is essential if the problem of protein malnutrition is to be solved in the future (Bogert et al., 1973; FAO, 1970b; Jones, 1974; Loosli, 1974; Pirie, 1975; Rusoff et al., 1980; and USDA, 1974).

Evaluation of Dietary Protein Quality

Food protein quality is dependent upon the quantity and bioavailability of the essential and non-essential amino acids. Nine of these amino acids (valine, leucine, isoleucine, threonine, lysine, methionine, phenylalaine, tryptophan, and histidine) must be present in higher concentrations for protein synthesis to occur in anabolic

situations: infancy, pre-school years, children, adolescence, pregnancy, lactation, and recovery from disease than in adult men or non-pregnant, non-lactating women. The ultimate value of protein quality is measured by its ability to support growth and maintenance in the form of cellular synthesis. Proteins of plant origin, such as wheat and oil-seed legumes, are usually required at higher levels of intake than those of animal origin, such as milk, meat, and eggs, to support an adequate protein nutritional status within an individual (Bodwell, 1977; and Jansen, 1978).

The objective of a biological evaluation or bioassay is to measure the efficiency of the biological utilization of dietary proteins as sources of essential amino acids. A bioassay has two possible applications: to rank protein foods according to their efficiency of utilization under a set of standard conditions (indicates the nutritional potential of the proteins), and to measure the efficiency of protein utilization as sources of nitrogen and essential amino acids for meeting the amino acid requirements of humans and animals (measures potential and physiological performance) (Friedman, 1975; and Lachance et al., 1977).

The biological methods presently used for assessing the nutritive value of protein are the Biological Value (BV), Net Protein Utilization (NPU), Slope Ratio Assay (SRA), and the Protein Efficiency Ratio (PER). All of these biological methods involve time consuming, costly, and cumbersome animal feeding studies. The procedures are based on the use of body nitrogen retention or body weight gain of rats when consuming a protein or nitrogen source for predicting the

nutritive value of proteins for humans. Some of these methods do not account for tissue protein maintenance and digestibility (Friedman, 1975; Hackler, 1977; and McLaughlan, 1972).

A simplified technique for evaluating protein quality is the amino acid score (chemical score) method. The amino acid score method requires knowledge of the amino acid composition of the proteins as well as the amino acid requirements of the individual or animal. The procedures involve expressing the amount of each indepensible amino acid in the test protein as a percentage of that in whole egg (100%) or some other reference protein. A lower chemical score represents a lesser amount of that amino acid relative to the amount required. The amino acid score method is satisfactory for rating proteins that differ widely in quality and has the advantage of being strictly an analytical procedure. The procedure does not consider amino acid availability or digestibility in the intact protein prior to hydrolysis. With the development of ion exchange chromatography, the amino acid distribution in a protein product can be measured with an animo acid analyzer. The amino acid composition of a protein hydrolyzate can be determined within 2 to 4 hours. Only a very small sample is required for the analysis. As a relatively rapid and less expensive measurement of protein quality, the amino acid score method is an in vitro analysis but is not truly indicative of the growth or maintenance potential of the protein source (Friedman, 1975; Jansen, 1978; and Lachance et al., 1977).

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VI. PESTICIDE RESIDUES IN FRESHWATER MUSSEL FLOUR

Composite samples from each location were examined for the presence of pesticide residues and polychlorinated biphenyls (PCB) in the FWM flour. Of the 46 possible pesticide residues (Appendix D) that can be detected with the procedures used, only 8 were detected (Table 16). The organochlorines found in the flour were benzene hexachloride (BHC), Heptachlor, Heptachlor Epoxide, Aldrin, Dieldrin, pp'DDE (a metabolite of DDT) and Endosulfan-1. The only organophosphate detected in the flour was Methyl Parathion.

With two exceptions, the pesticide concentrations (PPM) were below the "Action Levels" found in the Code of Federal Regulations (Anon., 1979a). These were BHC and Methyl Parathion, found in the New Johnsonville composite and Endosulfan-1 found in material from Athens, AL. The U.S. Food and Drug Administration has not set allowable limits for endosulfan-1 in edible fish tissues and shellfish. Polychlorinated biphenyls identified as Arochlor 1254 was detected in the flour from three out of the four locations. The highest level was detected in the flour from Athens, AL, and no residue was detected at Rome, TN. All values for PCB in the various composite flours were below the maximum levels allowed by federal standards.

Khan et al. (1976) reported that the use of freeze-drying techniques could lower the concentration of PCB in shrimp and egg. The results of the study by Khan et al. indicated at least 40% reduction in Arochlor 1254 in shrimp homogenate. This is of potential significance in the future processing techniques to be utilized for FWM by reducing toxic levels of pesticides and PCB.

Table 16. Pesticide residues in freshwater mussels

Pesticides	Athens, AL	Rome, TN	New Johnsonville, TN North	New Johnsonville, TN South	"Action Levels"[a]
	ppm				
Benzene Hexachloride (BHC)	0.1	Traces	0.5	0.01	0.1
Heptaclor	0.1	Traces	0.08	ND	0.3
Heptachlor Epoxide	ND	ND	ND	0.16	0.3
Aldrin	0.06	ND	0.07	0.06	0.3
Methyl Parathion	0.09	0.4	0.26	0.35	0.3
pp'DDE	3.0	ND	0.03	0.4	5.0
Dieldrin	ND	ND	0.04	0.02	0.3
Endosulfan-1	3.2	ND	ND	ND	---
Arochlor 1254 (PCB)	0.2	ND	0.034	0.05	10.0

[a]Action levels for poisonous or deleterious substances in human food and animal food--Code of Federal Regulations (Anon., 1979a).

ND = None Detected.

VIII. METAL CONTENT IN FRESHWATER MUSSEL AND SURFACE RIVER WATER MUSSEL

The F-ratios for the analysis of variance for metals found in freshwater mussel (FWM) flour and surface river water (SRW) are presented in Tables 19 and 20. The metals measured in both samples were copper, chromium, manganese, magnesium, potassium, calcium, iron, sodium, zinc, aluminum, lead, nickel, mercury and cadmium.

The copper content in the FWM flour was affected at the 0.05 level by location. Contents of chromium, manganese, magnesium, sodium, zinc and aluminum found in the FWM flours were affected by location at the 0.01 level. None of the metals, except copper, were significantly different for the time of harvest. Significant differences were observed in most of the metals for the interaction of the variables.

The concentrations of metals in SRW also varied significantly over location (0.01 level). Time of harvest, except for iron, also introduced significant variation in metal concentrations as well as the interaction, location x time of harvest.

The mean metal content of FWM flour and SRW by location are tabulated in Tables 21 and 22, respectively. Copper content for FWM flour by location ranged from a low of 0.0125 mg/g (Athens, AL) to a high of 0.0149 mg/g (New Johnsonville, TN, North) with an average of 0.0134 mg/g. Copper content in SRW samples ranged from a low of 0.0124 mg/l (Athens, AL) to a high of 0.0234 mg/l (Rome, TN) with an average of 0.0177 mg/l. There were no significant differences among the locations for copper in FWM flour; however, significant differences in copper content of SRW samples due to location were

Table 19. F-ratios of analysis of variance for metals found in freshwater mussel flour

Source	d.f.	Copper	Chromium	Manganese	Magnesium	Potassium	Calcium	Iron	Sodium	Zinc	Aluminum
Total	15	---	---	---	---	---	---	---	---	---	---
A. Location	3	3.48*	8.54**	7.66**	21.07**	0.91^{ns}	1.77^{ns}	1.69^{ns}	46.02**	13.53**	19.04**
B. Time of Harvest	3	4.23*	1.43^{ns}	2.43^{ns}	1.78^{ns}	0.94^{ns}	2.56^{ns}	2.56^{ns}	2.28^{ns}	0.61^{ns}	1.03^{ns}
A x B	9	1.37^{ns}	2.94*	2.23^{ns}	2.83*	0.58^{ns}	0.45^{ns}	2.52^{ns}	1.55^{ns}	3.20*	3.44*
Residual Error (Mean Square)	16	0.000003	0.000001	3.961429	0.009326	0.017380	378.026647	0.143456	0.025876	0.001054	0.011351

^{ns}Not significant at the 0.05 level.

*Significant at the 0.05 level.

**Significant at the 0.01 level.

Table 20. Analysis of variance for metals found in surface river water

Source	d.f.	Copper	Manganese	Magnesium	Potassium	Calcium	Iron	Sodium	Zinc	Aluminum
					F-ratios					
Total	31	---	---	---	---	---	---	---	---	---
A. Location	3	102.33**	37.64**	246.22**	40.15**	1811.85**	8.44**	43.10**	1082.25**	12.29**
B. Time of Harvest	3	13.39**	45.28**	2029.44**	26.62**	3937.47**	3.14^{ns}	5.90**	54.51**	33.78**
A x B	9	259.26**	38.37**	1956.62**	19.74**	1771.34**	3.44*	11.95**	177.75**	11.64**
					Mean square					
Residual Error	16	0.000002	0.000080	0.001578	0.018273	0.029494	0.000264	1.003525	0.000019	0.002216

*Significant at the 0.05 level.

**Significant at the 0.01 level.

^{ns}Not significant at the 0.05 level.

found (Table 20). Foster and Bates (1978) measured the effects of copper content in industrial wastes on the mussel fauna. These researchers found copper accumulation in the species tested was inversely related to body weight. The mussel fauna was seriously affected with mortalities during effluent exposures and with accumulated copper in the mussel tissues of 0.02 mg/g. Thus, Phillips (1976a; 1976b) observed that marine mussels should not be used as an indicator of contamination of the marine environment by copper effluent. He concluded that the copper uptake was affected by salinity, temperature changes, season and by the presence of other metals (zinc, cadmium, lead and copper). Foster and Bates (1978) did not take these variables into consideration. The average amount of copper found in natural waters in the United States is 0.015 mg/l (Anon., 1976b).

The mean chromium content for FWM flours by locations ranged from a low of 0.0014 mg/g (Rome, TN) to a high of 0.0042 mg/g (New Johnsonville, TN, South) with an average of 0.0031 mg/g for all locations (Table 21). There were significant differences among some of the locations. The concentration of chromium in the SRW was below the sensitivity limit of the atomic absorption spectrophotometer. For this reason, no quantitative data for this element can be reported.

The mean manganese content of FWM flours ranged from a low of 3.3501 mg/g at Athens, AL, to a high of 6.9402 mg/g at New Johnsonville, TN (North). The average for the four locations was 5.0896 mg/g. The manganese concentration was higher (0.01 level) in the FWM harvested from both sites at New Johnsonville, TN, than the FWM harvested at the other two locations. The manganese concentration in the SRW samples at any location was extremely low. The levels in FWM samples could

CHAPTER V

SUMMARY

As a result of an expanding world population, deficiencies in both the amount and quality of foods available are predicted in developing countries. Food production has not kept pace with population growth, causing severe protein-calorie malnutrition. Therefore, it is imperative that new sources of potential foodstuffs from currently unexploited resources be investigated.

An unexplored potential source of protein is available from the freshwater mussel (FWM), Megalonaias gigantea. Harvest of these freshwater shellfish by commercial shellers from the Tennessee River generates in excess of 909 metric tons of mussel flesh each year. Presently, the flesh is discarded. If the FWM could be shown to be safe and free from environmental contamination, a new and potentially nutritious food source could be made available to the public.

The FWM were collected at four locations, three on the Tennessee River (Athens, AL; New Johnsonville, TN, South; New Johnsonville, TN, North) and one on the Cumberland River (Rome, TN). Samples of mussels and surface river water (SRW) were collected from each location and shipped to the laboratory. A minimum of 12 mussels (1200 g of mussel flesh) and 1 SRW sample (700 ml) were collected at each location at four separate times during the 1980 fall season.

The microbiological examination of the FWM and SRW samples used three bacteriological analyses: total aerobic plate counts (TPC),

total coliform counts (TCC), and fecal streptococci counts (FSC). The counts on FWM were affected at the 0.01 level by location, time of harvest and the interaction, location x time of harvest. The time of harvest for TCC, however, had no significant effect at the 0.05 level.

The three bacteriological indices showed small variations in mean log values for FWM among the locations: TPC values (5.0 to 6.1 cells per g), TCC values (3.3 to 3.7 cells per g), and FSC values (0.0 to 0.1 cells per g). Small variations were observed in the mean log values among the locations for SRW. The three bacteriological indices were: TPC values (2.9 to 3.5 cells per ml), TCC values (0.7 to 1.6 cells per ml), and FSC (0.0 to 0.1 cells per ml). In comparison to market ground beef which was found to contain a TPC of 10^7 cells/ml (Westhoff and Feldstein, 1976), the microbiological quality of FWM is quite acceptable for human consumption.

The biological oxygen demand (BOD) of the SRW was affected at the 0.01 level by the four locations, time of harvest and the interaction, location x time of harvest. The chemical oxygen demand (COD) was affected at the 0.05 level by location but was not significantly affected by the time of harvest and the interaction, location x time of harvest. The mean BOD value for location ranged from 1.30 to 8.20 mg/l. The BOD of SRW from New Johnsonville (South) was highest at 8.20 mg/l and Athens, AL, had the lowest BOD with 1.30 mg/l. The mean COD value for location ranged from 1.09 to 18.19 mg/l. The COD of SRW from New Johnsonville (South) was highest, at 18.19 mg/l, and Rome, TN, had the lowest COD with 1.09 mg/l.

The FWM were freeze dried to form a dry FWM concentrate. This concentrate was broken down with a mortar and pestle to form a flour.

The proximate composition of the FWM flour and flesh varied at the 0.01 level due to the four locations, time of harvest and the interaction, location x time of harvest. The FWM flour on the average contained 4.09% moisture, 40.71% protein, 3.70% crude fat, 14.77% ash, and 36.74% nitrogen free extract. The FWM flesh contained 84.41% moisture, 6.29% protein, 0.65% crude fat, 2.20% ash and 5.83% nitrogen free extract. Variation between locations in the composition of the FWM flour and flesh could have resulted from seasonal varieties in the habitat, the age of mussel, food supply and sex of the mussel.

Seven short chain volatile fatty acids (VFA) were identified in freshwater mussel flour from the four locations. These acids were acetic, propionic, butyric, isovaleric, valeric, caproic and heptanoic acids. The concentrations of VFA from the four locations were different at the 0.01 level. Acetic and propionic acids were the predominant VFA in the flour which contained 47.29 and 40.30 mg/100g of flour, respectively.

Lipid content determined by the chloroform methanol extraction method in the FWM flour was affected by location at the 0.01 level. The percentage of lipid ranged from a low of 4.91 at Athens, AL, to a high of 9.38 at Rome, TN. The Rome, TN, location was significant by higher than the other three locations.

A wide variety of long chain fatty acids were detected in the extracted FWM flour lipids by gas liquid chromatography of methyl esters. In the FWM flour lipid, 7 fatty acids (C16:0, C16:1, C17:8, C17:0, C18:3, C18:1, C21:1) in the range of 3.70 to 24.88% accounted for 65% of the total fatty acid content. The two dominant fatty acids were C16:0 and C16:1, which accounted for 32% of the total fatty acid

content. The lipid of the FWM flour consisted of at least 50% unsaturated fatty acids.

Of the 47 possible pesticide residues that are detected with the procedure used, only 8 were found in the composite flour samples. These were benzene hexachloride (BHC), heptachlor, heptachlor epoxide, aldrin, dieldrin, pp'DDE, endosulfan-1 and methyl parathion. With two exceptions, the pesticide concentration (PPM) was below the "Action Levels" found in the Code of Federal Regulations. Polychlorinated biphenyl (PCB), identified as Arochlor 1254, was detected in the flour from three out of the four locations. PCB was below the maximum levels allowed by federal standards.

The metals measured in FWM flour and SRW were copper, chromium, manganese, magnesium, potassium, calcium, iron, sodium, zinc, aluminum, lead nickel, mercury and cadmium. The FWM had a relatively high content of iron, calcium, and manganese. SRW samples showed relatively low concentration of all elements tested.

The amino acid analysis showed that FWM contained at least 17 amino acids including the essential amino acids. Glutamic acid, aspartic acid, arginine, lysine, leucine, glycine valine and alanine were the major amino acids present.

The Hunter L color values of FWM flour were not significantly different between the four locations. Hunter "a" and "b" color values of the flour were affected (0.01 level) by location, time of harvest and the interaction, location x time of harvest.

A 62-member sensory panel evaluated three chowder soups made with freshwater mussels (Megalonaias gigantea), saltwater clams

(Mercenaria mercenaria) and a control consisting of all ingredients except the freshwater mussels or saltwater clams. An 8-point hedonic scale where 1 indicated "like extremely" and 8, "dislike extremely" was used to assess overall acceptability. The control soup had the highest score, while scores for saltwater clams and freshwater mussel chowders followed in succession. The scores ranged from 2.79 for the control chowder to 4.11 for the FWM chowder. The toughness of the muscular foot was considered with its high content of connective tissue as one factor in the scores of the freshwater mussels.

Gel electrophoresis showed the protein from FWM was a complex mixture of proteinaceous species which ranged from about 10,000 to 70,000 daltons in molecular weight. The source of the protein material and its treatment before analysis markedly affected the electrophoretic distribution of bands in the gels. Particularly, storage of FWM flour at room temperature decreased the number of bands that appeared.

From all indications, FWM could be a usable adjunct in the food supply, particularly as protein supplement in a dry flour form. While fish protein concentrate did not prove successful in the American markets, a large consumer market exists in Asian and African countries. Despite the lack of appeal to American tastes, freshwater mussels as a flour or even canned fresh, could have a place in the export trade American palates slowly become re-educated to the advantages of little-known food sources. Additional research is needed to develop processing techniques which take into account the inherent toughness of the mussel, as well as difficulties with shucking. New ideas are also necessary as to how this product should be utilized.

REFERENCES

Anon. 1971. "Pesticide Analytical Manual." Vol. 1. Food and Drug Administration. U.S. Dept. of Health and Human Services, Washington, DC.

Anon. 1976a. "Model 1200 Destainer Instruction Manual." Bio-Rad Laboratories, Richmond, CA.

Anon. 1976b. "Quality Criteria for Water." U.S. Environmental Protection Agency, Washington, DC.

Anon. 1977a. "A Comprehensive Review of the Commercial Oyster Industries in the United States." U.S. Dept. of Commerce, Washington, DC.

Anon. 1977b. "The Molluscan Shellfish Industries and Water Quality: Problems and Opportunities." U.S. Dept. of Commerce, Washington, DC.

Anon. 1979a. Action levels for poisonous or deleterious substances in human food and animal feed. U.S. Food and Drug Administration, Washington, DC.

Anon. 1979b. Depuration process cleanses polluted shellfish. BioScience 26(6):384.

Anon. 1981a. "Fisheries of the United States 1980." National Marine Fisheries Service, U.S. Dept. of Commerce, Washington, DC.

Anon. 1981b. Mussel Culture. Impact-TVA-Natural Resource and the Environment 4(5):1.

AOAC. 1980. "Official Methods of Analysis." 13th ed. Association of Official Analytical Chemists, Washington, DC.

AOAC. 1973. "Official and Tentative Methods." 3rd ed. American Oil Chemists' Society, Chicago, IL.

Aoyama, I., Inoue, Y. and Inoue, Y. 1978. Experimental study on the concentration process of trace elements through a food chain from the viewpoint of nutrition ecology. Water Res. 12:831.

Armstrong, F. A. J. and Uthe, J. F. 1971. Semi-automated determination of mercury in animal tissue. At. Absorp. Newsl. 10(5):101.

Barnes, R. D. 1963. "Invertebrate Zoology." W. B. Saunders Company, Philadelphia, PA.

Bedford, J. W., Roelofs, E. W. and Zabik, M. J. 1968. The freshwater mussel as a biological monitor of pesticide concentration in a lotic environment. Limnol. Oceanog. 13:118.

Bedford, J. W. and Zabik, M. J. 1973. Bioactive compounds in the aquatic environment: uptake and loss of DDT and dieldrin by freshwater mussels. Arch. Environ. Contam. Toxicol. 1(2):97.

Beleau, M. H., Heidelbaugh, N. D. and Van Dyke, D. 1975. Open-ocean farming and human foods. Food Technol. 29(12):27.

Bodwell, C. E., ed. 1977. "Evaluation of Proteins for Humans." AVI Pubishing Company, Inc., Westport, CT.

Boeapple, J. F. and Coker, R. E. 1912. Mussel resources of the Holston and Clinch Rivers of Eastern Tennessee. Bur. of Fish. Doc. 765, Washington, DC.

Bogert, L. J., Briggs, G. M. and Calloway, D. H. 1973. "Nutrition and Physical Fitness." W. B. Saunders Co., Philadelphia, PA.

Brice, J. W. and Lewis, R. B. 1979. Mapping of mussel (Unionidae) communities in large streams. Amer. Midland Nat. 191(2):454.

Burch, J. B. 1975. "Freshwater Unionacean Clams (Mollusca: Pelecypoda) of North America." Malacological Publications, Hamburg, MI.

Churchill, E. P., Jr. and Lewis, S. I. 1924. Food and feeding in freshwater mussels. Bull. U.S. Bur. Fish. 39:437.

Coker, R. E. 1919. Fresh-water mussels and mussel industries of the United States. Bull. U.S. Bur. Fish. 36:11.

Coker, R. E. 1921. Natural history and propagation of fresh-water mussels. Bull. U.S. Bur. Fish. 37:75.

Cook, S. F. 1946. A reconsideration of shell mounds with respect to population and nutrition. Amer. Antiquity 12:50.

Cossa, D. and Bourget, E. 1980. Trace elements in Mytilus edulis L. from the estuary and Gulf of St. Lawrence, Canada: lead and cadmium concentration. Environ. Poll. (Series A) 23:1.

Davies, I. M. and Pirie, J. M. 1980. Evaluation of a "mussel watch" project for heavy metals in Scottish coastal waters. Mar. Biol. 57:87.

Dietz, T. H. 1979. Uptake of sodium and chloride by freshwater mussels. Can. J. Zool. 57:156.

Dietz, T. H. and Findlay, A. M. 1980. Ion-stimulated ATPase activity and NACl uptake in the gills of freshwater mussels. Can. J. Zool. 58:917.

Doull, J., Klaassen, C. D. and Amdur, M. O., eds. 1980. "Casarett and Doull's Toxicology, The Basic Science of Poisons." 2nd ed. Macmillan Publishing Co., New York.

Dunn, B. P. and Stich, H. F. 1976. Release of the carcinogen benzo(a) pyrene from environmentally contaminated mussels. Bull. Environ. Contam. Toxicol. 15(4):398.

Emmel, R. H., Sotera, J. J. and Stux, L. R. 1977. "Atomic Absorption Manual--Standard Conditions for Flame Operations," Vol. 1. Instrumentation Laboratory, Inc., Wilmington, ME.

Establier, R. 1969. Seasonal variation in the composition of the mussel, Vegus gallina L. Investigation Pesquera 33(1):7.

EPA. 1979. "Methods for Chemical Analysis of Water and Wastes. EPA-600 4-79-020," Environmental Monitoring and Support Laboratory. U.S. Environmental Protection Agency, Cincinnati, OH.

Exler, J. and Weihrauch, J. L. 1977. Comprehensive evaluation of fatty acids in foods. XII. Shellfish. J. Am. Dietet. Assoc. 71:518.

FAO. 1970a. Amino acid content of foods and biological data on proteins. FAO Nutr. Stud. 24, Rome.

FAO. 1970b. "Lives in Peril: Protein and the Child." World Food Problems 12, Rome.

FAO/WHO. 1973. "Energy and Protein Requirements." FAO Nutr. Meeting Report, Series 52, Rome.

Fennema, O. R., ed. 1976. "Principles of Food Science. Part I, Food Chemistry." Marcel Dekker, Inc., New York.

Finch, R. 1977. Whatever happned to fish protein concentrate? Prospects for success still not very bright. Food Technol. 31(5):44.

Foster, R. B. and Bates, J. M. 1978. Use of freshwater mussels to monitor point source industrial discharge. Environ. Sci. Technol. 12(8):958.

Frazier, W. C. and Westhoff, D. C. 1979. "Food Microbiology." 3rd ed. McGraw-Hill Book Co., New York.

Friedman, M., ed. 1975. "Protein Nutritional Quality of Foods and Feeds, Part 1: Assay Methods, Biological, Biochemical, Chemical." Marcel Dekker, Inc., New York.

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

Furr, A. K., Parkinson, T. F., Ryther, J., Bache, C. A., Gutenmann, W. H., Pakkala, I. S. and Lish, D. J. 1981. Concentrations of elements in a marine food chain culture in sewage wastewater. Bull. Environ. Contam. Toxicol. 26:54.

Garreis, M. J. and Pittman, F. A. 1981. "Heavy Metal, Polychlorinated Biphenyl, and Pesticide Levels in Oysters (Crassostrea virginica), 1979, Maryland Oyster Spot Survey." Water Management Administration, Dept. of Health and Mental Hygiene, Baltimore, MD.

Gerry, R. W. 1980. Ground dried whole mussels as a calcium supplement for chicken rations. Poul. Sci. 59:2365.

Geyer, H. G. 1972. Environmental standards required in the production of aquatic animals for food. J.A.V.M.A. 161(11):1484.

Grant, B. F. 1976. Endrin toxicity and distribution in freshwater: A review. Bull. Environ. Contam. Toxicol. 15(3):283.

Hackler, L. R. 1977. Methods of measuring protein quality: A review of bioassay procedures. Cereal Chem. 54(4):984.

Hansen, P. D. 1980. Uptake and transfer of the chlorinated hydrocarbon lindane (γ-BHC) in a laboratory freshwater food chain. Environ. Pollut. (Series A) 21:97.

Harrigan, W. F. and McCance, M. E. 1976. "Laboratory Methods in Food and Dairy Microbiology." Academic Press, New York.

Hayes, J., ed. 1981. "Yearbook of Agriculture." U.S. Dept. of Agriculture, Washington, DC.

Helwig, J. T. and Council, K. A., eds. 1979. "Statistical Analysis System (SAS) User's Guide," 1979 ed. SAS Institute, Inc., Raleigh, NC.

Hendricks, C. W. 1972. Enteric bacterial growth rates in river water. Appl. Microbiol. 24(2):168.

Hendron, R. 1981. Private communication. The University of Tennessee, Knoxville, TN.

Hildebrand, S. G., Strand, R. H. and Huckabee, J. W. 1980. Mercury accumulation in fish and invertebrates of the North Fork Holston River, Virginia and Tennessee. J. Environ. Qual. 9(3):393.

Hobden, D. J. 1970. The catalase of a freshwater bivalve. Can. J. Zool. 48(2):201.

Huebner, J. D. 1980. Seasonal variation in two species of unionid clams from Manitoba, Canada: biomass. Can. J. Zool. 58:1980.

Humason, G. L. 1966. "Animal Tissue Techniques." 2nd ed. W. H. Freeman and Co., San Francisco, CA.

Hurlburt, C. G. 1979. Frozen cultured mussels introduced by New England mariculture firm. Quick Frozen Foods 41(7):36.

Imlay, M. J. 1973. Effects of potassium on survival and distribution of freshwater mussels. Malacologia 12:97-113.

Isom, B. G. 1969. The mussel resources of the Tennessee River. Malacologia 7:397-425.

Isom, B. G., Yokley, P., Jr. and Gooch, C. H. 1973. Mussels of the Elk River basin in Alabama and Tennessee, 1965. Amer. Midland Nat. 89(2):437.

Jansen, G. R. 1978. Biological evaluation of protein quality. Food Technol. 32:52.

Jones, A. 1974. "World Protein Resources." Halsted Press, New York.

Jones, J. G. 1971. Studies on freshwater bacteria: Factors which influence the population and its activity. J. Ecology 59(2):593.

Kahn, S. G. 1981. World hunger: An overview. Food Technol. 35(9):93.

Khan, M. A., Rao, M. R. and Novak, A. F. 1976. Reduction of polychlorinated biphenyls in shrimp and eggs by freeze-drying techniques. J. Food Sci. 41:1137.

Kraemer, L. R. 1979. Corbicula (Bivalvia:Sphaeriacea) vs. indigenous mussels (Bivalvia:Unionacea) in U.S. rivers: A hard case for interspecific competition? Amer. Zool. 19:1085.

Lachance, P. A., Bressani, R., and Elias, L. G. 1977. Shorter protein bioassays. Food Technol. 31:82.

Langston, W. J. 1978. Persistence of polychlorinated biphenyls in marine bivalves. Mar. Biol. 46:35.

Lee, G. F. and Wilson, W. 1969. Use of chemical composition of freshwater chamshells as indicators of paleohydrologic conditions. Ecology 50(6):990.

Lee, Y. B., Richansrud, D. A., Hagberg, E. C. and Forsythe, R. H. 1976. Detection of various nonmeat extenders in meat products. J. Food Sci. 41:589.

Lin, K. T. D. 1977. Use of low-salt, alkaline buffer in the elution of basic amino acids from a single-column amino acid analyzer. J. Chromatogr. 132:160.

Lippmann, M. and Schlesinger, R. B. 1979. "Chemical Contamination in the Human Environment." Oxford University Press, New York.

Loosli, J. K. 1974. New sources of protein for human and animal feeding. BioScience 24(1):26.

Lutz, R. A. and Incze, L. S. 1977. "A Comprehensive Review of the Commercial Mussel Industries in the United States." National Marine Fisheries Service, U.S. Dept. of Commerce, Washington, DC.

Mackie, G. L. 1978. Shell structure in freshwater Sphaeriaceae (Bivalvia:Heterodonta). Can. J. Zool. 56(1):1.

Mahaffey, K. R., Corneliussen, P. E., Jelinek, C. F. and Fiorino, J. A. 1975. Heavy metal exposure from foods. Environ. Health Perspec. 12:63.

Marable, N. L., Hinners, M. L., Hardison, N. W. and Kehrberg, N. L. 1980. Protein quality of supplements and meal replacements. J. Am. Dietet. Assoc. 77:270.

Matteson, M. R. 1953a. Fresh-water mussels used by Illinoian Indians of the Hopewell culture. Nautilus 66(4):130.

Matteson, M. R. 1953b. Fresh-water mussels used by Illinoian Indians of the Hopewell culture. Nautilus 67(1):25.

McLaughlan, J. M. 1972. Nutritional evaluation of proteins by biological methods. Cereal Sci. Today 17:162.

Melton, S. L., Moyers, R. E. and Playford, C. G. 1979. Lipids extracted from soy products by different procedures. J. Am. Oil Chemists' Soc. 56(4):489.

Melton, S. L., Black, J. M., Davis, G. W. and Backus, W. R. 1982. Flavor and selected chemical components of ground beef from steers backgrounded on pasture and feed corn up to 140 days. J. Food Sci. 47:699.

Munro, I. C. and Charbonneau, S. M. 1978. Environmental contaminants. Federation of American Societies for Experimental Biology--Fed. Proc. 37(12):2582.

Munson, P. J., Parmalee, P. W. and Yarnell, R. A. 1971. Subsistence ecology of Scovill, a terminal Middle Woodland village. Amer. Antiquity 36(4):410.

Needleman, S. B., ed. 1975. "Protein Sequence Determination: A Source Book of Methods and Techniques." 2nd ed. Springer-Verlag, New York.

Nelson, D. J., Rains, T. C. and Norris, J. A. 1966. High-purity calcium carbonate in freshwater clam shell. Science 152(3727):1368.

Neter, E., ed. 1970. "Recommended Procedure for the Examination of Sea Water and Shellfish." The American Public Health Association, Inc., Washington, DC.

Ostrander, J. and Dugan, L. R. 1961. A rapid, quantitative lipid extraction method. No. 50. American Meat Inst. Found., Chicago, IL.

Parmalee, P. W. 1955. Buttons, pearls, and fertilizer. Living Museum 17(1):453.

Parmalee, P. W. 1966. Oyster peals from fresh-water mussels. Living Museum 27(9):453.

Parmalee, P. W. and Klippel, W. E. 1974. Freshwater mussels as a prehistoric food resource. Amer. Antiquity 39(3):421.

Parmalee, P. W., Klippel, W. E. and Bogan, A. E. 1980. Notes on the prehistoric and present status of the naiad fauna of the Middle Cumberland River, Smith County, Tennessee. Nautilus 93(3):93.

Penke, B., Ferenzci, R. and Kovacs, K. 1974. A new acid hydrolysis method for determining tryptophan in peptides and proteins. Anal. Biochem. 60:45.

Pennak, R. W. 1978. "Fresh-water Invertebrates of the United States." John Wiley and Sons, New York.

Phillips, D. J. H. 1976a. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. Mar. Biol. 38:59.

Phillips, D. J. H. 1976b. The common mussel Mytilus edulis as an indicator of pollution by zinc, cadmium, lead and copper. II. Relationship of metals in the mussel to those discharged by industry. Mar. Biol. 38:71.

Phillips, D. J. H. 1977. The common mussel Mytilus edulis as an indicator of trace metals in Scandinavian waters. I. Zinc and cadmium. Mar. Biol. 43:283.

Pietrzak, J. E., Bates, J. M. and Scott, R. M. 1973. Constituents of unionid extrapallial fluid. I. Electrophoretic and immunological studies of protein components. Biol. Bull. 144:391.

Pirie, N. W. 1975. "Food Protein Sources." Cambridge University Press, Cambridge, England.

Poppensiek, G. C. 1972. Aquaculture and mariculture: protein potentials. J.A.V.M.A. 161(11):1467.

Price, J. F. and Schweigert, B. S., eds. 1978. "The Science of Meat and Meat Products." Food and Nutrition Press, Inc., Westport, CT.

Rand, M. C., Greebert, A. E. and Taras, M. J. 1976. "Standard Methods for the Examination of Water and Wastewater." 14th ed. The American Public Health Assoc., Inc., Washington, DC.

Renzoni, A. and Bucci, E. 1976. Bodily distribution, accumulation and excretion of mercury in a fresh-water mussel. Bull. Environ. Contam. Toxicol. 15(3):366.

Ritchie, T. P. 1977. "A Comprehensive Review of the Commercial Clam Industries in the United States." U.S. Dept. of Commerce, Washington, DC.

Ruppert, V. and Draughon, F. A. 1982. Private Communication. The University of Tennessee, Knoxville, TN.

Ryther, J. H. 1981. Mariculture, ocean ranching, and other culture-based fisheries. BioScience 31(3):223.

Rusoff, L. L., Blakeney, E. W., Jr. and Culley, D. D., Jr. 1980. Duckweeds (Lemnaceae Family): A potential source of protein and amino acids. J. Agric. Food Chem. 28(4):848.

Rusoff, J. H. 1981. Mariculture, ocean ranching, and other culture-based fisheries. BioScience 31(3):223.

Sathe, S. K. and Salynkhe, D. K. 1981. Solubilization and electrophoretic characterization of Great Northern Bean (Phaseolus vulgaris L.) Proteins. J. Food Sci. 46:82.

Satterlee, L. D. 1975. Improving utilization of animal by-products for human foods--A review. J. Anim. Sci. 4(2):687.

Schalie, H. V. D. 1938. Additional notes on the naiades (fresh-water mussels) of the lower Tennessee River. Amer. Midland Nat. 22:452.

Sidwell, V. D. and Ambrose, M. E. 1974. Nutritional and chemical evaluation of the protein of various finfish and shellfish. In "Protein Nutritional Quality of Foods and Feeds," Part 2. Ed. Friedman, M. Marcel Dekker, Inc., New York.

Sidwell, V. D., Bonnet, J. C. and Zook, E. G. 1973. Chemical and nutritive values of several fresh and canned finfish, crustaceans, and mollusks. Part I: Proximate composition, calcium, and phosphorus. Mar. Fish. Rev. 35(2):16.

Sidwell, V. D., Foncannon, P. R., Moore, N. S. and Bonnet, J. C. 1974. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish, and mollusks. I. Protein, fat, moisture, ash, carbohydrate, energy value, and cholesterol. Marine Fish. Rev. 36(3):21.

Sinclair, R. M. 1964. Clam pests in Tennessee water supplies. J. Amer. Water Works Assoc. 56(5):592.

Sinclair, R. M. and Ingram, W. M. 1961. A new record for the Asiatic clam in the United States, the Tennessee River. Nautilus 74(3):114.

Sinclair, R. M. and Isom, B. G. 1963. "Further Studies on the Introduced Asiatic Clam (Corbicula) in Tennessee." Tennessee Dept. of Public Health, Nashville, TN.

Smith, A. L., Green, R. H. and Lutz, A. 1975. Uptake of mercury by freshwater clams (family Unionidae). J. Fish Res. Board Can. 32(8):1297.

Smith, P., Jr., Ambrose, M. E. and Knobl, G. M., Jr. 1965. Possible interference of fats, carbohydrates, and salts in amino acid determinations in fish meals, fish protein concentrates, and mixed animal feeds. J. Agr. Food Chem. 13(3):266.

Speck, M. L., ed. 1976. "Compendium of Methods for the Microbiological Examination of Foods," American Public Health Assoc., Inc., Washington, DC.

Spinelli, J., Koury, B., Groninger, H., Jr. and Miller, R. 1977. Expanded uses for fish protein from underutilized species. Food Technol. 31(5):184.

Steel, R. G. D. and Torrie, J. H. 1960. "Principles and Procedures of Statistics." McGraw-Hill Publishing Co., New York.

Stern, E. M. and Felder, D. L. 1978. Identification of host fishes for four species of freshwater mussels (Bivalvia:Unionidae). Amer. Midland Nat. 100(1):233.

Teeny, F. M. 1975. Rapid method for the determination of mercury in fish tissue by atomic absorption spectroscopy. J. Agric. Food Chem. 23(4):668.

Thompson, B. 1982. Private communication. Mark B. Whitaker Water Plant, Knoxville, TN.

Ubbaonu, C. N. 1980. Effect of paraffin wax coating on fatty acids in country cured hams. Thesis for M.S. degree, The University of Tennessee Library, Knoxville, TN.

USDA. 1974. "The World Food Situation and Prospects to 1985." Foreign Agr. Econ. Rpts. 98, Washington, DC.

Uthe, J. F., Armstrong, F. A. J. and Stainton, M. P. 1970. Mercury determination in fish samples by wet digestion and flameless atomic absorption spectrophotometry. J. Fish Res. Board Can. 27:805.

Varanka, I. 1977. The effect of some pesticides on the rhythmic activity of adductor muscle of fresh-water mussel larvae. Act Biol. Acad. Sci. Hung. 28(3):317.

Varanka, I. 1978. Effect of some pesticides on the rhythmic adductor muscle activity of fresh-water mussel larvae. Acta Biol. Acad. Sci. Hung. 29(1):43.

Watt, K. and Merrill, A. L. 1963. Composition of foods: raw, processed, prepared. Agri. Handbook 8, U.S. Dept. of Agriculture, Washington, DC.

Weber, K. and Osborn, M. 1969. The reliability of molecular weight determinations by dodecysulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244(16):4406.

Weber, K. and Osborn, M. 1975. Proteins and sodium dodecylsufate: Molecular weight determination on polyacrylamide gels and related procedures. In "The Proteins," Vol. 1, p. 179. Ed. Neurath, H. and Hill, R. L. Academic Press, New York.

Wedin, W. F., Hodgson, H. J. and Jacobson, N. L. 1975. Utilizing plant and animal resources in producing human food. J. Anim. Sci. 41(2):667.

Weisz, P. B. 1968. "Elements of Zoology." McGraw-Hill Book Co., New York.

Wesson, J. B., Lindsay, R. C. and Stuiber, D. A. 1979. Discrimination of fish and seafood quality by consumer population. J. Food Sci. 44:878.

Westhoff, D. and Feldstein, F. 1976. Bacteriological analysis of ground beef. J. Milk Food Technol. 39(6):401.

Whitaker, J. R. and Tannenbaum, S. R. 1977. "Food Proteins." Avi Publishing Co., Westport, CT.

Wurtz, C. B. 1962. Zinc effects on fresh-water mollusks. Nautilus 76(2):53.

Zoellner, D. R. 1977. "Water Quality and Molluscan Shellfish: An Overview of the Problems and the Nature of Appropriate Federal Laws." U.S. Dept. of Commerce, Washington, DC.

Zook, E. G., Powell, J. J., Hackley, B. M., Emerson, J. A., Brooker, J. R. and Knobl, G. M., Jr. 1976. National marine fisheries service prelmiminary survey of selected seafood for mercury, lead, cadmium, chromium, and arsenic content. J. Agric. Food Chem. 24(1):47.