AQUACULTURE OF PACU (Piaractus mesopotamicus) AND A COMPARISON OF ITS QUALITY: MICROBIOLOGICAL, SENSORY AND PROXIMATE COMPOSITION

by

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(ABSTRACT)

Pacu (*Piaractus mesopotamicus*) initially weighing 72.0 g were fed three diets - a) 0.5% vegetable (zucchini), b) commercial 32% (P32) and c) commercial 36% (P36) protein diets for 24 weeks and their growth performance compared. Processing yields and proximate composition were determined following dressing of pacu. The microbiological quality of pond cultured pacu was compared to aquacultured hybrid striped bass, tilapia, and rainbow trout grown in pond and recirculating aquaculture systems. Sensorial analyses for differences in flavor, preference, and color were also determined. Protein concentration significantly influenced the weights, lengths, specific growth rate, feed conversion ratio, and protein efficiency ratio (p < 0.05). Diet insignificantly influenced the processing yields (p > 0.05). The moisture, protein and total lipid contents were significantly affected (p < 0.05) by the dietary protein. The indicative bacterial quality differed significantly for pacu as well as the water used for

culturing pacu (p < 0.05) among dietary treatments. Aquaculture production systems significantly influenced the indicative and pathogenic bacterial quality. *Listeria monocytogenes, Salmonella spp., Yersinia enterocolitica,* and *Escherichia coli* O157:H7 were not isolated from any of the sampled fish. The qualitative and quantitative results of *Clostridium botulinum* were influenced by the production system (p < 0.05). Flavor of pacu was comparable to that of hybrid striped bass, tilapia, and rainbow trout, but superior to catfish. Cooking significantly improved the color of the ground fish fillets.

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INTRODUCTION

The annual per capita seafood consumption in the United States is estimated at 16 pounds and is expected to rise to 20 pounds by the end of the century (Flick et al., 1991). The decline in America's natural fishery resources coupled with increased demand for seafood has led aquaculture to develop into a science relying on sophisticated technology. Further, many new species such as commom and grass carp, tilapia, etc. have been introduced into the United States and are now being grown extensively under artificial conditions.

Pacu (*Piaractus mesopotamicus*) is a freshwater fish indigenous to the Parana -Uruguai river system in Brazil. It is herbivorous and feeds on leaves, flowers, fruits, and seeds of superior plants. Pacu fed formulated diets can take up to a year to reach 1.2 Kg. The fast growth rate demonstrated by the fish coupled with its organoleptic qualities could be exploited to sustain the increasing aquaculture production.

Fish and fish products have long been considered a vehicle of foodborne bacterial and parasitic infections leading to human illnesses (Brown and Dorn, 1977). The Center for Disease Control and Prevention (CDCP) reported that seafoods account for 5% of the individual cases and 10% of all foodborne illness outbreaks in the United States, with most of the outbreaks resulting from the consumption of raw molluscan shellfish (Otwell, 1989). It is generally accepted that the number and types of bacteria associated with aquacultured fish is influenced directly by the environment in which they are grown (Nedoluha and Westhoff, 1995). Aquaculture systems tend to accumulate organic and inorganic materials that serve as an excellent substrate for bacterial proliferation, and hence deteriorate the bacterial quality of the water. Therefore, a careful monitoring of bacterial water quality would help in the production of safe and high quality seafood products.

In December 1997, the Food and Drug Administration (FDA) will require seafood processors to adopt a quality assurance program based on the hazard analysis critical control point (HACCP) concept. Hence, it is necessary for the aquaculture industry to monitor the microbial hazards that might be encountered during the growth of fish. Therefore, the objectives of this study were:

To determine the use of a low - cost agricultural product (zucchini) for raising pacu.
To determine the indicative and pathogenic microbial quality of pacu raised in an intensive aquaculture system.

3) To evaluate the effect of aquaculture production system on the indicative and pathogenic microbial quality of pacu and other aquacultured finfish.

4) To compare the sensory qualities of pacu with other aquacultured fish species.

SECTION I.

REVIEW OF LITERATURE

INTRODUCTION

World seafood supply has been estimated at about 100 million tons, with the world aquaculture production estimated at about 15 million tons. Asia accounts for more than 85% of the production, followed by Europe (7.3%), and then by North America (3.5%). Fish account for almost half (49.5%) of the world aquaculture production, followed by aquatic plants (27.7%), mollusks (18.2%), and crustaceans (4.1%). Inland fishes account for more than 95% of the world fish production (FAO, 1995). With a growing demand for seafood and a decline in the world's fishery resources, aquaculture seems to be the only hope to substantially increase the world seafood production and bridge the widening gap of supply and demand of seafood (Redmayne, 1989).

Aquaculture is defined as the rearing of aquatic organisms in controlled or semi controlled conditions (Stickney, 1996). Aquaculture possesses the potential advantage of providing high quality fishery products to the market place throughout the year (Johnsen, 1989). The practice of aquaculture can be traced back thousands of years, to at least 473 B. C., when FanLei from China wrote an account of carp culture. However, it has only been in the last three decades that aquaculture has undergone a transition from an ancient relatively primitive craft to a science relying on sophisticated technology (Allen, 1982a).

AQUACULTURE IN THE UNITED STATES

The United States contributes less than 3% of the total world aquaculture production, even though a high percentage of the world's farmed seafood production is targeted at its market. Seafood consumption in the United States has been increasing over the past few years with increased consumer awareness of the healthfulness and culinary attributes of seafood (Redmayne, 1989). The per capita consumption of seafood in the recent years has increased from 4.5 to 7 Kg/year (Stickney, 1994). Among the aquacultured fish species, channel catfish (*Ictalurus punctatus*) is produced and processed in the largest quantities, with the production mainly in the Delta region of Mississippi (Stickney, 1994). Production has increased from 10 million Kg in 1977 to 208 million Kg in 1992 (Silva and White, 1994).

Salmonids are cultured in nearly every state of the United States, but most of the trout production comes from the Haggerman valley of Idaho. Presently 25,000 metric tons of trout are produced in the United States.

Species	Common name	Production in 1993
		(Metric Tons)
Ictalurus punctatus	Channel catfish	224,874
Crassostrea virginica	American Oyster	74,611

Table 1. Aquaculture Production of Different Species in the United States (FAO, 1995)

Crassostrea gigas	Pacific Oyster	39,053
Procambarus clarkii	Crayfish	25,757
Oncorhynchus mykiss	Rainbow Trout	25,325
Salmo salar	Atlantic Salmon	10,750

EXOTIC SPECIES IN UNITED STATES AQUACULTURE

Several exotic species have been introduced and are now being farmed extensively in the United States. Some of these include freshwater shrimp (*Macrobrachium rosenbergii*), grass carp (*Ctenopharyngodon idella*), and several species of tilapia (*Oreochromis spp.*) (Stickney, 1994).

In recent years, there has been a growing interest in ornamental tropical freshwater fishes. The tropical fish industry is centered in Florida. The total sale of tropical fish in Florida during 1991 was placed at \$32.8 million dollars and the retail value of tropical fish in the United States has been estimated at between \$250 and \$700 million (Stickney, 1996). Efforts are now proceeding to culture some of the larger species of ornamental fishes as food fish to supplement the declining seafood production. One of the most promising candidates for achieving this goal is pacu, which is already abundant in its wild form and is extensively farmed in Brazil.

PACU (*Piaractus mesopotamicus*)

Pacu (Piaractus mesopotamicus) is a freshwater fish of the order Cypriniform, suborder Characoidei, and family Myleinae (Maristela and Sgarbieri, 1991) and was initially named by Berg in 1895 (Maristela and Sgarbieri, 1991). Pacu is a herbivorous fish, which preferentially feeds on leaves, flowers, fruits, and seeds of superior plants (Merola, 1988). It is indigenous to the Plate River Basin in South America (Borghetti and Canzi, 1993) yet migratory. This species inhabits the Parana - Uruguai river system and is thus found in all major rivers of the central - southern region of Brazil. It has small scales and weighs between 3 and 7 Kg at normal growth. It has been reported that a 18 Kg fish has been harvested from the wild. It has also been reported that the fish is simple to grow and requires low - cost feed. Pacu has been preferred over tilapia and catfish in informal taste tests time after time (Anonymous, 1994). Magalhaes (1931) referred to this species as being valuable and having good organoleptic qualities (Castagnolli and Donaldson, 1981). The construction of large hydroelectric dams on Parana - Uruguai river system is interfering with the migratory patterns of pacu, thus diminishing its biomass (Castagnolli and Donaldson, 1981). Its large size, eating habits, and high prolificity make it adequate for the practice of fish culture (Maristela and Sgarbieri, 1991). Intensive culture of pacu has been developed in the south, south - east, and west - central regions of Brazil, but the species had not been successfully spawned and reared in captivity until recently (Castagnolli and Donaldson, 1981). Most of the available literature on this

species deals with reproductive cycle, induced reproduction, and conditions for intensive cultivation (Maristela and Sgarbieri, 1991).

GROWTH REQUIREMENTS

Protein

Dietary protein is the single most important nutritional factor which influences the growth performance of fish and the cost of the feed; hence the success of aquaculture (Merola, 1988). It is generally accepted that the growth rates of fish increase with increasing levels of dietary protein. However, there is a limit to which dietary protein can be incorporated into aquaculture feeds beyond which there may not be any significant impact on yields. Hence, a careful consideration has to be given to the optimum level of protein in the diet to raise fish because the wastage of protein directly reflects on the increased cost of fish production. Most of the previous experiments involving pacu have been feasibility studies for their culture in cages and ponds. Experiments were also conducted to determine the optimal feeding rate and levels of protein in their diet. Merola (1988) tested the effect of three dietary levels of protein (30, 35, and 40%) and obtained superior yields and weight gains with the 35% protein diet. Carneiro et al. (1994a) reported better growth rates of pacu at the highest protein and lowest energy levels. Borghetti and Canzi (1993) studied the effect of water temperature and feeding rate on the

growth of pacu and noted a direct relationship between water temperature and the amount of feed.

Temperature

Water temperature is the single most important factor, other than food, affecting fish growth (Piper et al., 1982). In most warm water fishes, feeding activity starts when the temperature reaches 17 - 18°C and attains its maximum rate at 28 - 30°C. Borghetti and Canzi (1993) determined the effect of water temperature and feeding rate on the growth rate and feed utilization of pacu and noted that the higher the water temperature and feed, the better the growth rates and feed conversion. They also noted that a wastage of diet at low temperatures (19 - 20°C) when pacu were fed more than 1% of the biomass, owing to their reduced metabolic activity. Higher water temperatures also decreased the mean transit time through the gastrointestinal tract of pacu, but did not have any effect on the protein digestibility (Carneiro et al., 1994b).

Oxygen

Pacu is an obligate gill breather which can survive low oxygen concentrations of less than $0.5 \text{ mg } 1^{-1}$. These low oxygen (hypoxia) conditions are frequently encountered in the Parana - Uruguay river systems which pacu inhabit and under intensive and semi - intensive culture conditions with minimal water exchange. This ability of pacu to survive

low oxygen levels is due to the special behavioral and ecomorphological responses to reduced O₂ availability (Saint - Paul and Bernardinho, 1988).

Under conditions of hypoxia, pacu are capable of utilizing the oxygen - rich surface layer of the water for respiration. This mechanism is referred to as "aquatic surface respiration" (ASR), and is common among tropical fish species (Saint - Paul and Bernardinho, 1988).

The ecomorphological adaptations for pacu under reduced O_2 conditions include dermal swellings on the lower jaw, which has a hydrodynamic function of using the surface layer for gill respiration. These swellings continue to grow in size with on - going ASR showing an increase of 220% in 5 h. When the water is aerated, the lip surface area decreases by about 60% in 3 h (Saint - Paul and Bernardinho, 1988).

Locomotory activity of pacu is affected by O_2 concentration and surface access. Activity increases with hypoxia if the fish has surface access and decreases if surface access is denied, for example by a floating macrophyte cover. Both vertical and horizontal locomotory activity increase in the search for surface access for ASR (Saint -Paul and Bernardinho, 1988).

Reproduction

A single 5 Kg female pacu can produce between 0.5 - 1 million larvae under natural conditions. The reason is that the ripening of the gonads requires a synergistic stimulus of several environmental factors, such as conductivity, water level and rainfall. Pacu

spawn usually during the months of November - March when the water temperatures are 26°C (Saint - Paul, 1986).

Several attempts have been made for artificial reproduction and larval rearing of pacu. Castagnolli and Donaldson (1981) were the first to successfully spawn and rear pacu in captivity. Ovulation was induced by using a priming injection of 0.2 mg/Kg partially purified gonadotropin SG - G100 followed at 8 h by injecting an extract of 20 mg/Kg acetone - dried chum salmon pituitary powder. Spermiation in pacu was induced using a similar primer followed by 14 mg/Kg acetone - dried chum salmon pituitary powder.

Godinho and Godinho (1986) induced pacu to ovulate and spermiate using crude carp pituitary extract. They injected female pacu with 0.5 and 5 mg of dried pituitaries per Kg of body weight with a time interval of 18 h, and the male pacu with a single dose of 0.5 mg of dried pituitary per kg of body weight.

The use of luteinizing hormone - releasing hormone (LHRH) for induced final maturation and ovulation of female pacu was tested by Carolsfeld et al. (1988) with promising results. They concluded that a minimum dose of 50 - 100µg/Kg would reliably induce final maturation and ovulation in female pacu. They also noted that a single injection of 100µg/Kg dose or 50µg/Kg applied as two injections were effective in inducing ovulation. Human chorionic gonadotropin (HCG) and salmon pituitary extract (SPE) were also used to induce spawning in female pacu by Romagosa et al. (1990).

They noted 57.9% and 84.0% of female pacu responded positively to the HCG and SPE treatments respectively.

Aquaculture

Pacu have been traditionally grown in earthen ponds, however, they are now extensively cultured in floating cages made from scrap metal structures near the Itaip Binacional hydroelectric plant located between Paraguay and Brazil. Currently there are about 100 floating cages of various designs covering an area of approximately 1000 m², with more permit requests for such cages. Polyculture with other species is not practiced due to pacu's habit of fin nipping.

Pacu fed under extensive systems in earthen ponds, can take up to two years to attain 1.2 Kg. Cultured pacus fed formulated diets reach 1.2 - 1.5 Kg (1 Kg = 2.2 lbs) in 12 months. Market size is at least a kilo and farm - gate prices now command 1.50 - 2.00 per kilo with a consumer - end price reaching 2.70.

HAZARDS ASSOCIATED WITH THE CONSUMPTION OF SEAFOOD

Seafood may be contaminated with various microbiological agents, parasites, and natural toxins which could affect its quality and safety. The biological hazards can be categorized into three groups: 1) those that cause disease in healthy adults 2) those that usually do not cause disease in healthy adults, but can cause illness in special disadvantaged population groups and 3) those that are of uncertain pathogenecity to humans. Illness due to natural toxins are both highly localized and species - associated (Ahmed, 1992).

Illnesses implicating seafood as the vehicle account for approximately 5% of the individual illnesses and 10% of all foodborne illness outbreaks per year (Otwell, 1989; Bean and Griffin, 1990). The Food and Drug Administration (FDA) estimates show that seafoods are ten times safer than poultry products. A higher risk of disease is associated with the consumption of raw and partially cooked molluscan shellfish than with the consumption of fish (Archer, 1989).

Microbiological Quality of Aquacultured Fish

The aquaculture industry has an excellent record of producing safe and high quality fishery products (Ward, 1989; Fernandes et al.,1997). It is generally accepted that the environment can influence the number and types of bacteria associated with the skin, gills, and intestines of finfish (Nedoluha and Westhoff, 1995; Nickelson II and Finne, 1992). Hence, the microbiological quality of the growing water could influence the microbiological "quality" of fish and ultimately the processed products (Ward, 1989).

Fish may be aquacultured in open (flow - through, pens, cages, raceways etc.), semi - closed or closed (recirculating) systems. Davis and Goulder (1993) determined that the bacteriological quality of the growing water deteriorates when it flows through fish farms. They also noted an enhanced microbial metabolism due to enzymatic breakdown of organic polymers to the oligomeric and monomeric molecules which can be

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subsequently utilized by the bacteria. Hence, the success of aquaculture in providing safe fishery products is dependent on the efficiency of the system employed to maintain and/or improve the bacteriological quality of water coming into the farm.

Nedoluha and Westhoff (1995) examined the quality and quantity of microflora of aquacultured striped bass (Morone saxatilis) grown in indoor flow - through tanks and noted standard plate counts ranging from 3 - 5 log CFU/cm² and anaerobic counts ranging from 2 - 6 log CFU/cm². The predominant groups of bacteria isolated were Aeromonas spp. (18%), Flavobacterium/Cytophaga species (15%), Comamonadaceae (15%), Plesiomonas shigelloides (13%), Moraxellaceae (6%), and Bacillus spp. (4%). Food borne pathogens Staphylococcus aureus and Vibrio spp. were also isolated. McAdams et al. (1996) compared the general microbial quality, qualitative and quantitative levels of human pathogens in rainbow trout reared in different aquaculture systems. They noted standard plate counts ranging from 3 - 6 log CFU/g, coliforms (3.09 - 543 MPN/g), fecal coliforms (Not detectable - 4.98 MPN/g), and E. coli (Not - detectable - 3.58 MPN/g). Food borne pathogens Listeria monocytogenes (0.36 - 4.83 MPN/g) and Clostridium botulinum (1.12 - 21 MPN/g) were isolated with incidence levels ranging from 25 - 90% and 60 - 90% respectively. No Salomonella spp., Yersinia enterocolitica and Vibrio *cholerae* were isolated. No significant differences were observed in the bacteriological quality of fish reared in flow through and recirculating aquaculture systems.

Recirculating systems have been gaining popularity in recent years due to increasing land prices, water shortages and governmental regulation on the effluents from aquaculture facilities (Stickney, 1994). Constant and favorable environments for more efficient fish growth can be achieved through these systems, though they often have the disadvantage of deteriorating the microbiological quality of water. Such systems tend to accumulate inorganic materials dissolved in water, which eventually serve as an excellent substrate for microbial proliferation (Davis and Goulder, 1993; Austin et al., 1985).

Foodborne Pathogens in Aquacultured Fish

Fish and fish products have been associated with a number of human illnesses and are a vehicle for foodborne bacterial infections and intoxications (Brown and Dorn, 1977). Some of the pathogens that have been isolated from fish and have the capability of causing human illnesses include *Salmonella spp.*, *Edwardsiella tarda*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Clostridium botulinum*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Vibrio spp.*, and *Campylobacter fetus* subsp. *jejuni*.

Listeria spp.

History

An organism closely resembling *Listeria monocytogenes* was described as early as 1891. Murray et al. (1926) isolated the organism from the livers of sick rabbits and guinea pigs and named it *Bacterium monocytogenes* as it produced monocytosis as one of

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the symptoms. Subsequently, Pirie isolated the identical bacterium from the livers of several gerbils and named it *Listerella hepatolytica* (Bahk and Marth, 1990). When the common identity of *B. monocytogenes* and *L. hepatolytica* was established, the name was changed to *Listeria monocytogenes* by Pirie in 1940 (Lovett, 1989).

Morphology

Listeria monocytogenes is a small (1.0 - 2.0 μ m X 0.5 μ m), Gram - positive, aerobic, non - spore forming, non - acid - fast, diphtheroid - like rod with round ends (Bahk and Marth, 1990). The organism is flagellated and exhibits motility in a characteristic tumbling or slightly rotary fashion (Lovett, 1989). The bacterium is catalase - positive, Voges - Proskauer - positive, hydrolyzes esculin, and can produce β hemolysis on blood agar (Bahk and Marth, 1990).

Growth Characteristics

Listeria monocytogenes can grow over a wide range of temperatures. It grows under refrigeration temperatures of 1 - 5°C to 47°C or higher, with optimal growth between 30 - 37°C (Donnelly et al., 1992). Growth at 0°C has also been reported. The organism can withstand repeated freeze - thaw cycles and high salt concentrations (0 -10% NaCl) (Lovett, 1989). Doyle (1988) reported that the organism can survive for 5 days in 20 - 30% NaCl. Survival for more than 100 days has been observed in 10.5 - 30% NaCl at 4°C (Lovett, 1989). The organism can grow over a wide pH range (5.2 - 9.6); and it has been shown to be quite resistant to alkaline pH. The organism is also capable of surviving a low water activity (<0.92) (Garland, 1995).

Ecology

Listeria monocytogenes is ubiquitous and has been isolated from numerous environmental sources (Donnelly et al., 1992). It has been isolated from both cultivated and uncultivated lands, meadows, pastures, forests, and wildlife feeding grounds (Weis and Seeliger, 1975). Decaying moist vegetation favors the support of *L. monocytogenes* implying its saprophytic existence (Welshimer and Donker - Voet, 1971).

A variety of animals - house pets, rodents, amphibians, fish, and insects can harbor *L. monocytogenes*, but domestic farm animals are often identified as reservoirs. Avian species such as domestic fowl, songbirds, birds of prey, and scavengers are also known to harbor *L. monocytogenes* (Brackett, 1988). Fenlon (1985) suggested that wild birds may be the vectors responsible for inoculating silage. Silage harbors *L. monocytogenes* sometimes in populations greater than 12,000 cells/g and can lead to listeriosis in domestic animals and humans.

Listeria monocytogenes has been recovered from raw and treated sewage and from abattoir effluents and poultry packing plants. It can be isolated from surface waters, including rivers and lakes and canals (Brackett, 1988). Colburn et al. (1990) detected *Listeria spp.* in 81% of the fresh water samples of which the predominant species was *L. monocytogenes*. The organism has been isolated from 62% of all the fresh or low -

salinity water samples collected in tributaries draining into Humboldt - Arcata Bay, California. Listeria spp. was also recovered from 30.4% of the sediment samples and L. monocytogenes from 17.4% of the samples. It was reported that the incidence of *Listeria* spp. was lower in marine waters (33%) when compared to fresh waters (81%). Watkins and Sleath (1980) isolated L. monocytogenes from every sample of sewage, river water, and effluents of cattle and poultry processing plants examined. The numbers isolated were higher than the counts obtained for Salmonellae thus making it a potential public health concern. Survival time studies for sewage sludge sprayed onto agricultural land indicated that L. monocytogenes could survive for longer periods than Salmonellas. Jemmi and Keusch (1994) analyzed the occurrence of Listeria spp. in water samples from three rainbow trout farms in Switzerland and isolated *Listeria* from 2.3 - 13.8% of the samples from farms using ground water and 31.6% of the samples from farms using river water flowing through agricultural land. Based on the above studies, it can be concluded that dispersion of *L. monocytogenes* is mainly due to contamination of the environment.

Incidence of Listeria monocytogenes in Foods

Listeria monocytogenes is a widespread organism which has been isolated from a variety of foods such as dairy products including pasteurized milk and soft cheeses, eggs, coleslaw, vegetables, shellfish, raw fish, raw meat, poultry, frozen fish, and smoked, fermented, and marinated fish (Dillon,1994; Garland,1995). Hartemink and Georgsson (1991) determined the incidence of *Listeria spp.* in seafood salads, raw and smoked fish

and noted a higher incidence of *Listeria spp*. (32%) in seafood salads than in fish. *Listeria spp*. was also found in 29% of the smoked fish samples. Dillon et al. (1994) isolated *Listeria spp*. from 16.7% of the smoked fish samples of which the incidence levels in hot and cold smoked samples were 25.4% and 6% respectively. Of all the *Listeria spp*. isolated, 18.3% were *L. monocytogenes*. Farber (1991) determined the incidence levels of *L. monocytogenes* in fishery products at the wholesale and retail levels to be 9 - 50% and 20 - 25% respectively.

Pathology

L. monocytogenes is widespread in the environment and humans can be exposed to the bacterium in various ways, though many persons remain symptomless (Bahk and Marth, 1990). Subpopulations who could develop the disease which sometimes can be life threatening include pregnant women, newborns and infants, and adults with a compromised immune system (Marth, 1988). *Listeria monocytogenes* produces a series of toxins - hemolytic, lipolytic, hemorrhagic and pyrogenic which are involved in the disease process (Schlech, 1988).

The cell wall of *Listeria monocytogenes* also contributes to its pathogenecity. The virulence of the organism comes from a water soluble toxic polysaccharide from the cell wall which is capable of inducing lymphomenia and granulocytosis. The protein and carbohydrate extracts of the cell wall are antigenic and pyrogenic and are also capable of inducing lymphomenia and granulocytosis. Granulocytosis can also be caused by a

fractionated glycine lysate which increases the virulence of the organism. There are at least 11 serotypes, however only 3 serotypes cause 90% of the clinical infections - types Ia, Ib, and IVb. Types Ia and Ib are more common in neonates, where as, type IV b is more prevalent in those infected at or after birth (Bahk and Marth, 1990).

Five forms of listeriosis can be caused after infection with *L. monocytogenes* pregnancy infections, granulomatosis infantiseptica, sepsis, meningoencephalitis, and focal infections. Infection in pregnant women leads to the infection of the fetus which may cause damage to the embryo, abortion or stillbirth. Granulomatosis infantiseptica (infection of the newborn) is pathologically characterized by involvement of numerous organs which develop nodules ranging in size from a pinhead to a millet seed. Symptoms in the newborn include respiratory distress, heart failure, difficult and forced respiration, cyanosis, refusal to drink, vomiting, convulsions, soft whimpering, early discharge of meconium, and mucus stools. Meningitic encephalitis develops in newborns and in older persons, usually men more than 50 years old. The fatality rate is 70% for patients who are either untreated or treated too late. *Listeria monocytogenes* can also invade the eye and skin and cause conjunctivitis and skin lesions respectively (Bahk and Marth, 1990).

Epidemiology

During 1933 - 69, there were 986 bacteriologically confirmed cases reported to the Centers for Disease Control and Prevention. The first major foodborne outbreak of listeriosis in North America occurred in 1981. There were 41 confirmed cases, of which 7

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were adults and 34 were perinatal. The causative organism was *L. monocytogenes* serotype IVb and the probable vehicle was determined to be coleslaw made from cabbage grown on a field fertilized with manure of listeric sheep (Bahk and Marth, 1990).

The second outbreak occurred in 1983 in Massachusetts. There were 49 confirmed cases, of which 7 were newborns, 42 were adults, and 14 died. The causative organism was *L. monocytogenes* serotype IVb and the probable vehicle was pasteurized milk (Bahk and Marth, 1990).

The most recent major United States outbreak of listeriosis in 1985, in Los Angeles and Orange Counties, California, comprised more than 100 confirmed cases of which more than 90 were infants. The causative agent was again determined to be *L*. *monocytogenes* serotype IVb and the vehicle was determined to be Mexican - style soft cheese (Lovett, 1989).

Salmonella spp.

History

Salmonellae, a name coined by Lignieres in 1900 in honor of D. E. Salmon, an American bacteriologist who characterized the hog cholera bacillus, are prominent members of the family Enterobacteriaceae (D'Aoust, 1989). *Salmonella* is a generic name applied to a group of biochemically and serologically related bacteria (Flowers, 1982). Nearly 2,300 *Salmonella* serotypes are recognized and all of them are believed to be pathogenic to humans (Doyle and Cliver, 1990). In the 1950's the Food and Drug Administration (FDA) recognized the importance of the organism as a cause of food borne illness and established a center for the identification of *Salmonella* cultures at the Division of Microbiology in Washington, D. C. Since 1974, the FDA's Minneapolis Center for Microbiological Investigations (MCMI) has been responsible for confirmation and serotyping of *Salmonella* cultures originally isolated by FDA field laboratories (Wagner and McLaughlin, 1986).

Morphology

Salmonellae are Gram - negative, non - spore forming rods capable of growing both aerobically and anaerobically (Doyle and Cliver, 1990). They usually utilize citrate as a sole carbon source; and rarely ferment lactose or sucrose. *Salmonellae* regularly ferment glucose with the production of gas except for *S. typhi*, generally produce hydrogen sulfide gas, decarboxylate lysine, and ornithine but are urease negative and do not produce indole (Flowers et al., 1992). The organism is peritrichously flagellated with the exception of *Salmonella pullorum*, *S. gallinarum*, and a type of *S. arizonae* which are non - motile (Doyle and Cliver, 1990).

Growth Characteristics

Salmonella spp. grow within a temperature range of 5 - 47°C. Growth is very slow at the extremes and more rapid in the optimal range of 35 - 37°C. Studies indicate

that *Salmonella* is not very resistant to both refrigerated temperatures ($<2^{\circ}$ C) and high temperatures ($>60^{\circ}$ C). *Salmonella* can grow within a pH range of 4 - 9 with a pH optima of 6.5 - 7.5. The tolerance of the organism to extreme pH conditions is dependent on temperature; the closer to the optimal temperature for growth, the more tolerant the organism is for adverse pH conditions. Although, *Salmonella* can grow under both aerobic and anaerobic conditions, growth can be inhibited by an oxidation - reduction potential below - 30mV. The growth of *Salmonella* is also inhibited by 3 - 4% NaCl (D'Aoust, 1989).

Ecology

Salmonella inhabits the intestinal tracts and associated organs of humans and most farm and wild animals, birds, reptiles, amphibians, and arthropods. *Salmonellae* are mostly non - host adapted, i.e., they are carried by a variety of animals; less than 1% of the *Salmonella* serotypes are host adapted. They usually cause little or no disease in their hosts, but are excreted in large number in their feces which is an important source of contamination of surface waters (Doyle and Cliver, 1990).

Incidence of Salmonella in Water

The pollution of natural waters has increased the detection frequency and persistence of *Salmonella* in areas affected by sewage discharges, with a subsequent health hazard to human population. *Salmonella* has been isolated from natural waters as

well as from polluted water. Arvanitidou et al. (1995) isolated 17 (19.8%) Salmonella strains from 86 surface (lake and river) water samples. Salmonella were found in 16.7% and 20.3% of lake and river samples respectively. Dutka and Bell (1973) isolated Salmonella from 32% of moderately polluted water samples. Jimenez et al. (1988) studied the survival and activity of Salmonella in tropical fresh water in situ and noted that activity of *Salmonella typhimurium*, as measured by acridine orange staining, ranged form 35 - 65%. They also calculated the time for S. typhimurium to decrease by 90% to be 130 h. Morinigo et al. (1989) studied the viability of Salmonella spp. in natural waters. They noted that the T_{90} (time required to obtain a decrease of 90% of the initial microbial concentration) values for Salmonella spp. varied between 6 - 12.5 minutes. A low survival percentage was noted, with a mortality rate higher than 96%. These findings indicate a strong negative effect of raw freshwater on Salmonella populations. Phelps (1991) isolated Salmonellae from water samples and silver carp raised in a waste - water aquaculture system. Salmonella was isolated from 21.3% (21/90) of the waste water samples and 3.2% (2/61) of the fish samples. The relationship between fish and Salmonella has been described by several scientists; some believe that fish are possible carriers of *Salmonella* which are harbored in their intestines for relatively short periods of time and some believe that fish get actively infected by *Salmonella*. Bocek et al. (1992) reported that silver carp could harbor S. typhimurium for 14 days in the intestine. Baker et al. (1982) inoculated Salmonella typhimurium into Tilapia aurea and water pools
fertilized with swine waste and were able to isolate the organism up to 32 days after inoculation. The organism was never recovered from the flesh of the fish, but was isolated from viscera and epithelium.

Incidence of *Salmonella* **in Foods**

Salmonella surveillance conducted by the FDA during 1974 - 85 indicated that two - thirds of the Salmonella positive food samples were of foreign origin, whereas only one - third were domestic. Most of the Salmonella positive samples were isolated from fresh seafood that was being imported. The most frequently isolated serotype was *S*. *weltevreden* in the imported samples (18.3%) (Wagner and McLaughlin, 1986). Nambiar and Iyer (1991) isolated Salmonella from both fresh and frozen fish samples with the incidence levels being 5.76% and 8.66% respectively. Salmonella spp. was also isolated from 11.1% of fresh tilapia samples.

Pathology

The disease caused by *Salmonella* is generally called "salmonellosis". The most severe form of salmonellosis is typhoid fever, which is caused by *S. typhi*. Fortunately, this disease is infrequent in the United States. The reservoir of this organism is humans and hence, the incidence of typhoid fever implies fecal contamination of water or food. The organism gets into the food or water via raw sewage or by direct contamination by a food handler who has typhoid fever and sheds the organism in feces. Symptoms of typhoid fever include septicemia, high fever, headache, constipation, vomiting, and diarrhea. The second most severe form of salmonellosis in humans is enteric fever caused by *S. paratyphi* A, B and C. Symptoms of enteric fever are similar to those of typhoid fever but less severe. The most common form of salmonellosis in the United States is the gastroenteritis syndrome, symptoms of which include diarrhea, abdominal pain, chills, fever, vomiting, dehydration, and headache. This form of illness is more common in the elderly and very young and the mortality rate is 0.1 - 0.2% (Doyle and Cliver, 1990).

Epidemiology

The number of reported cases of human foodborne salmonellosis in the United States has shown a steady increase since the 1960s. Estimates of this illness vary in the country ranging from 740,000 to 5,300,000 cases annually (Flowers et al., 1992). During the period 1973 - 87, *Salmonella* accounted for 28% of the foodborne disease outbreaks and 45% of the cases where precooked roast beef was implicated as the probable vehicle (Bean and Griffin, 1990). Human salmonellosis is more common in children, the elderly, and immunocompromised individuals (Doyle and Cliver, 1990). In 1965, a major outbreak of salmonellosis occurred in Riverside, California where there were more than 18,000 cases. The organism involved was *Salmonella typhimurium* which was transmitted through the city water system (Greenberg and Ongerth, 1966; Ross et al., 1966). Salmonellosis was reported from a resort hotel in Puerto Rico in July 1986, where 16% of the people complained of acute diarrhea (CDC, 1986). In 1985, there were more

than 16,000 laboratory confirmed cases of salmonellosis in which pasteurized milk was implicated as the vehicle and the causative agent was *Salmonella typhimurium* (D'Aoust, 1990).

Clostridium botulinum

History

The word "botulism" comes from the Latin word for sausage (botulus). Van Ermengen (1986) first isolated the organism from inadequately cured ham from Ellezelles, Belgium and named the organism *Bacillus botulinus* as the patients were suffering from the same disease symptoms as blood sausage poisoning. The name of the organism was later changed to *Clostridium botulinum* in 1924 by Bengston (Hatheway, 1993; Pierson and Reddy, 1988).

Morphology

Clostridium botulinum is an obligate, anaerobic non spore - forming rod, Gram positive, sporulating bacteria. The bacteria biosynthesizes a protein (botulinum toxin) that produces a characteristic neurotoxicity. The botulinum toxin (botulin) can be produced by a variety of clostridia. The neurotoxins produced by various organisms can be serologically classified into seven toxin types: A, B, C, D, E, F, and G. All type A strains are proteolytic and all type E strains are non - proteolytic. Types B and F both contain some proteolytic and non - proteolytic strains. Types A, B, E, and F are involved in human botulism; type G has never been associated with an outbreak but has been isolated in cases of sudden and unexpected death in humans. Botulism is currently classified into four categories: (1) classical food borne botulism, and intoxication caused by the ingestion of preformed botulinal toxin in the contaminated food; (2) wound botulism, which results from the elaboration of botulinal toxin *in vivo* after growth of *C*. *botulinum* in an infected wound; (3) infant botulism, in which botulinal toxin is elaborated *in vivo* in the intestinal tract of an infant who has been colonized with *C. botulinum*; and (4) an "undetermined" classification of botulism for those cases involving individuals older than 12 months in which no food or wound source is implicated (Pierson and Reddy, 1988).

Ecology

Clostridium botulinum is found widely distributed throughout the land and coastal waters of North America. The western states of the United States, including Great Plains states, mainly harbor type A while the Mississippi valley and Atlantic states contain mainly type B. Type E is predominantly found in the Great Lakes region in the shoreline, soil, and sediment samples (Dodds,1993a). The organism can be isolated from a variety of sources such as soil, marine and lake sediments, feces and carcasses of birds and animals, human autopsy specimens, rotting vegetation, and foods (Pierson and Reddy, 1988).

Incidence of *Clostridium botulinum* in Foods

The incidence of foodborne botulism continues to be much higher than other types of botulism (Dodds, 1993c). Incidence of *C. botulinum* has been reported from fruits and vegetables, meats and poultry, dairy products, honey, fish, shellfish, and smoked fish. Eklund et al. (1981) reported two botulism outbreaks in juvenile salmon at the Washington State Elokomin Hatchery in 1979 and 1980 and the other at the Oregon State Klaskanine Hatchery in 1980 which resulted in an estimated loss of 1.25 million juvenile salmon raised in earth - bottom ponds. Symptoms of botulism in fish included loss of equilibrium of the fish, swimming on one side and then on the other and inability to swim against the water current. Symptoms persisted for many hours in some fish whereas death occurred rapidly in others. *Clostridium botulinum* type E toxin was isolated from stomach and intestinal contents of the morbid fish or from pond sediments which contained 75,000 type E organisms per gram. Cann et al. (1975) determined the incidence levels of C. botulinum in trout farms and trout. They reported the organism in 13 of the 17 farms with the incidence levels ranging from 2.9 - 100%. Incidence of *Clostridium botulinum* has also been identified from sediment samples of freshwater environments. Venkateswaran et al. (1989) isolated C. botulinum spores from 10 of the 36 (28%) sediment samples tested from the Ohta River, Japan. The incidence of the organism in the whole fish and viscera was 9.4 and 11.0% respectively. Incidence of *Clostridium* botulinum has also been reported from smoked fish (Christiansen et al., 1967; Pace and

Krumbiegel, 1973), surimi (Rhodehamel et al., 1991), and vacuum - packed fish (Dodds, 1993b). Rhodehamel et al. (1991) isolated *Clostridium botulinum* spores from 7 of 565 test portions (1.7%) in raw menhaden surimi.

Vacuum packaging provides the consumer with high quality and convenient fish products, but there is also a concern of increased risk from the growth and toxin production of *C. botulinum* type E in the package if it was stored at a temperature greater than 4°C. Under reduced oxygen conditions, *Clostridium botulinum* might have a competitive advantage over the normal psychrotrophic bacteria associated with the fish and produce the botulinal toxin (Garren et al., 1995). They also noted that botulinal toxin was detected in the packages stored at 10°C after 6 days of storage, but spoilage of the fish preceded the toxin production. These results are in contrast to the findings of Eklund (1982) who artificially inoculated 2, 3 and 4 logs of type E spores and noticed that toxin production occurred even when the inoculated salmon were acceptable when stored under 60 and 90% CO₂ modified atmospheric conditions at 10°C.

Clostridium botulinum is also reported to survive the smoking process. Christiansen et al. (1967) artificially inoculated in loin muscle with 10⁶ type E spores and smoked the fish to an internal temperature of 180°F (82.2°C) for 30 minutes. The smoked fish were sealed in plastic bags, and incubated at room temperature for 7 days. The authors noted that type E toxin was found in all the fish that had a brine concentration of less than 3%. In 1963 an outbreak of botulism was reported in which vacuum packed smoked white - fish chubs were incriminated. Two persons died after they consumed these smoked fish (Pace and Krumbiegel, 1973).

Pathology

The botulinum toxin is a neurotoxin with a molecular weight of 150 - 167 kDa, each consisting of a heavy chain and a light chain joined by a disulfide linkage (Hatheway, 1992). The toxin is produced during growth within the clostridial cell (Hauschild, 1989) as a single peptide with a relatively low biological activity, requiring clevage by a proteolytic enzyme to form the highly active di - chain molecule. The neurotoxins exist, in culture, as complexes with non - toxic proteins. The toxin in the complex is stable under acidic conditions, but the complex dissociates under slightly alkaline conditions and the biological activity is readily inactivated in this state (Hatheway, 1992).

Botulinum neurotoxin acts by blocking release of acetylcholine at the neuromuscular junction in three step process: (1) the toxin molecule binds to receptors on the nerve ending; (2) the toxin molecule or a portion of it, is internalized; and (3) within the nerve cell, the toxin interferes with the release of acetylcholine by an unknown mechanism (Hatheway, 1992).

Symptoms of botulism include gastrointestinal problems, fatigue, and muscular illness, which are soon followed by ocular effects such as droopy eyelids, sluggish response of pupils to light, and blurred and double vision. The musculature controlling

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the limbs and respiration become progressively paralyzed, with death within 3 - 5 days due to respiratory failure (Sugiyama, 1990).

Infant botulism, which results from the production of botulinum toxin in the infant's intestine after ingestion and outgrowth *in situ* of ingested *C. botulinum* spores, affects younger children with 90% of the reported cases in the United States being in the age group of 2 weeks - 6 months. The disease is characterized by constipation, general weakness, lethargy, lack of facial expression, irritability, and ocular dysfunctions. The only food implicated in infant botulism is honey. The toxin involved in most of the infant botulinum cases were type A or B.

Epidemiology

During 1971 - 89, there were 272 outbreaks and 597 cases of botulism in the United States of which 20% were reported from Alaska (Hauschild, 1993). Most of the outbreaks in the United States have been caused by type A toxin in which the vehicle was fruits and vegetables in 70% of the cases (Sugiyama, 1990). Fruits accounted for 97% of the cases and the remaining 3% were caused by vegetables in this food category (Hauschild, 1993).

There were 39 outbreaks of botulism in the United States involving fish, of which 25 occurred in Alaska alone. Similarly, 22 out of 37 outbreaks from meats were reported from Alaska. Type E toxin was associated with more than 65% of the outbreaks reported from Alaska in which fish were incriminated as the vehicle. Type E was isolated 75%

(38/51) of the times in *Clostridium botulinum* types found in Alaska of which fish and sea mammals (seal, whale, and walrus) accounted for 36 (>95%) of the isolates, thus indicating that type E is the predominant environmental type in Alaska (Hauschild, 1993).

In the mainland United States, type E is indigenous to the Great Lakes region. The first known cases of botulism deriving from fish caught in the Great Lakes was reported in 1960 where vacuum packed ciscoes were incriminated (Pace and Krumbiegel, 1973).

Yersinia spp.

History

In 1939, Schleifstein and Coleman first isolated a bacterium from human infections that they thought resembled *Bacillus lignieri* and *Pasturella pseudotuberculosis*. Over the next 20 years the same organism was isolated sporadically mostly from children with enteritis, which was then called *Bacterium enterocoliticum*. Frederiksen collected the strains of the organism and found them to be sufficiently different from *Yersinia pseudotuberculosis* to justify definition of a new species but with enough resemblance to be included in the same genus. The genus *Yersinia* was proposed in honor of a French bacteriologist, Yersin (Schiemann, 1989).

Morphology

Yersinia is classified in the family Enterobacteriaceae and demonstrates the characteristics that define the family: Gram - negative bacilli that are usually nitrate - reductase - positive, fermentative, oxidase - negative, and facultative with respect to oxygen requirements. *Yersinia* is usually urease - positive and exhibits motility at 25°C but not at 35°C (Schiemann and Wauters, 1992). *Yersinia enterocolitica* is classified approximately into 60 serogroups on the basis of O antigens. Not all of the strains of the organism are pathogenic to man; the pathogenic strains belong to only a few serogroups namely O:3, O:5, O:8, O:27 (predominant in the United States) and O:9 (Kapperud, 1991).

Growth Characteristics

Yersinia is a psychrotrophic food borne pathogen. It can grow at low (0°C) as well as high temperatures (44°C) with an optimum of 32 - 34°C. It is able to multiply at temperatures approaching 0°C, a circumstance which means that it can grow in refrigerated foods. Growth of *Yersinia enterocolitica* has been reported in raw and cooked meat, as well as milk at low temperatures (Kapperud, 1991)

Yersinia is more resistant to high pH than any other Gram - negative bacteria (Schiemann and Wauters, 1992; Schiemann, 1989). It can grow in a pH range of 4.6 - 9.0, with an optimum pH of 7 - 8. The organism grows in the presence of 5% NaCl but not 7% NaCl (Doyle and Cliver, 1990).

Ecology

Yersinia enterocolitica and *Y. pseudotuberculosis* - like bacteria are frequently encountered in terrestrial and freshwater ecosystems, animals, foods, and water. The bacteria has been isolated from all the vertebrate species examined, though the pig is the only animal consumed by man which regularly harbors pathogenic *Y. enterocolitica* (Schiemann, 1989; Kapperud, 1991).

Arvanitidou et al. (1995) determined the incidence levels of *Yersinia spp*. in river and lake waters in Northern Greece and isolated *Yersinia intermedia* from 10.8% of the river water and 8.3% of the lake water samples. Aleksic et al. (1987) reported the incidence of *Yersinia rhodei spp*. from human and dog feces and surface waters of Germany. They hypothesized that the natural habitat of the organism is water, leading to fecal carriage in dogs and humans and possibly an occasional infection. Bercovier et al. (1984) reported the incidence of *Yersinia aldovae* from drinking waters and river waters of Norway and Czechoslovakia.

Incidence of Yersinia in Foods

In foods, a broad spectrum of bacteria belonging to several species within the genus *Yersinia*, and a number of serogroups within the species *Y. enterocolitica* can be expected (Kapperud, 1991). Milk, milk products, meat and poultry products are the major foods that may be contaminated with *Y. enterocolitica*. The presence of the bacterium in milk indicates contamination from water, soil or equipment or

recontamination from a raw milk source. In a recent survey of various foods, Greenwood (1990) reported that 22% (of 572) of pasteurized milk samples, 15% (of 150) of cooked chill meals, and 22% (of 77) of coleslaw samples were positive for Yersinias (Schofield, 1992). Aulisio et al. (1983) reported versiniosis associated with the consumption of commercial tofu (soyabean curd) contaminated with Y. enterocolitica. A survey of the manufacturing site of the incriminated tofu yielded 112 different strains of Y. enterocolitica, of which 2 were pathogenic O:8 strains. The incidence of Yersinia spp. has also been reported from fresh and smoked fish (Bercovier, 1984; Bruce and Drysdale, 1988). Bruce and Drysdale (1988) determined the incidence levels of *Yersinia* in fresh and smoked salmon, rainbow trout and mussels and noted that 47.5% of smoked salmon, 50% of fresh salmon, 55.6% of rainbow trout and 47.8% of mussels were positive. All the Yersinia isolated from fresh and smoked salmon were classified as Yersinia enterocolitica O:14 and one O:15 which have never been associated with pathogenecity. Toora et al. (1994) surveyed ready - to - eat foods and pork and found the Yersinia enterocolitica incidence was 12.6% (15/119).

Pathology

The disease caused by *Yersinia enterocolitica* is called as yersiniosis. *Yersinia enterocolitica* produces a heat stable enterotoxin. The toxin is highly resistant to pH within a pH range of 2.2 - 8.0, stable during storage for longer periods and highly resistant

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to enzymatic degradation (Schiemann, 1989). The toxin is unusual in that it is produced at 25°C or below, but not at body temperature (37°C) (Doyle and Cliver, 1990).

Symptoms of yersiniosis include severe abdominal pain that suggests an appendicitis - like attack, fever, diarrhea, headache, and vomiting. The symptoms are much more severe in young adults and children. The incubation period for yersiniosis is 24 - 36 h and the duration of illness is usually 1 - 3 days (Doyle and Cliver, 1990).

Epidemiology

Significant food associated outbreaks of yersiniosis have occurred in the United States but it does not appear to be as great a problem as in Canada and Europe (Doyle and Cliver, 1990). The first outbreak occurred in upstate New York where more than 220 children became ill and 36 were hospitalized. The causative agent was established as *Yersinia enterocolitica* and chocolate milk as the vehicle of transmission.

In December 1981 - February 1982, an outbreak of yersiniosis occurred in Seattle, Washington. The vehicle was tofu that had been packaged in untreated spring water contaminated with *Y. enterocolitica*.

In 1982 there was a three - state outbreak in Tennessee, Arkansas and Mississippi linked to drinking pasteurized milk brought in plastic carrying cases used to carry the milk after it was bottled. The carrying cases were previously set in mud and manure in a pig farm and brought back to the plant and superficially washed. Investigators from FDA isolated a very unusual serotype of *Yersinia* from the carrying cases and showed that the pig farm was the source of the organism.

Coliforms, Fecal Coliforms and Escherichia coli

History

Escherichia coli was first described by Theodor Escherich in 1885. It is a predominant bacterium of the facultative anaerobic normal flora of the intestine of warm blooded animals, and plays an important role in maintaining intestinal physiology. Escherich (1887) observed the ubiquity of the organism in human stools and Shardinger (1892) suggested that members of this species be used as an index of fecal pollution (Hitchins et al., 1992).

Morphology

The coliform group includes aerobic and facultatively anaerobic, Gram negative, non - spore forming rods that ferment lactose, forming acid and gas within 48 hr at 35° C. The fecal coliform group includes organisms that grow in the gastrointestinal tract of humans and warm - blooded animals. It includes members of at least three genera: *Escherichia, Klebsiella* and *Enterobacter*.

Escherichia coli is a Gram - negative, peritrichously flagellated non - spore forming rod. It ferments lactose and is usually aerogenic producing gas from glucose (Doyle and Cliver, 1990). There are principally four different groups of *E. coli* that have been implicated in foodborne disease outbreaks of which enterohaemorrhagic *E. coli* (*E. coli* O157:H7) is the most important in terms of foodborne disease (Doyle and Padhye, 1989).

Growth Characteristics of E. coli

E. coli O157:H7 grows rapidly between 30 and 42° C. The organism is shown to survive well in ground beef during frozen storage at - 20°C for 9 months. The organism is more sensitive to heat than *Salmonella* with a D - value of 9.6 minutes at 64.3°C (Doyle and Padhye, 1989).

Ecology

Fecal coliforms originate from natural sources such as birds, land animals, soils, and plants. They are a major component of storm water runoff and sewage discharges (Chai et al., 1994).

Several studies have been conducted to determine the levels of coliforms, fecal coliforms and *Escherichia coli* and the counts ranged from very few (<3MPN/100 ml) to very high (>5000MPN/100 ml) depending on the season (Chai et al., 1994; Foster et al., 1977). Indicator bacterial counts showed a cyclic pattern with maximum levels in autumn and minimum levels in spring. Survival studies for these bacteria indicates a negative effect of freshwater on the indicator bacteria (Lopez - Torres, 1988; McFeters et al., 1974). However, these bacteria would revive their growth when the water conditions get

nutritive with phosphates, total phosphorus, nitrates, raw sewage, and other industrial effluents (Lopez - Torres, 1987). Eventually, they could become a part of the natural flora of the freshwater environment and pose potential health - risk to humans and other aquatic organisms.

Since 1914, the United States has used coliforms as the standard indicator of human pathogens in recreational waters. However, recently the United States Environmental Protection Agency recommended that specifically "fecal coliforms should be used as the indicator organism for evaluating the microbiological suitability of recreational waters (Lopez - Torres et al., 1988). The National Shellfish Sanitation Program (NSSP) has designated 14 fecal coliforms most probable number (MPN)/100 ml of growing water as acceptable for shellfish harvesting (Chai, 1994).

Incidence of Escherichia coli in Foods

E. coli O157:H7 has been isolated from a variety of dairy, meat and fishery products, with dairy cattle considered the reservoir of the organism (Doyle, 1985; Doyle and Padhye, 1989). Samadpour et al. (1994) in a retail survey of fresh beef, lamb, pork, poultry, and seafood from grocery stores in Seattle, Washington isolated Shiga - like toxin producing *Escherichia coli* from 14 (23%) of 60 beef samples, 9 (18%) of 51 pork samples, 10 (48%) of 21 lamb samples, 5 (63%) of 8 veal samples, 4 (12%) of 33 chicken samples, 1 (7%) of 15 turkey samples, 6 (10%) of 62 fish samples, and 2 (5%) of 44 products from the Puget Sound seafood markets in Seattle, Washington and found only 12 (4.3%) of 287 samples positive for *E. coli*. Foster et al. (1977) isolated *E. coli* from 30 (4.7%) of the 597 fresh and frozen seafood products. Ayulo et al. (1994) in a survey of finfish and shellfish isolated *E. coli* from 66 (37.7%) of the 175 samples tested. Chai et al. (1990) determined the microbiological quality of soft shell clams from the Chesapeake Bay and isolated low levels of *E. coli* (<27/100g). The isolation of *Escherichia coli* has also been reported from smoked fish. Okafor and Nzeako (1985) isolated *E. coli* from 40% of the smoked *Clarias lazera* fish, which is a dominant freshwater food fish in Nigeria.

Pathology

There are five kinds of *E. coli* which have been implicated in foodborne disease outbreaks - Enteropathogenic *E. coli* (EPEC), Enteroinvasive *E. coli* (EIEC), Enterotoxigenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAggEC) and Enterohemorrhagic *E. coli* (EHEC). Of these, EHEC is probably the most important in terms of foodborne disease. This type of *E. coli* is also known as *E. coli* O157:H7 (Jay, 1996).

E. coli O157:H7 causes hemorrhagic colitis, hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura. Virulence of the organism comes from the "verotoxins", which is very similar to Shiga toxin. When injected, in mice, the toxin goes

to the colon, ulcerates the epithelial layer, and ultimately causes bleeding in the gut (Doyle and Cliver, 1990).

Epidemiology

Before 1982, *E. coli* O157:H7 was identified only once in the United States at the Centers for Disease Control (Doyle, 1985). Two food associated outbreaks of hemorrhagic colitis occurred in the states of Oregon and Michigan where ground beef sandwiches of a restaurant chain were implicated. *E. coli* O157:H7 was isolated from a frozen, raw ground beef patty from the same lot of meat implicated in one of the outbreaks (Doyle and Padhye, 1989).

Consumption of hamburgers was associated with diarrheal illness in a nursing home in the United States in 1984. There were 19 cases of hemorrhagic colitis, 1 case of HUS, and 4 deaths. The vehicle was *E. coli* O157:H7 which probably survived the cooking procedure of the hamburger (Doyle and Padhye, 1989).

In 1985, a widespread outbreak of hemorrhagic colitis was identified in East Angola. There were 24 cases and 1 death and the probable vehicle of infection was potatoes prepared in a home kitchen (Doyle and Padhye, 1989).

Sporadic cases of hemorrhagic colitis have been reported from Alberta, Canada. In 1984, there were 64 confirmed cases of *E. coli* O157:H7 illness and the hamburger was suggested as a possible source in 12 cases (Doyle and Padhye, 1989).

REFERENCES

Abeyta, Jr., C. 1983. Bacteriological Quality of Fresh Seafood Products from Seattle Retail Markets. J. Food Prot. 46(10): 901 - 909.

Ahmed, F. E. 1992. Review: Assessing and Managing Risk Due to Consumption of Seafood Contaminated with Micro - Organisms, Parasites and Natural Toxins in the US. Int. J. Food Sci. Tech. 27: 243 - 260.

Aleksic, S., Steigerwalt, A. G., Bockemuhl, J., Huntley - Carter, G. P., and Brenner, D.
J. 1987. *Yersinia rohdei spp.* Isolated from Human and Dog Feces and Surface Water.
Int. J. Syst. Bacteriol. 37(4):327 - 332.

Allen, D. A. 1982. Introduction. In *Bacteria Associated with Freshwater Fish Farming*, *with Emphasis on the Fish Pathogen*, *Aeromonas salmonicida*. Ph. D Thesis. University of Maryland.

Anonymous. 1994. Cold Chain Could Yet Bring Warm Comfort to Roller Coaster Salmon Industry. Quick Frozen Foods International (October). 96 - 99.

Archer, D. 1989. FDA Estimates Prepared in Support of Legislative Hearings to Address Seafood Safety. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Washington, D. C.

Arvanitidou, M., Stathopoulos, G. A., Constantinidis, T. C., and Katsouyannopoulos,V. 1995. The Occurrence of *Salmonella, Campylobacter* and *Yersinia spp.* in River andLake Waters. Microbiol. Res. 150: 153 - 158.

Asakawa, Y., Akahane, S., Kagata, N., and Noguchi, M. 1973. Two Community Outbreaks of Human Infection with *Yersinia enterocolitica*. J. Hyg., Camb. 71: 715 -723.

Aulisio, C. C. G., Stanfield, J. T., Weagant, S. D., and Hill, W. E. 1983. Yersiniosis Associated with Tofu Consumption: Serological, Biochemical and Pathogenecity Studies of *Yersinia enterocolitica* Isolates. J. Food Prot. (46)3:226 - 230.

Austin, B. and Allen - Austin, D. 1985. Microbial Quality of Water in Intensive Fish Rearing. J. Appl. Bacteriol. Symposium Supplement. 207S - 226S.

Ayulo, A. M. R., Machado, R. A., and Scussel, V. M. 1994. Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in Fish and Seafood from the Southern Region of Brazil. Int. J. Food Microbiol. 24: 171 - 178.

Bahk, J. and Marth, E. M. 1990. Listeriosis and *Listeria monocytogenes*. pp 248 -257. In *Foodborne Diseases*. Cliver, D. O. (Ed.), Academic Press Inc., New York, NY.

Baross, J. and Liston, J. 1970. Occurrence of *Vibrio parahaemolyticus* and Related Hemolytic Vibrios in Marine Environments of Washington State. Appl. Microbiol. 20(2): 179 - 186.

Bean, N. H. and Griffin, P. M. 1990. Foodborne Disease Outbreaks in the United States, 1973 - 1987: Pathogens, Vehicles, and Trends. J. Food Prot. 53(9): 804 - 817.

Bercovier, H., Steigerwalt, A. G., Guiyoule, A., Huntley - Carter, G., and Brenner, D. J.
1984. *Yersinia aldovae* (Formerly *Yersinia enterocolitica* - Like Group X2): A New
Species of Enterobacteriaceae Isolated from Aquatic Ecosystems. Int. J. Syst.
Bacteriol. 34(2):166 - 172.

Bockemuhl, J., Roch, K., Wohlers, B., Aleksic, V., and Aleksic, S. 1986. Seasonal Distribution of Facultatively Enteropathogenic *Vibrios (Vibrio cholerae, Vibrio mimicus, Vibrio parahaemolyticus)* in the Freshwater of the Elbe River at Hamburg. J. Appl. Bacteriol. 60: 435 - 442.

Borghetti, J. R. and Canzi, C. 1993. The Effect of Water Temperature and Feeding Rate on the Growth Rate of Pacu (*Piaractus mesopotamicus*) Raised in Cages. Aquaculture. 93 - 101.

Brackett, R. E. 1988. Presence and Persistence of *Listeria monocytogenes* in Food and Water. Food Technol. 42. 162 - 164, 178.

Bruce, J. and Drysdale, E. M. 1989. *Yersinia enterocolitica* in Fish. J. Appl. Bacteriol. 67(6): xviii - xix.

Cann, D. C. and Taylor, L. Y. 1979. The Control of the Botulism Hazard in Hot -Smoked Trout and Mackerel. J. Food Technol. 14: 123 - 129.

Cann, D. C., Taylor, L. Y., and Hobbs, G. 1975. The Incidence of *Clostridium botulinum* in Farmed Trout Raised in Great Britain. J. Appl. Bacteriol. 39: 331 - 336.

Carneiro, D. J., Rantin, F. T., Dias, T. C. R., and Malheiros, E. B. 1994a. Interaction Between Temperature and Dietary Levels of Protein and Energy in Pacu (*Piaractus mesopotamicus*). I. The Effects on Growth and Body Composition. Abstracts/ Aquaculture. 124: 127 - 131.

Carneiro, D. J., Rantin, F. T., Dias, T. C. R., and Malheiros, E. B. 1994b. Interaction Between Temperature and Dietary Levels of Protein and Energy in Pacu (*Piaractus mesopotamicus*). II. Effects on Digestibility of Protein and Transit Time Through the Gastrointestinal Tract. Abstracts/ Aquaculture. 124: 127 - 131. Carolsfeld, J., Ramos, S. M., Ormanezi, R., Gomes, J. H., Barbosa, J. M., and Harvey,
B., 1988. Analysis of Protocols for Application of LHRH Analog for Induced Final
Maturation and Ovulation of Female Pacu (*Piaractus mesopotamicus*, Holmberg 1887).
Aquaculture. 74: 49 - 55.

Castagnolli, N. and Donaldson, E. M, 1991. Induced Ovulation and Rearing of the Pacu (*Colossoma mitrei*). Aquaculture. 25: 275 - 280.

Chai, T., Han, T., and Cockey, R. R. 1994. Microbiological Quality of Shellfish -Growing Waters in Chesapeake Bay. J. Food Prot. 57(3): 229 - 234.

Chai, T., Han, T., Cockey, R. R., and Henry, P. C. 1990. Microbiological Studies of Chesapeake Bay Soft - Shell Clams (*Mya arenaria*). J. Food Prot. 53(12): 1052 - 1057.

Christiansen, L. E., Deffner, J., Foster, E. M., and Sugiyama, H. 1967. Survival and Outgrowth of *Clostridium botulinum* type E Spores in Smoked Fish. Appl. Microbiol. 16: 133 - 137.

Colburn, K. G., Kaysner, C. A., Abeyta, Jr., C., and Wekell, M. M. 1990. *Listeria* Species in a California Coast Estuarine Environment. Appl. Environ. Microbiol. 56(7): 2007 - 2011.

D'Aoust, J. 1989. *Salmonella*. pp. 328 - 445. In *Foodborne Bacterial Pathogens*. Doyle, M. P. (Ed.). Marcel Dekker Inc., New York, NY.

Davis, S. H. and Goulder, R. 1993. Deterioration in Bacteriological Quality of Water Through Fish Farms. J. Appl. Bacteriol. 74. 336 - 339. Depaola, A., Presnell, M. W., Motes, Jr., M. L., McPhearson, R. M., Twedt, R. M., Becker, R. E., and Zywno, S. 1983. Non - O1 *Vibrio cholerae* in Shellfish, Sediment and Waters of the U. S. Gulf Coast. J. Food Prot. 46(9): 802 - 806.

Dillon, R., Patel, T., and Ratnam, S. 1994. Occurrence of *Listeria* in Hot and Cold Smoked Seafood Products. Int. J. Food Microbiol. 22: 73 - 77.

Dodds, K. L. 1993a. *Clostridium botulinum* in the Environment. pp. 21 - 51. In *Clostridium botulinum: Ecology and Control in Foods*. Hatheway, C. L. and Dodds, K. L. (Eds). Marcel Dekker Inc., New York, NY.

Dodds, K. L. 1993b. *Clostridium botulinum* in Foods. pp. 53 - 68. In *Clostridium botulinum: Ecology and Control in Foods*. Hatheway, C. L. and Dodds, K. L. (Eds). Marcel Dekker Inc., New York, NY.

Dodds, K. L. 1993c. Worldwide Incidence and Ecology of Infant Botulism. pp 105 -117. In *Clostridium botulinum: Ecology and Control in Foods*. Hatheway, C. L. and Dodds, K. L. (Eds). Marcel Dekker Inc., New York, NY.

Donnelly, C. W., Brackett, R. E., Doores S., Lee, W. H., and Lovett, J. 1992. *Listeria*. pp. 637 - 663. In *Compendium of Methods for the Microbiological Examination of Foods*. 3 rd Ed. Vanderzant C. and Splitoesser, D. F. (Eds.). American Public Health Association, Washington, D. C.

Doyle, M. P. 1985. Food - Borne Pathogens of Recent Concern. Ann. Rev. Nutr. 5: 25 - 41.

Doyle, M. P. 1988. Effect Of Environmental and Processing Conditions on *Listeria monocytogenes*. Food Technol. 42. 169 - 171.

Doyle, M. P. and Cliver, D. O. 1990. *Escherichia coli*. pp. 210 - 215. In *Foodborne Diseases*. Cliver, D. O. (Ed.). Academic Press Inc., New York, NY.

Doyle, M. P. and Cliver, D. O. 1990. *Salmonella*. pp. 186 - 204. In *Foodborne Diseases*. Cliver, D. O. (Ed.). Academic Press Inc., New York, NY.

Doyle, M. P. and Cliver, D. O. 1990. *Vibrio*. pp. 242 - 245. In *Foodborne Diseases*. Cliver, D. O. (Ed.). Academic Press Inc., New York, NY.

Doyle, M. P. and Cliver, D. O. 1990. *Yerisinia enterocolitica*. pp. 224 - 228. In *Foodborne Diseases*. Cliver, D. O. (Ed.). Academic Press Inc., New York, NY.

Doyle, M. P. and Padhye, V. P. 1989. *Escherichia coli*. pp. 236 - 281. In *Foodborne Bacterial Pathogens*. Doyle, M. P. (Ed.). Marcel Dekker Inc., New York, NY.

Eklund, M. W. 1982. Significance of *Clostridium botulinum* in Fishery Products Preserved Short of Sterilization. Food Technol. 36(12):107 - 112.

Eklund, M. W., Peterson, M. E., Poysky, F. T., Peck, L. W., and Conrad, J. F. 1982.Botulism in Juvenile Coho Salmon (*Oncorhynchus kisutch*) in the United States.Aquaculture. 27(1):1 - 11.

Eklund, M. W., Poysky, F. T., Peterson, M. E., Peck, L. W., and Brunson, W. D. 1984. Type E Botulism in Salmonids and Conditions Contributing to Outbreaks. Aquaculture. 41(4): 293 - 309.

Farber, J. M. 1991. *Listeria monocytogenes* in Fish Products. J. Food Prot. 54(12): 922 - 924.

Fernandes, C. F., Flick, G. J., Silva, J. L., and McCaskey, T. A. 1997. Quality of Aquacultured Fresh Catfish Fillets II. *E. coli* O157:H7, *Campylobacter, Vibrio, Plesiomonas*, and *Klebsiella*. *In Press*.

Fishbein, M., Mehlman, I. J., and Pitcher, J. 1970. Isolation of *Vibrio parahaemolyticus* from the Processed Meat of Chesapeake Bay Blue Crabs. Appl. Microbiol. 20(2): 176 - 178.

Flowers, R. S. 1982. *Salmonella*. In "Bacteria Associated with Foodborne Diseases".Food Technol. 36(4): 182 - 185.

Flowers, R. S., D'Aoust J., Andrews, W. H., and Bailey J. S. 1992. Salmonella. pp. 371 - 421. In Compendium of Methods for the Microbiological Examination of Foods. 3rd
Ed. Vanderzant C. and Splitoesser, D. F. (Eds.). American Public Health Association, Washington D. C.

Food and Agriculture Organization. 1995. Aquaculture Production Statistics. Fishery Information, Data and Statistics Service; Food and Agriculture Organization of the United Nations, Rome.

Foster, J. F., Fowler, J. L. and Dacey, J. 1977. A Microbial Survey of Various Fresh and Frozen Seafood Products. J. Food Prot. 40(5): 300 - 303.

Garland, C. D. 1995. Microbiological Quality of Aquaculture Products with Special Reference to *Listeria monocytogenes* in Atlantic Salmon. Food Australia. 47(12): 559 - 563.

Garren, D. M., Harrison, M. A., and Huang, Y. 1995. Growth and Production of Toxin of *Clostridium botulinum* type E in Rainbow Trout Under Various Storage Conditions. J. Food Prot. 58(8): 863 - 866.

Godinho, H. P. and Godinho, A. L., 1986. Induced Spawning of the Pacu, *Colossoma mitrei* (Berg 1895), by Hypophysation with Crude Carp Pituitary Extract. Aquaculture. 55: 69 - 73.

Hartemink, R. and Georgsson, F. 1991. Incidence of *Listeria* Species in Seafood and Seafood Salads. Int. J. Food Microbiol. 12: 189 - 196.

Hatheway, C. L. 1993. *Clostridium botulinum* and Other Clostridia that Produce
Botulinum Neurotoxin. pp. 3 - 20. In *Clostridium botulinum: Ecology and Control in Foods*. Hatheway, C. L. and Dodds, K. L. (Eds). Marcel Dekker Inc., New York.

Hauschild, A. H. W. 1989. *Clostridium botulinum*. pp. 112 - 189. In *Clostridium botulinum: Ecology and Control in Foods*. Hatheway, C. L. and Dodds, K. L. (Eds).Marcel Dekker Inc., New York, NY.

Hauschild, A. H. W. 1993. Epidemiology of Human Foodborne Botulism. pp 69 - 104.In *Clostridium botulinum: Ecology and Control in Foods*. Hatheway, C. L. and Dodds,K. L. (Eds). Marcel Dekker Inc., New York, NY.

Hitchins, A. D., Hartman, P. A., and Todd, E. C. D. 1992. Coliforms - *Escherichia coli* and its Toxins. Pp. 325 - 369. In *Compendium of Methods for the Microbiological Examination of Foods*. 3 rd Ed. Vanderzant C. and Splitoesser, D. F. (Eds.). American Public Health Association, Washington D. C.

Huss, H. H., Pedersen, A., and Cann, D. C. 1974. The Incidence of *Clostridium botulinum* in Danish Trout Farms II. Measures to Reduce Contamination of the Fish. J. Food Technol. 9(4): 451 - 458.

Jay, J. M. 1996. Foodborne Gastroenteritis Caused by *Escherichia coli*. pp 527 - 543. In *Modern Food Microbiology*. 3rd ed. Chapman and Hall, New York, NY. Jemmi, T. and Keusch, A. 1994. Occurrence of *Listeria monocytogenes* in Freshwater Fish Farms and Fish - Smoking Plants. Food Microbiol. 11: 309 - 316.

Kaper, J., Lockman, H., Colwell, R. R., and Joseph, S. W. 1979. Ecology, Serology, and Enterotoxin Production of *Vibrio cholera* in Chesapeake Bay. Appl. Environ.Microbiol. 37(1): 91 - 103.

Kapperud, G. 1991. *Yerisinia enterocolitica* in Food Hygiene. Int. J. Food Microbiol. 12: 53 - 66.

Kautter, D. A., Solomon, H. M., Lake, D. E., Bernard, D. T., and Mills, D. C. 1992. *Clostridium botulinum* and its Toxins. pp. 605 - 621. In *Compendium of Methods for the Microbiological Examination of Foods.* 3rd Ed. Vanderzant C. and Splitoesser, D. F. (Eds.). American Public Health Association, Washington D. C.

Kaysner, C. A., Abeyta, Jr., C., Wekell, M. M., Depaola, Jr., A., Stott, R. F., and Leitch, J. M. 1987. Incidence of *Vibrio cholera* from Estuaries of the United States West Coast. Appl. Environ. Microbiol. 53(6): 1344 - 1348.

Kaysner, C. A., Abeyta, Jr., C., Wekell, M. M., Depaola, Jr., A., Stott, R. F., and Leitch, J. M. 1987. Virulent Strains of *Vibrio vulnificus* Isolated from Estuaries of the United States West Coast. Appl. Environ. Microbiol. 53(6): 1349 - 1351.

Kelly, M. T. 1982. Effect of Temperature and Salinity on *Vibrio* (Beneckea) *vulnificus* Occurrence in a Gulf Coast Environment. Appl. Environ. Microbiol. 44(4): 820 - 824.

Kenyon, J. E., Gillies, D. C., Piexoto, D. R., and Austin, B. 1984. *Vibrio cholerae* (Non - O1) Isolated from California Coastal Waters. Appl. Environ. Microbiol. 46(5): 1232 - 1233.

Kenyon, J. E., Piexoto, D. R., Austin, B., and Gillies, D. C. 1984. Seasonal Variation in Numbers of *Vibrio cholerae* (Non - O1) Isolated from California Coastal Waters.Appl. Environ. Microbiol. 47(6): 1243 - 1245.

Lopez - Torres, A. J., Prieto, L., and Hazen, T. C. 1988. Comparison of the In Situ Survival and Activity of *Klebsiella pneumoniae* and *Escherichia coli* in Tropical Marine Environments. Microb. Ecol. 15: 41 - 57.

Lopez - Torres A. J., Hazen, T. C., and Toranzos, G. A. 1988. Distribution and In Situ Survival and Activity of *Klebsiella pneumoniae* and *Escherichia coli* in a Tropical Rain Forest Watershed. Current Microbiology. 15: 213 - 218.

Lovett, J. 1988. Isolation and Enumeration of *Listeria monocytogenes*. Food Technol. 42(4): 172 - 175.

Lovett, J. 1989. *Listeria monocytogenes*. pp. 284 - 310. In *Foodborne Bacterial Pathogens*. Doyle, M. P. (Ed.). Marcel Dekker Inc., New York, NY.

Madden, J. M. 1982. *Vibrio*. In "Bacteria Associated with Foodborne Diseases". Food Technol. 36(4): 191 - 192.

Madden, J. M., McCardall, B. A., and Morris Jr., J. G. 1989. *Vibrio cholerae*. pp. 525 - 542. In *Foodborne Bacterial Pathogens*. Doyle, M. P. (Ed.). Marcel Dekker Inc., New York, NY.

Machado, M. G. S. and V. C. Sgarbieri, 1991. Partial Characterization of Proteins from Pacu (*Colossoma mitrei*, Berg 1895). J. Agric. Food Chem. 39: 1715 - 1718.

Marth, E. H. 1988. Disease Characteristics of *Listeria monocytogenes*. Food Technol. 42(4): 165 - 168.

McFeters, G. A., Bissonnette, G. K., Jezeski, J. J., Thomson, C. A., and Stuart, D. G. 1974. Comparative Survival of Indicator Bacteria and Enteric Pathogens in Well Water. Appl. Microbiol. 27(6): 823 - 829.

Merola, N. 1988. Effects of Three Dietary Protein Levels on the Growth of Pacu, *Colossoma mitrei*, Berg, in Cages. Aquacult. Fish. Manmgt. 19: 145 - 150.

Murray, E. G. D., Webb, R. A., and Swann, M. B. R. 1926. A Disease of Rabbits Characterized by a Large Mononuclear Leucocytosis Caused by a Hitherto Undescribed Bacillus *Bacterium monocytogenes* (N.Sp.). J. Pathol. Bacteriol. 29: 407 - 439.

Nedoluha, P. C. and Westhoff, D. 1995. Microbiological Analysis of Striped Bass (*Morone saxatilis*) Grown in Flow - Through Tanks. J. Food Prot. 58(12): 1363 - 1368.

Nickelson II, R. and Finne, G. 1992. Fish, Crustaceans, and Precooked Seafoods. pp 875 - 895. In *Compendium of Methods for the Microbiological Examination of Foods.* 3 rd Ed. Vanderzant C. and Splitoesser, D. F. (Eds.). American Public Health Association, Washington D. C.

Okafor, N. and Nzeako, B. C. 1985. Microbial Flora of Fresh and Smoked Fish from Nigerian Fresh - Water. Food Microbiol. 2(1): 71 - 75

Oliver, J. D., Warner, R. A., and Cleland, D. R. 1982. Distribution and Ecology of *Vibrio vulnificus* and Other Lactose - Fermenting Marine *Vibrios* in Coastal Waters of the Southeastern United States. Appl. Environ. Microbiol. 44(6): 1404 - 1414.

Otwell, W. S. 1989. Regulatory Status of Aquacultured Products. Food Technol. 43(11): 103 - 105.

Pace, P. J. and Krumbiegel, E. R. 1973. *Clostridium botulinum* and Smoked Fish Production: 1963 - 1972. J. Milk Food Technol. 36(1): 42 - 47.

Pierson, M. D. and Reddy, N. R. 1982. *Clostridium botulinum*. In "Bacteria Associated with Foodborne Diseases". Food Technol. 36(4): 196 - 198.

Piper, R. G., Mcelwain, I. B., Orme, L. E., Mc Craren, Fowler, L. G., and Leonard, J.
R., 1982. Fish Hatchery Management, 2nd Ed. United States Department of the Interior,
Fish and Wildlife Service, Washington, D. C., pp. 210 - 211

Redmayne, P. C. 1989. World Aquaculture Developments. Food Technol. 43(11): 80 - 81.

Rhodehamel, E. J., Solomon, H. M., Lilly, Jr., T., Kautter, D. A., and Peeler, J. T.
1991. Incidence and Heat Resistance of *Clostridium botulinum* Type E Spores in
Menhaden Surimi. J. Food Sci. 56(6):1562 - 1563, 1592.

Roberts, N. C., Siebeling, R. J., Kaper, J. B., and Bradford, Jr., H. B. 1982. *Vibrios* in the Louisiana Gulf Coast Environment. Microbial Ecology. 8: 299 - 312.

Romagosa, E., DePaiva, P., and Godinho, H. M. 1990. Pattern Of Oocyte Diameter Frequency Distribution in Females of the Pacu, *Piaractus mesopotamicus* (Holmberg 1887) (=*Colossoma mitrei* Berg 1895), Induced to Spawn. Aquaculture 86: 105 - 110.

Ruple, A. D. and Cook, D. W. 1992. *Vibrio vulnificus* and Indicator Bacteria inShellstock and Commercially Processed Oysters from the Gulf Coast. J. Food Prot. 55(9): 667 - 671.

Saint - Paul, U. and Bernardinho G. 1988. Behavioral and Ecomorphological Responses of Neotropical Pacu *Piaractus mesopotamicus* (Teleosti, Serrasalmidae) to Oxygen -Deficient Waters. Exp Biol. 48: 19 - 26.

Saint - Paul, U. 1986. Potential for Aquaculture of South American Freshwater Fishes: A Review. Aquaculture. 54: 205 - 240.

Samadpour, M., Ongerth, J. E., Liston, J., Tran, N., Nguyen, D., Whittam, T. S.,
Wilson, R. A., and Tarr, P. I. 1994. Occurrence of Shiga - Like Toxin - Producing *Escherichia coli* in Retail Fresh Seafood, Beef, Lamb, Pork, and Poultry from Grocery Stores in Seattle, Washington. Appl. Environ. Microbiol. 60(3): 1038 - 1040.

Sarkar, B. L., Balakrishnair, G., Banerjee, A. K., and Pal, C. 1985. Seasonal Distribution of *Vibrio parahaemolyticus* in Freshwater Environs and in Association with Freshwater Fishes in Calcutta. Appl. Environ. Microbiol. 49: 132 - 136.

Schiemann, D. A. 1989. Yerisinia enterocolitica and Yerisinia pseudotuberculosis. pp.
601 - 672. In Foodborne Bacterial Pathogens. Doyle, M. P. (Ed.). Marcel Dekker Inc., New York, NY.

Schiemann, D. A. and Wauters, G. 1992. *Yersinia* pp. 433 - 450. In *Compendium of Methods for the Microbiological Examination of Foods*. 3 rd Ed. Vanderzant C. and Splitoesser, D. F. (Eds.). American Public Health Association, Washington D. C.

Schlech III, W. F. 1988. Virulence Characteristics of *Listeria monocytogenes*. Food Technol. 42(4): 176 - 178.

Schofield, G. M. 1992. Emerging Food - Borne Pathogens and their Significance in Chilled Foods. J. Appl. Bacteriol. 72: 267 - 273.

Stickney, R. R. 1994. Introduction In *Principles Of Aquaculture*. pp 1 - 44. John Wiley and Sons Inc., New York, NY.

Stickney, R. R. 1996. The Growth Years Following 1970. In *Aquaculture in the United States - A Historical Perspective*. pp. 225 - 297. John Wiley and Sons Inc., New York, NY.

Sugiyama, H. Botulism. pp. 108 - 125. 1990. In *Foodborne Diseases*. Cliver, D. O. (Ed.). Academic Press Inc., New York, NY.

Tamplin, M., Rodrick, G. E., Blake, N. J., and Cuba, T. 1982. Isolation and Characterization of *Vibrio vulnificus* from Two Florida Estuaries. Appl. Environ. Microbiol. 44(6): 1466 - 1470.

Toora, S., Budu - Amoaka, E., Ablett, R. F., and Smith, J. 1994. Isolation of *Yersinia enterocolitica* from Ready - to - Eat Foods and Pork by a Simple Two Step Procedure. Food Microbiol. 11: 369 - 374.

Venkateswaran, K., Kiiyukia, C., Takaki, M., Nakano, H., Matsuda, H., Kawakami, H., and Hashimoto, H. 1989. Characterization of Toxigenic *Vibrios* Isolated from the Freshwater Environment of Hiroshima, Japan. Appl. Environ. Microbiol. 55(10): 2613 - 2618.

Ward, D. R. 1989. Microbiology of Aquaculture Products. Food Technol. 43(11): 82 - 84.

Watkins, J. and Sleath, K. P. 1981. Isolation and Enumeration of *Listeria monocytogenes* from Sewage, Sewage Sludge, and River Water. J. Appl. Bacteriol. 50: 1 - 9.

Weis, J. and Seeliger, H. P. R. 1975. Incidence of *Listeria monocytogenes* in Nature. Appl. Microbiol. 30(1): 29 - 32.

Weismann, J. B., Dewitt, W. E., Thompson, J., Muchnick, C. N., Portnoy, B. L.,Feeley, J. C., and Gangarosa, E. J. 1975. A Case of Cholera in Texas. Am. J.Epidemiol. 100(6): 487 - 498.

Welshimer, H. J. and Donker - Voet, J. 1971. *Listeria monocytogenes* in Nature. Appl. Microbiol. 21(3): 516 - 519.

Wilson, R., Lieb, S., Roberts, A., Stryker, S., Janowski, H., Gunn, R., Davis, B., Riddle,
C. F., Barrett, T., Morris, Jr., J., and Blake, P. A. 1981. Non - O Group 1 *Vibrio cholerae* Gastroenteritis Associated with Eating Raw Oysters. Am. J. Epidemiol. 114(2): 293 - 299.

Wong, H., Ting, S., and Shieh, W. 1992. Incidence of Toxigenic *Vibrios* in Foods Available in Taiwan. J. Appl. Bacteriol. 73: 197 - 202.

SECTION II.

Effect Of Dietary Protein Content On Growth Performance and Proximate Composition Of Aquacultured Pacu (*Piaractus mesopotamicus*)

by

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ABSTRACT

Pacus (Piaractus mesopotamicus) were fed three diets varying in protein (range 0.5 -36%) for 24 weeks and their growth rate, processing yield, and proximate composition compared. They were fed adlibitum twice daily one of three diets (a) zucchini diet (0.5% protein wet weight), (b) commercial P32 diet (32% protein), and (c) commercial P36 diet (36% protein). The weight and length of each fish were recorded at four week intervals. At the end of 24 weeks, the fish were sacrificed and dressed to determine yields during various stages of manual processing viz. edible (e.g., eviscerated, gilled, deheaded fillet w/ rib cage, fillet w/o rib cage, skinless fillet) and non - edible (e.g., viscera, gills, head, frame, rib cage, skin) portions. Skinless fillets from pacu were used to compare the influence of dietary protein on proximate composition. The final yields of pacu in the zucchini, P32 and P36 diets were 5.00 kg/m³, 8.93 kg/m³ and 7.09 kg/m³ respectively and were significantly different (p < 0.05). Protein concentration in the diet significantly influenced specific growth rate (p < 0.05), protein efficiency ratio (p < 0.05) and feed conversion ratio (p < 0.05). There was no significant difference (p > 0.05) in yields of pacu fed P32

and P36 protein diets, but the yields were significantly higher than the pacu fed zucchini diet. The processing yields were determined following dressing. Pacu fed either P32 or P36 diets did not significantly influence the yield of the edible portions (p < 0.05) yields. However, for the non - edible portions, only the viscera (p < 0.05) and gill (p < 0.05) yields were significantly influenced by dietary protein level. The moisture, protein, and lipid content of the fillets ranged from 76.4 to 82.4% (wet weight basis), 17.2 - 21.1%, and 0.6 - 2.3% respectively and were significantly different (p < 0.05) for the diets. Based on the findings, it was concluded that the dietary composition of feed can be manipulated to alter the proximate composition of aquacultured pacu.

Key Words: pacu, aquaculture, dietary protein, proximate composition, fish, nutrition

INTRODUCTION

Pacu (*Piaractus mesopotamicus*) is a freshwater fish indigenous to the Plate River Basin in South America (Borghetti and Canzi, 1993). It is a herbivorous fish and feeds preferentially on leaves, flowers, fruits and seeds of superior plants. It is normally small scaled and has a weight range of 3 - 7 Kg under normal growth conditions (Saint - Paul, 1986). However, pacu weighing 18 Kg have been harvested from the wild (Merola and DeSouza, 1988).

Preliminary experiments involving pacu have yielded encouraging results, which generated interest among aquaculturists to culture the fish under intensive and controlled conditions. Fed under intensive systems, pacu can take up to 2 years to attain 1.2 Kg in earthen ponds. However, on formulated diets they can reach 1.2 - 1.5 Kg in 12 months. Pacu is now being aquacultured intensively in the south, south - east, and west - central regions of Brazil.

Dietary protein is the single most important nutritional factor that influences the growth performance of fish and feed cost; hence the success of aquaculture. It is generally accepted that the growth rates of fish increase with increasing levels of dietary protein. However, there is a limit to which dietary protein could be incorporated into aquaculture feeds beyond which there may not be any significant impact on yields. To raise fish economically, dietary protein levels need to be considered, because cost of fish production is dependent on protein utilization.

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Most of the previous research involving pacu were feasibility studies for their culture in cages, ponds, etc. and to determine the optimal feeding rate and levels of protein in their diet. Merola (1988) tested the effect of three dietary levels of protein (30, 35, and 40%) and obtained superior yields and weight gains with the 35% protein diet. Carneiro et al. (1994a) reported better growth rates of pacu at the highest protein and lowest energy levels. Carneiro et al. (1994b) noted an increase in the mean transit time through the gastro - intestinal tract. They also noted increasing protein digestibility with increasing energy levels at low crude protein levels. Borghetti and Canzi (1993) studied the effect of water temperature and feeding rate on the growth of pacu and noted a direct relationship between water temperature and the amount of feed. Higher water temperatures led pacu to consume more feed and resulted in better yields and lower temperatures led to feed waste and poor yields.

The prohibitive cost of high energy commercial feeds has led aquaculturists to use agricultural (e.g., coffee pulp, rice, sorghum) and agro - industrial wastes (effluents from paper mills, bio - gas plants) as direct feeds or incorporated as components of commercial feeds for raising fish (Fagberno and Arowosoge, 1991). Successful results have been achieved by many investigators using such products without affecting the color, flavor and taste of the fish.

Kaur et al. (1987) used biogas slurry to treat carp tanks and noted 3.5 times higher yields. Additional supplementing with rice and ground - nut cake increased the yields by

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5.8 times more than the control, which received no biogas slurry. Ufodike and Matty (1983) fed carp with different levels of cassava and rice and noted a slightly higher utilization of protein and better growth rates. Mahadevaswamy and Venkataraman (1988) utilized rabbit droppings for production of Indian carp (*Labeo rohita*) and common carp. They noted slightly lower yields than those on commercial feed, but, were successful in considerably reducing the production costs. Govindan (1989) utilized treated municipal wastewater for the culture of Indian and Common carp (*Cyprinus carpio*) and obtained yields of 6352 and 1285 Kg/ha/year respectively.

Christensen (1981) studied the feasibility of coffee pulp as a source of protein to culture common carp and catfish (*Clarias mossambicus*) which resulted in a decline in specific growth rates from 1.65 to 0.34 g/day in the case of common carp and by more than 45% in catfish. Fagberno and Arowosoge (1991a) fed catfish (*Clarias isheriensis*) with isoproteic diets using yellow maize as the control (37% crude protein) and supplemental feeds containing 25% of cassava peel, yam peel, plantain peel and maize chaff and observed poorer yields, specific growth rates, protein efficiency ratios and feed conversion ratios than the control. Fagberno and Arowosoge (1991b) tested different levels of coffee pulp (0, 10, 20 and 30%) as a replacement for yellow maize in culturing catfish. They too noted significantly poorer yields, growth rates and feed conversion ratios in the experimental diets.

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The objectives of this experiment were to compare the growth rates, fillet yields and proximate composition of pacu fed three different diets - commercial 32% protein diet (32P), commercial 36% protein diet, (36P) and a 0.5% protein vegetable diet (zucchini). Zucchini was included in the study to determine the feasibility of utilizing low - cost agricultural products as an alternative feed.

MATERIALS AND METHODS

Transportation and Distribution of fish to treatments

Pacu (*Piaractus mesopotamicus*) fingerlings with an initial weight of approximately 45g were procured from Ekkwill Fishery Resources, Tampa, FL and transported to the Virginia Tech Aquaculture Center, Blacksburg, VA. The fingerlings were grown to a weight of approximately 72 g, then randomly distributed into six 1165 L fiberglass tanks for a period of 24 weeks, and stocked at an initial stocking density of 1.28 kg/m3. The experiment involved three treatments (diets) - a) 32% Protein Big Strike Fish Feed (SSC 50822061, Southern States Co - Op. Inc., Richmond, VA) (32P), b) 36% Protein Catfish Fingerling Feed (SSC 338200; Southern States Co - Op. Inc.) (36P), and c) a vegetable diet - zucchini (0.5% Protein) with two replicates per treatment. The fish were fed twice daily adlibitum for a period of 24 weeks.

Water Conditions During Growth Period

Temperature, dissolved oxygen, total ammonia nitrogen, absorbance and pH were measured daily before the first feeding. Measurements of alkalinity, hardness, nitrite and nitrate were performed once a week. Total ammonia nitrogen, nitrites, nitrates and absorbance were measured according to manufacturer's instructions with a spectrophotometer DR/2000 (HACH Company, Loveland, CO), and alkalinity and hardness through titration (Greenberg et al., 1992). A portable dissolved oxygen meter (Yellow Springs Instrument, Yellowsprings, OH) was used to measure dissolved oxygen (mg/ml) and temperature (°C). The hydrogen ion (H⁺ ion) concentration in all the tanks was measured using a Hach pen (HACH Company, Loveland, CO) pH meter.

Fish Physical Properties

The weights and lengths of all the fish were recorded every 4 weeks. The fish were anaesthetized with tricaine methane sulfonate (MS - 222, Argent Chemical Laboratories, Redmond, WA). Weights were recorded to the nearest 0.1 g and lengths to the nearest mm.

The growth parameters specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR) were calculated using the following formulae:

SGR = [(log_e final body weight - log_e initial body weight) / Time in days] \times 100

FCR = Food Intake / Weight gain

PER = Weight gain / Protein Intake

Manual Processing of Pacu

At the end of 24 weeks of growth period, 10 fish from each diet treatment were randomly selected and manually processed into skinless fillets. The yields during various stages of dressing were determined. Pacu fed zucchini were not filleted as they were too small.

Pacu fillets without rib cage contained numerous bones throughout the muscle tissue. Radiographs were taken to determine the locations of the bones in the fillets.

Radiography of Pacu

A mature pacu was obtained from the recirculation system and anaesthetized with tricaine methane sulfonate (MS - 222, Sigma Chemical Company, St. Louis, MO) at a concentration of 100 mg/liter of water. While under anesthesia, the fish was positioned for a standard radiographic projection in the lateral position. Radiographic images were captured using Kodak Lanex fine screen TMGRA film exposed with a conventional x - ray machine at a MAS of 6.3 and a kvp of 46. The fish was then euthanized with a overdose exposure to the MS - 222 and immediately frozen. After the fish was thoroughly frozen, the fish was serially sectioned every 0.5 inches from nose to tail with an electric bandsaw. The body sections were placed in a progressive order on a cold dissection tray and transported to the radiology laboratory. The sections were transferred to the dorsal surface of a cardboard holder containing Kodak EBI film, which was then placed with the sections into a Faxitron 43805N x - ray system (Hewlett - Packard) and exposed for 1 minute at 50 kvp.

Proximate Composition

Moisture

The moisture contents in the skinless pacu fillets were determined using the standard convection air oven method (AOAC, 1984a). About 10 g (9.5 to 10.5 g) of the sample was dried in an aluminum dish with lid. The moisture was determined in a standard forced air convection oven (Hotpack model 14512, Hotpack Corp. Philadelphia, PA) with the temperature was set at 100 - 102°C for 16 - 18 h.

Protein

The protein content in skinless fillets of pacu was determined using the Association of Official Analytical Chemists (AOAC) method for the determination of protein in meat and meat products (AOAC, 1984b). Two grams of the sample were weighed in a N - free filter paper (Whatman International Ltd., Maidstone, England) and transferred to a 250 ml digestion tube. Two or three boiling stones, 2 catalyst tablets, 15 ml H₂SO₄, and 3 ml of 30 - 35% H₂O₂ (Fisher Scientific, Fairlawn, NJ) were added and digested using a block digestor at 410°C for 45 minutes. Approximately 50 - 75 ml of H₂O was added after cooling the tubes for 10 min. The contents of the tube were then distilled using a Kjeldahl distillation unit (Labconco Corp., Kansas City, MO). Approximately 100 - 125 ml of the distillate was collected into a 250 ml flask containing 25 ml H₃BO₃ and titrated with 0.2N HCl to a neutral gray endpoint. The volume of 0.2N

HCl required to complete the reaction was recorded. The percent protein in the sample was calculated using the formula:

% Protein = $(V_A - V_B) \times 1.4007 \times N \times 6.25/g$ sample where

 $V_A - V_B =$ vol. standard acid required for sample and blank respectively; 1.4007 = milliequiv. wt N × 100(%); N = normality of std. acid; and 6.25 = protein factor for meat products (16%).

Total Lipid

About 1.0 - 5.0 g of the sample was suspended in 8.0 ml of 2.0 M sodium acetate and the total moisture was adjusted to 32.0 ml with distilled, deionized (dd) water. Total lipid extraction was initiated by adding 80.0 ml of methanol (Fisher Scientific, Fairlawn, NJ) and 40.0 ml of chloroform (Fisher Scientific). The mixture was shaken (@ 250 rpm) overnight (14 h at 24°C) on an orbital Shaker Model # G - 33 (New Brunswick Scientific, Edison, NJ). Extraction was continued with 40.0 ml of chlorform and completed with 40.0 ml of dd water. During each extraction step, the contents were shaken (@ 250 rpm) for 0.5 h. The mixture was centrifuged with a Sorvall H6000A rotor in a Sorvall RC 3B Plus Centrifuge (Sorvall Instr. Inc., Newtown, CT) (@ $650 \times G$, 10 min @ 10° C). The mixture was allowed to temper (@ 25° C, 30 min) and subsequently aliquots of samples were dispensed with a Hamilton Micro Lab 900 dispenser (Hamilton, Las Vegas, NV) for total lipid. The chloroform was evaporated (@ 60° C, under N₂ flow) in a Multivap Analytical Evaporator (Organomation Association Inc., Berlin, MA), then dried in an oven (@100°C, 0.5 h) and subsequently cooled in a dessicator and weighed. In house quality control samples were also run for lipid analysis.

STATISTICAL DESIGN AND ANALYSES

The means weight, length and growth performance (SGR, FCR, PER) at each time point for all fishes in the treatment were analyzed as a split-plot treatment design with randomized complete blocks using SAS procedures (SAS, 1989). The three diets represented a whole plot while the time of evaluation (0, 4, 8, 12, 16, 20, and 24 weeks) was the sub-plot. The data were analyzed using analysis of variance (ANOVA) followed by least square means for comparison among treatments.

The processing yields (edible and non-edible) were analyzed as completely randomized design, with diets as two treatments. Ten fish from each diet group were selected randomly and the fish represented the replicate.

The responses for proximate composition (moisture, protein and total lipid) were analyzed as completely randomized design with diets (P32, P36 and zucchini) as the treatments. About a pound of fillet was ground from each treatment and sub-sampled three times for each treatment. The values were analyzed using ANOVA followed by the least square means for treatments (SAS, 1989).

RESULTS

Yield

The mean weights of pacu measured at 4 week intervals in the three treatment groups are given in table 1 and figure 1. At the end of the 24 weeks, the final mean weights of the fish were 484, 424, and 139 g for the P32, P36 and zucchini diets respectively. The average weights of pacu from the P32 and P36 diets were significantly higher than those obtained for the zucchini diet (p < 0.05). However, the final yields for P32 and P36 were not significantly different (p > 0.05).

Lengths

The average lengths of pacu measured at 4 week intervals in the three treatment groups are given in table 2 and figure 2. The final lengths of pacu at the end of the 24 week period were 295, 281, and 199 mm for P32, P36 and zucchini diets respectively and were significantly different (p<0.05). The average lengths of pacu from the P32 and P36 diets were significantly higher than those obtained for the zucchini diet (p < 0.05). However, the final lengths for P32 and P36 were not significantly different (p > 0.05).

Growth rates

Specific Growth Rate

The specific growth rates (SGR) for the three treatment groups is given in table 3. The SGR's were significantly different among the treatment groups (p < 0.05). Pacu fed the P32 diet performed better than pacu fed either the P36 or zucchini diets.

The observed daily gain in the pacu was the highest (1.13 g/day) in P32 and is comparable to the SGR's obtained for pacu grown in cages (Merola, 1988; Merola and DeSouza, 1988). The highest SGR was obtained in the first 4 weeks for the P32 and P36 diets, whereas a negative SGR (weight loss in fish) was observed in pacu fed zucchini. This negative value may be a reflection of the fish requiring time to adjust to the unfamiliar feed. The SGR showed a slight decline in subsequent weeks in the P32 and P36 (p > 0.05) diets and an increase of SGR in fish on zucchini diet (p < 0.05). During weeks 12 - 16, the fish on zucchini diet had the highest SGR but was not significantly different from the other treatments (p > 0.05). During the final four weeks (20 - 24) of the study, there were no significant differences between the SGR's for all treatments. The SGR's showed a gradual decline in the P32 and P36 diet treatments with time, whereas the SGR showed a bell - shaped in the zucchini group. Zucchini was chosen as a representative low - protein, high - carbohydrate feed since pacu are herbivores and their normal diet includes plants and leaves. Additionally, zucchini are readily available and can serve as a model for various by - products of fruit and vegetable processing operations which could be converted into a low - cost feed.

Protein Efficiency Ratio

The protein efficiency ratios (PER) for the three diets are given in table 4. The PER was significantly different among the treatments (p < 0.05). Pacu fed zucchini had a significantly higher PER (4.5) than pacu on the concentrated protein diets. Pacu on the

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P36 diet had a slightly higher PER (2.00) and hence, performed better than pacu on the P32 (1.96) feed.

Feed Conversion Ratio

The feed conversion ratios (FCR) for all the treatment groups are given in table 5. The FCR was significantly different among the treatments (p < 0.05). However, the P32 and P36 diets did not show any differences in the FCR (p > 0.05). A significantly poor FCR (56.5) was observed in pacu fed zucchini (p < 0.05) and gradually increased with time, indicating a poor feed conversion.

The pacu on the P36 diet with the lowest mean FCR (1.58) performed better than the P32, which had an FCR of 1.81. The FCR seemed to be constant during the growth period for both the P32 and P36 feeds. A sharp rise in FCR was observed in these two treatments in the final 4 weeks where the fish almost required 1.5 - 2 times more feed intake to gain 1 g in weight.

Fillet Yields

The yields of the edible and non - edible portions of pacu are contained in table 6. The pacu grown on zucchini were not processed as they were too small to be filleted. The protein content of the feed significantly influenced the yields of edible (eviscerated) and non - edible (viscera and gill) portions of pacu (p < 0.05). The average fillet yields without skin for the P32 and P36 diets were 35.1 and 34.0% respectively and were not significantly different (p > 0.05).

Proximate Composition

The proximate composition (percent moisture, protein, and total lipid) are given in table 7. Among the treatments, fillets of pacu grown on the P32 diet had the highest protein (20.75 - 21.09%) and lipid content (3.68 - 3.78%) and the lowest moisture content (76.21 - 76.62%). Pacu fed zucchini had the lowest protein (17.41 - 17.86%) and lipid (0.51 - 0.62%) content and the highest water content (82.40 - 82.50%). Pacu on the P36 diet contained intermediate moisture (77.90 - 78.27%), protein (17.20 - 20.28%) and total lipid (2.34 - 2.75%) contents. The moisture and total lipid contents were significantly different among all three diets (p<0.05). There were no significant differences in the protein content of fish grown on the P32 and P36 diets, but both the treatments were different from fish on the zucchini diet.

Radiography of Pacu

The lateral view radiograph (figure 3) was typical of teleost fish previously reported in the literature (Gosline, 1948; Miller and Tucker, 1979; Smith and Smith, 1994). The bones of the skull, vertebral column and fins, as well as large, two chambered swim bladder were clearly evident. Upon closer examination, numerous small bones were observed in the dorsal musculature of the body. Images of the serial body sections (figure 4) clearly demonstrated these bones free in the center of the musculature of the dorsum.

DISCUSSION

The growth performance of pacu for the experiment was comparable to that obtained by other investigators (Merola, 1988 and Merola and DeSouza, 1988) who compared the effects of water temperature and stocking density on growth. The results could be improved by changing from restricted (adlibitum) feeding to unlimited and continuous feeding through manual or automatic feeders.

Carneiro et al. (1984a) and Cantelmo and DeSouza (1987a,b) obtained increased growth rates with increases in protein content of the diet. Based on the results of this study, pacu fed 32% dietary protein performed superior than pacu fed 36% dietary protein. This difference was attributed to lower feed consumption by fish on the P36 diet than their counterparts on the P32 diet. Pacu fed zucchini had unlimited access to the feed but were unable to grow because of the low protein content of the feed (~0.5%). Even though pacu grew slightly on the zucchini diet, improved growth rates could be obtained by supplementing the feed with a protein source.

According to Stansby (1962) and the proximate composition results of this study, pacu could be classified as a fish with a low fat (<15%) and high protein (>15%) content. These results completely contradict the results obtained by Machado and Sgarbieri (1991) who classified pacu as a fish of high fat (>15%) and low protein (<15%). However, total lipid and moisture exhibit great variability in fish muscle, as compared to lesser variability shown by protein content (Machado and Sgarbieri, 1991). Further, factors such as age, physiological state, season of harvest of the fish and the composition of the diet greatly influence the proximate composition of the fish.

Stansby (1962) and Thurston et al. (1959) reported an inverse relationship between water and lipid contents and that relationship is also evident in this study. The inverse relationship between water content and size of the fish, as reported by Suzuki (1981) was also demonstrated in this study.

Since the bones are present throughout the fillets, production of a boneless fillet is virtually impossible. It is possible that some of the bones could soften if an appropriate thermal process was applied at the processing level (e.g., battered fish fillet) or within the home or foodservice establishment. If the bones are not affected by heat, it is possible that pacu could be processed with a meat/bone separator for the production of a minced product. The minced meat could then be used in the production of a fish stick or portion. Since the growth rate of pacu is relatively fast, minced pacu may be an economically viable product.

The success of raising fish under controlled conditions depends greatly on the provision of a cost effective diet, which is nutritionally rich, particularly in dietary protein. It is generally accepted that protein represents a major cost of commercial feeds. Hence, a better understanding of the dietary protein requirements of the fish is critical to the success of aquaculture (Merola, 1988; Borghetti and Canzi, 1993).

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CONCLUSIONS

Based on the results of this study, it was concluded that pacu fed commercial feeds performed better than those on unprocessed agricultural products. This superior growth was attributed to the higher nutritive value of the commercial feeds. However, pacu can be raised on low - cost agricultural products with promising results if supplemented with a source of protein. Since most American consumers prefer fish that are white, light flavored, and boneless, pacu may not have high acceptability in the market place. However, pacu processed into minced meat could be an economically viable product.

REFERENCES

Association of Official Analytical Chemists (AOAC). 1984a. Meat and Meat Products. Method 24.003. In *Official Methods of Analysis of the AOAC*. 14th ed. Ellis, R. L. (Ed.). AOAC, Arlington, VA.

Association of Official Analytical Chemists (AOAC). 1984b. Meat and Meat Products. Method 24.038. In *Official Methods of Analysis of the AOAC*. 14th ed. Ellis, R. L. (Ed.). AOAC, Arlington, VA.

Borghetti, J. R. and Canzi, C. 1993. The Effect of Water Temperature and Feeding Rate on the Growth Rate of Pacu (*Piaractus mesopotamicus*) Raised in Cages. Aquaculture. 61: 93 - 101.

Carneiro, G. J., Rantin, F. T., Dias, T. C. R., and Malheiros, E. B. 1994a. Interaction Between Temperature and Dietary Levels of Protein and Energy in Pacu (*Piaractus mesopotamicus*). I. The Effects on Growth and Body Composition. Abstracts / Aquaculture. 124: 127 - 131.

Carneiro, G. J., Rantin, F. T., Dias, T. C. R., and Malheiros, E. B. 1994b. Interaction Between Temperature and Dietary Levels of Protein and Energy in Pacu (*Piaractus mesopotamicus*). II. Effects on Digestibility of Protein and Mean Transit Time Through Gastrointestinal Tract. Abstracts/Aquaculture. 124: 127 - 131.

Christensen, M. S. 1981. Preliminary Tests on the Suitability of Coffee Pulp in the Diets of Common Carp (*Cyprinus carpio* L.) and Catfish (*Clarias mossambicus* Peters). Aquaculture. 25: 235 - 242.

Fagberno, O. A. and Arowosoge, I. A. 1991a. Replacement Value of Some HouseholdWastes as Energy Substitutes in Low - Cost Diets for Rearing Catfish in South - WesternNigeria. Bioresource Technol. 37: 197 - 203.

Fagberno, O. A. and Arowosoge, I. A. 1991b. Growth Response and Nutrient
Digestibility by *Clarias isheriensis* (Sydenham, 1980) Fed Varying Levels of Dietary
Coffee Pulp as Replacement for Maize in Low - Cost Diets. Bioresource Technol. 37:
253 - 258.

Gosline, W. A. 1948. Some Possible Uses of X - rays in Icthyology and Fisheries Research. Copeia: 58 - 61.

Govindan, V. S. 1989. Food and Feed Production from Municipal Wastewater Treatment. Biological Wastes. 30: 169 - 179.

Kaur, K., Sehgal, G. K., and Sehgal, H. S. 1987. Efficacy of Biogas Slurry in Carp *Cyprinus carpio* Var. *communis* (Linn.), Culture - Effects on Survival and Growth.Biological Wastes. 22: 139 - 146.

Machado, M. G. S. and Sgarbieri, V. C. 1991. Partial Characterization of Proteins from Pacu (*Colossoma mitrei*, Berg 1895). J. Agric. Food Chem. 39: 1715 - 1718.

Mahadevaswamy, M. and Venkataraman, L. V. 1988. Integrated Utilization of Rabbit Droppings for Biogas and Fish Production. Biological Wastes. 25: 249 - 256.

Merola, N. 1988. Effects of Three Dietary Protein Levels on the Growth of Pacu, *Colossoma mitrei*, Berg, in Cages. Aquacult. Fish Manage. 19: 145 - 150.

Merola, N. and DeSouza, J. H. 1988. Preliminary Studies on the Culture of the Pacu *Colossoma mitrei*, in Floating Cages: Effect of Stocking Density and Feeding Rate on Growth Performance. Aquaculture. 68: 243 - 248.

Miller, J. M. and Tucker, J. W. 1979. X - radiography of the Larval and Juvenile Fishes. Copeia: 535 - 538.

Saint - Paul, U. 1986. Potential For Aquaculture of South American Freshwater Fishes: A Review. Aquaculture. 54: 205 - 240.

SAS. 1989. SAS/STAT Guide for Personal Computers, Version 6.0 Ed. SAS Institute, Cary, NC.

Smith, S. A. and Smith, B. J. 1994. Xeroradiographic and Radiographic Anatomy of the Channel Catfish, *Ictalurus punctatus*. Veterinary radiology and Ultrasound. 35: 384 -389.

Stansby, M. E. 1962. Proximate Composition of Fish. In *Fish in Nutrition*; Heen E., Kreuzer, R., Eds.; Fishing News: London. pp 55 - 56.

Suzuki, T. 1981. Characteristics of Fish Meat and Fish Protein. pp 5 - 7. In *Fish and Krill Protein*; Applied Science Publishers: London.

Thurston, C. E., Stansby, M. E., Karrick, N. L., Miyauchi, D. T., and Clegg, W. C. 1959. Composition of Certain Species of Freshwater Fish II. Comparative Data for 21 Species of Lake and River Fish. Food Res. 24: 493.

Ufodike, E. B. C. and Matty, A. J. 1983. Growth Responses and Nutrient Digestibility in Mirror Carp (*Cyprinus carpio*) Fed Different Levels of Cassava and Rice. Aquaculture. 31: 41 - 50.

Time	Pacu Weight (g)			
(weeks)	0.5% Vegetable Diet	32% Comm. Diet	36% Comm. Diet	
0	72.50 ^a	72.55 ^a	72.50 ^a	
4	69.80 ^a	111.85 ^a	110.70 ^a	
8	76.25 ^a	169.85 ^{b, c, f}	150.70 ^{c, f}	
12	84.35 ^a	225.65 ^b	202.20 ^{b, c}	
16	115.50 ^a	328.90 ^d	290.35 ^d	
20	118.60 ^a	429.70 ^e	378.40 ^{d, e}	
24	138.85 ^f	484.05 ^e	423.85 ^e	

Table 1. Effect Of Dietary Protein On Mean Body Weight Of Pacu (Piaractusmesopotamicus)

Mean values in rows and columns followed by the same letter are not significantly

different (p>0.05) (Standard Error \pm 20.57)



Figure 1. Effect of Dietary Protein on the Weights of Pacu (Piaractus mesopotamicus)

Time	Pacu Length (mm)			
(weeks)	0.5% Vegetable Diet	32% Comm. Diet	36% Comm. Diet	
0	155.95 ^{a.}	155.95 ^a	156.00 ^a	
4	165.35 ^a	182.70 ^{a, b, f}	182.25 ^{a, b, f}	
8	168.50 ^a	211.55 ^{b, c, f}	203.15 ^{b, c, f}	
12	173.65 ^a	231.10 ^{c, d, g}	228.10 ^{c, d, g}	
16	184.25 ^a	250.55 ^d	237.75 ^d	
20	183.65 ^a	267.71 ^{d, e}	258.00 ^{d, e}	
24	199.40 ^{f, g}	294.55 ^e	281.15 ^e	

 Table 2. Effect of Dietary Protein on the Lengths (mm) of Pacu (*Piaractus mesopotamicus*)

Mean values in rows and columns followed by the same letter are not significantly different ($p{<}0.05$) (Standard Error \pm 11.90)



Figure 2. Effect of Dietary Protein on the Lengths of Pacu (Piaractus mesopotamicus)

Time	Specific Growth Rates			
(weeks)	0.5% Vegetable Diet	32% Comm. Diet	36% Comm. Diet	
0 - 4	- 0.14 ^f	1.55 ^a	1.51 ^a	
4 - 8	0.32 ^{c, f}	1.49 ^a	1.10 ^a	
8 - 12	0.34 ^{c, f}	1.00 ^b	1.03 ^a	
12 - 16	1.04 ^{a, e}	1.36 ^a	1.29 ^a	
16 - 20	0.11 ^{c, f}	0.96 ^{b, e}	0.91 ^{b, d, e}	
20 - 24	0.52 ^c	0.43 ^{c, d}	0.42 ^{c, d}	

 Table 3. Effect of Dietary Protein on the Specific Growth Rate of Pacu (*Piaractus mesopotamicus*)

Means followed by the same letter in rows and columns are not significantly different (p > 0.05) (Standard error ± 0.172)

Time	Protein Efficiency Ratio			
(weeks)	0.5% Vegetable Diet	32% Comm. Diet	36% Comm. Diet	
0 - 4	- 3.22 ^d	1.86 ^{a, c}	1.68 ^{a, c}	
4 - 8	4.29 ^{a, c}	2.50 ^{a, c}	2.34 ^{a, c}	
8 - 12	4.74 ^c	2.24 ^{a, c}	2.69 ^{a, c}	
12 - 16	10.82 ^e	2.34 ^{a, c}	2.63 ^{a, c}	
16 - 20	1.72 ^a	1.84 ^{a, c}	1.57 ^{a, c}	
20 - 24	8.67 ^e	1.03 ^{a, b}	1.09 ^{a, b}	

 Table 4. Effect of Dietary Protein on the Protein Efficiency Ratio of Pacu (*Piaractus mesopotamicus*)

Means followed by the same letter in rows and columns are not significantly different (p > 0.05) (Standard error ± 1.105)

Time	Feed Conversion Ratios			
(weeks)	0.5% Vegetable diet	32% Comm. Diet	36% Comm. Diet	
0 - 4	- 75.66 ^{a, c}	1.68 ^{a, b}	1.66 ^{a, b}	
4 - 8	50.75 ^b	1.25 ^{a, b}	1.23 ^{a, b}	
8 - 12	80.21 ^b	1.42 ^{a, b}	1.08 ^{a, b}	
12 - 16	18.58 ^{a, b}	1.36 ^{a, b}	1.10 ^{a, b}	
16 - 20	216.12 ^d	1.71 ^{a, b}	1.84 ^{a, b}	
20 - 24	24.19 ^{a, b}	3.49 ^{a, b}	2.60 ^{a, b}	

Table 5. Effect of Diet on the Feed Conversion Ratio of Pacu (Piaractus mesopotamicus)

Means followed by the same letter in rows and columns are not significantly different (p > 0.05) (Standard error ± 39.99)

	32% Protein Diet		36% Protein Diet	
EDIBLE	Mean \pm SD	Range	Mean \pm SD	Range
Whole fish	100 ± 0.00 $^{\rm a}$	100 - 100	100 ± 0.00 $^{\rm a}$	100 - 100
Eviscerated fish	88.5 ± 1.15 ^a	86.2 - 90.0	91.7 ± 2.83 ^b	86.9 - 95.3
Degilled fish	86.3 ± 1.89 ^a	83.3 - 88.4	86.5 ± 2.00^{a}	82.6 - 88.9
Deheaded fish	68.8 ± 2.51 ^a	66.4 - 73.5	68.2 ± 1.56 ^a	65.5 - 71.3
Fillet with rib cage	56.8 ± 1.89 ^a	54.7 - 60.7	55.4 ± 2.23 ^a	51.3 - 58.3
Fillet without rib cage	45.3 ± 1.67 ^a	40.8 - 46.4	44.9 ± 1.68 ^a	42.6 - 46.9
Fillet without skin	35.1 ± 2.10 ^a	31.9 - 37.4	34.0 ± 2.39^{a}	30.1 - 38.6
NON EDIBLE				
Viscera	11.4 ± 1.15 ^a	9.9 - 13.7	8.3 ± 2.83 ^b	5.5 - 13.0
Gills	2.2 ± 1.16^{a}	0.5 - 3.9	5.4 ± 2.83 ^b	0.7 - 8.8
Head	17.4 ± 1.51 ^a	14.1 - 19.3	18.3 ± 1.26 ^a	17.1 - 20.3
Frame	11.9 ± 2.62 ^a	9.7 - 18.8	12.7 ± 2.32 ^a	9.5 - 17.6
Rib Cage	11.5 ± 2.10^{a}	8.6 - 15.2	10.5 ± 1.84 $^{\rm a}$	7.2 - 13.1
Skin	10.2 ± 1.94 ^a	7.8 - 13.6	10.8 ± 2.28 ^a	8.2 - 16.3

 Table 6. Effect of Different Levels of Protein on the Processing Yields of Aquacultured

 Pacu (*Piaractus mesopotamicus*)

Means with the same letter in rows are not significantly different (p > 0.05)

Table 7. Effect of Dietary Protein on the Proximate Composition of Skinless Pacu(*Piaractus mesopotamicus*) Fillets

Proximate Composition of Pacu fed Different Levels of Protein			
	0.5% Vegetable diet	32% Comm. Diet	36% Comm. Diet
Moisture	82.4 °	76.4 ^a	78.0 ^b
Protein	17.7 ^b	20.9 ^a	19.1 ^a
Lipid	0.6 ^c	3.4 ^a	2.6 ^b

Means with same letter in rows are not significantly different (p > 0.05) (Standard errors:

moisture \pm 0.115, protein \pm 0.619, and lipid \pm 0.077)



Figure 3. Lateral view of Pacu (Piaractus mesopotamicus) Showing the Bony Structure of Skeleton



Figure 4. Radiograph of a Body Section Showing Main Body Skeleton and Additional Bones in the Dorsal Musculature

SECTION III.

Effect Of Diet On The Indicative And Pathogenic Microbiological Quality Of Aquacultured Pacu (*Piaractus mesopotamicus*)

by

S. Pullela, C. F. Fernandes, G. J. Flick, G. S. Libey, S. A. Smith, and C. W. Coale.

ABSTRACT

The qualitative and quantitative numbers of bacteria were determined on water used for culturing pacu as well as on pacu (Piaractus mesopotamicus) following processing. Pacu fingerlings weighing approximately 72 g were fed three different diets (a) zucchini (0.5% protein); (b) commercial aquaculture feed P32 (32% protein); and (c) commercial aquaculture feed P36 (36% protein) and raised for 24 weeks. Microbial analyses on growing waters included aerobic counts, psychrotrophic counts, total and fecal coliform counts as well as selected pathogens (Listeria monocytogenes, Salmonella spp., Yerisinia enterocolitica, Escherichia coli O157:H7, and Clostridium botulinum) were assayed at 6 week intervals. At 24 weeks, twenty pacu were randomly selected, gutted and analyzed for pathogens using AOAC procedures. Five fish from the pathogen analyses were used for analyzing the indicative microbial quality using 3MTMPetrifilmTM. The mean counts (range) for aerobes, psychrotrophes, total coliforms, fecal coliforms, and Escherichia coli ranged between 5.05 (3.72 - 6.81 log CFU/g), 5.05 (2.49 - 6.81 log CFU/g), 2.66 (0.85 -4.21 log CFU/g), 2.92 (0.85 - 4.00 log CFU/g), and 0.13 (0.00 - 0.20 CFU/g) respectively. The indicative microbial quality differed significantly (p<0.05) among the treatments, except for *E. coli* which was not significantly different (p>0.05). *Listeria monocytogenes, Salmonella spp., Yerisinia enterocolitica, E. coli* O157:H7 and *Clostridium botulinum* were not isolated from the sampled fish. Pacu grown on P32 and P36 diets exceeded the International Commission on Microbiological Specification for Foods (ICMSF) limits for fecal coliform counts for freshwater fish (M=400/g) and hence were concluded to be of unacceptable bacterial quality.

INTRODUCTION

The demand for seafood products had been rising over the past few years with increased awareness of the health benefits associated with consumption of fish. Fish are low in saturated fat and cholesterol, and provide a good source of protein. The annual per capita consumption of fish in the United States is close to sixteen pounds and is expected to rise to twenty pounds by the end of the century (Flick et al., 1991).

The decline in America's fishery resources coupled with growing demand for seafood has lead aquaculturists to introduce and grow several new species under controlled environments, examples of which include tilapia (*Oreochromis spp.*) and grass carp (*Ctenopharyngodon idella*), which were introduced into the United States to control aquatic weeds (Stickney, 1996). Hybrid striped bass, with its fast growth rates (1.7 pounds/year and 4.8 pounds/2 years), commanding price (\$2.50/pound) and excellent sensory attributes (white flesh, mild taste, and low fat content) is gaining popularity among the aquaculturists and consumers (Rawles et al., 1997). Pacu is another exotic fish, which has been billed as simple to grow, cheap to feed, and delicious to eat (Anonymous, 1994).

Pacu (*Piaractus mesopotamicus*) is a herbivorous freshwater fish indigenous to the Parana - Uruguay river system in South America. The abundance of lakes and reservoirs in this region has generated interest among the aquaculturists to grow the fish under artificial conditions. The fish is now being intensively aquacultured in the south, south -

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east, and west - central regions of Brazil. Fed under intensive systems, pacu can take up to 2 years to attain 1.2 Kg in earthen ponds, but the time to attain the same yields could be greatly reduced by feeding pacu commercially formulated diets.

Most of the available literature on pacu includes growing the fish under different systems (cages, ponds, tanks etc.) and under different feeding regimes. Merola (1988) obtained superior yields and weight gains with a 35% protein diet. Carneiro et al. (1994a) reported better growth rates of pacu at the highest protein and lowest energy levels. Carneiro et al. (1994b) noted an increase in the mean transit time of the feed through the gastro - intestinal tract. They also noted increasing protein digestibility with increasing energy levels at low crude protein levels. Borghetti and Canzi (1993) reported that higher water temperatures led pacu to consume more feed and resulted in improved yields while lower temperatures led to feed wastage and poor yields.

Fish and fish products have long been considered a vehicle of foodborne bacterial and parasitic infections leading to human illnesses (Brown and Dorn, 1977). The Center for Disease Control and Prevention (CDCP) reported that seafoods account for 5% of the individual cases and 10% of all foodborne illness outbreaks in the United States, with most of the outbreaks resulting from the consumption of raw molluscan shellfish (Otwell, 1989 and Ahmed, 1992). In December 1997, the Food and Drug Administration (FDA) will require seafood processors to adopt a quality assurance program based on the hazard analysis and critical control point (HACCP) concept. The aquaculture industry in the United States has an excellent record of producing safe and high quality fishery products (Fernandes et al., 1997). It is generally accepted that the number and types of bacteria associated with fish is influenced directly by the environment in which they are grown (Nedoluha and Westhoff, 1995; Ward, 1989). Aquaculture systems (both closed and recirculating) often deteriorate the bacterial quality of water by accumulating organic and inorganic materials, which eventually serves as an excellent substrate for bacterial proliferation (Davis and Goulder, 1993; Austin et al., 1985). Hence, careful monitoring of bacterial water quality would help in the production of safe and high quality seafood products.

The purpose of this study was to determine the overall microbial quality of aquacultured pacu. These parameters included indicative bacterial quality, the incidence levels and quantity of foodborne bacteria pathogens (*Listeria monocytogenes, Salmonella spp., Yersinia enterocolitica, Escherichia coli* O157:H7, and *Clostridium botulinum*) in the fish and rearing water.

MATERIALS AND METHODS

Collecting and transporting fish samples

Pacu were fed three different diets - a) commercial 32% Protein (P32) diet (SSC 50822061, Southern States Co - Op. Inc., Richmond, VA), b) commercial 36% Protein (P36) diet (SSC 338200, Southern States Co - Op Inc.), and zucchini diet (0.5% Protein) and grown for a period of 24 weeks in six 1165 L fiber glass tanks with no external

filtration system. There were 2 tanks (replicates) per treatment. At the end of 24 weeks, 10 fish per tank (20 per treatment) were randomly selected, wrapped individually in sterile polythene bags, placed on ice (thermal shock to kill the fish) and transported to the laboratory.

Microbiological analyses of fish

Sample Preparation

Upon receipt of the fish, they were aseptically gutted, weighed and equal volume of cold (0 to 1°C) sterile 15 mM Butterfield's phosphate buffer was added. Each fish was massaged by hand for 2 minutes, removed aseptically with sterile forceps, and the rinse held on ice until microbiological analyses were performed. All microbial analyses were performed within 8 h of receiving the sample (Swanson et al., 1992).

Aerobic and Psychrotrophic counts

The rinse from the fish was diluted in 15 mM Butterfield's phosphate buffer and appropriate dilutions plated on $3M^{TM}$ PetrifilmTM Aerobic Count (PAC) plates (The 3M Corp., Healthcare Division, Minneapolis, MN) in duplicate. For aerobic and psychrotrophic counts, the plates were incubated at $35 \pm 2^{\circ}$ C for 48 h and at $21 \pm 2^{\circ}$ C for 96 h, and all red colonies were enumerated.

E. coli, Total Coliform and Fecal Coliform counts

The wash from the whole gutted pacu was diluted in 15 mM Butterfield's phosphate buffer and appropriate dilutions were enumerated on 3M[™] Petrifilm[™] *E. coli*

count plates and coliform count plates (The 3M Corp., Healthcare Division) in duplicate. Each dilution was plated onto four different plates - one set for the total coliforms and the other for fecal coliforms. *E. coli* were also plated in duplicate and incubated at $35 \pm 2^{\circ}$ C for 24 to 48 h (Swanson et al., 1992) and all the blue colonies with gas were counted as*E. coli* cells. The coliform count plates for enumerating total coliforms and fecal coliforms were incubated for 48 h at $35 \pm 2^{\circ}$ C and $44 \pm 2^{\circ}$ C respectively. All the red colonies with gas were counted as coliforms.

Indicative microbial quality of rearing water

Collection of Water samples

The water samples for the indicative microbiological quality were collected in sterile glass bottles. For the isolation and identification of pathogens, water samples were concentrated using the techniques described in the Standard Methods for the Examination of Water and Waste Water (Greenberg et al., 1992). Six gauze pads (Johnson and Johnson Medical Inc., Arlington, Texas) of 4 in. \times 4 in. area were sterilized at 121°C for 35 min. and placed in the fish tanks for 2 days. After exposure, the gauze pads were aseptically transferred into sterile polythene bags using sterile forceps, placed on ice and transported to the laboratory (Greenberg et al., 1992).

Heterotrophic and Psychrotrophic counts

The water samples were diluted in 15 mM phosphate buffer and appropriate dilutions were plated onto Standard Methods Agar (Difco Laboratories, Detroit, MI) in
duplicate using the pour plate method. The plates were then incubated at $35 \pm 2^{\circ}$ C for 48 h and $21 \pm 2^{\circ}$ C for 96 h for heterotrophic and psychrotrophic counts respectively (Greenberg et al., 1992).

Total Coliform Counts

A five - tube, three dilution (10.0, 1.0, and 0.1 ml) most probable number (MPN) technique was followed for enumerating total coliforms. Appropriate dilutions were inoculated into double strength Lauryl Tryptose (LST) Broth (Difco Laboratories) and the tubes incubated at $35 \pm 2^{\circ}$ C. Following incubation, the tubes were tested for gas production, heavy growth, and acid production at 24 h and 48 h. Three mm loopful of the presumptive culture was transferred to a test tube containing 10 ml of Brilliant Green Lactose Bile (BGLB) Broth (Difco Laboratories) and incubated at $35 \pm 2^{\circ}$ C for 48 h. Gas production in any tube was considered as a positive reaction for coliforms and the MPN calculated as described in the Bacteriological Analytical Manual (Greenberg et al., 1992).

Fecal Coliform Counts

Fecal coliforms in the water were enumerated using a 5 tube - 3 dilution (10.0, 1.0, and 0.1 ml) fermentation technique using A - 1 Medium (Difco Laboratories) Appropriate dilutions were inoculated into double strength A - 1 Medium and incubated at $35 \pm 2^{\circ}$ C for 3 h. The tubes were then transferred to a water bath at $44.5 \pm 2^{\circ}$ C and incubated for an additional 21 h. Gas production in any tube with in 24 h or less was considered as a positive reaction for coliforms of fecal origin and MPN calculated (Greenberg et al, 1992).

Escherichia coli

The LST tubes with gas were gently agitated and a 3 mm loopful of suspension was transferred to EC Broth (Difco Laboratories). The tubes were examined for gas production at 24 h and 48 h. A loopful of suspension from gassing EC broth tube was streaked onto Levine's Eosin - Methylene Blue (L - EMB) agar (Difco Laboratories) and incubated at $35 \pm 2^{\circ}$ C for 18 - 24 h. After incubation, suspect *E. coli* colonies were transferred onto Plate Count Agar (PCA) slants (Difco Laboratories) and incubated at $35 \pm 2^{\circ}$ C for 18 - 24 h and biochemically tested for ++--or -+--IMViC (Indole, Methyl - red, Voges - Proskauer, Citrate) patterns and gas production from lactose. The MPN's were calculated using the methods described in the Bacteriological Analytical Manual (Hitchins et al., 1995).

Listeria monocytogenes

Twenty - five ml of the rinse solution and 225 ml of University of Vermont Medium (UVM) (Difco Laboratories) were added into a sterile stomacher 400 bag. Following mixing, the contents were pre-enriched at $30 \pm 2^{\circ}$ C for 24 h. Following preenrichment, 0.1 ml of the UVM culture was pipetted into 10 ml of Fraser's secondary enrichment broth (Difco Laboratories), and incubated at $35 \pm 2^{\circ}$ C for 24 and 40 h. Enriched Fraser's broth was streaked onto Modified Oxford Agar (Difco Laboratories, Detroit, MI) and incubated at $35 \pm 2^{\circ}$ C and examined for typical round *Listeria* colonies surrounded by a black zone. Presumptive *Listeria* colonies were confirmed using API *Listeria* strips (bioMe`rieux Vitek, Hazelwood, MO) (Hitchins, 1995). *Listeria monocytogenes* ATCC 7644 was used as a control during the isolating procedure to confirm microbiological tests.

Salmonella spp.

Twenty - five ml of the rinse solution was added to 225 ml of Lactose Broth (Difco Laboratories) and enriched primarily at $35 \pm 2^{\circ}$ C for 24 h. Secondary enrichment was carried out by transferring 1 ml of the incubated sample mixture to 10 ml of secondary enrichment (Selenite Cystine) broth (Difco Laboratories) and the mixture incubated at $35 \pm 2^{\circ}$ C for 24 h. To isolate suspect colonies, the culture was streaked onto Xylose Lysine Desoxycholate Citrate (XLD) and Bismuth Sulfite (BS) Agar (Difco Laboratories) and incubated at $35 \pm 2^{\circ}$ C for 24 h. Suspect colonies were subjected to preliminary biochemical tests using Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA). To determine the genus and species Rapid 20E test kits (bioMe`rieux Vitek) were used (Andrews et al., 1995). *Salmonella typhimurium* ATCC 19585 was carried through the experiments to ensure the accuracy of media reactions.

Yersinia enterocolitica

An enrichment was carried out using 25 ml of the sample and 225 ml of Peptone Sorbitol Bile Broth (PSBB) and incubated at 4°C for 10 days. Following enrichment, the enriched broth was transferred to 1.0 ml of 0.5% KOH in 0.5% saline and immediately streaked onto MacConkey's Agar and incubated at 35 ± 2 °C for 48 h. Pink suspect colonies were transferred to Lysine Arginine Agar, Esculin Bile Agar, and Christensen's Urea Agar slants for preliminary biochemical testing. To determine the genus and species, API Rapid 20E test kits were used (bioMe`rieux Vitek) (Weagant et al., 1995). *Yersinia enterocolitica* ATCC 9610 was used as a control to confirm microbiological tests.

Clostridium botulinum

Enrichment was carried out using 25 g equivalents of the sample and 225 g of Cooked Meat Medium and incubated at $35 \pm 2^{\circ}$ C for 7 days. Following pre-enrichment, 1.0 ml of the enriched medium was treated with 1.0 ml of absolute ethanol for 1 h and subsequently streaked onto Anaerobic Egg Yolk Agar and incubated under anaerobic conditions using anaerobe jars and BBL® GasPak PlusTM anaerobic system envelopes with a paladium catalyst (Becton Dickinson, Cockeysville, MD) at $35 \pm 2^{\circ}$ C for 48 h. The plates were then checked for typical *C. botulinum* colonies and the positive cultures were checked for toxin production using the mouse bioassay. For each sample tested, three mice were used: one receiving a heat treated sample, one receiving a trypsinized sample, and one receiving an untreated sample (Solomon et al., 1995). *Clostridium botulinum* Beluga E was used as a control during toxicity testing.

Escherichia coli O157: H7

The pacu sample (25.0 g) was enriched in modified EC Broth (225 ml) (Difco Labs.) containing 20 µg/ml of filtered sterilized (0.2 µm: Nalgene Co., Rochester, NY) sodium novobiocin (Sigma Chemical Co., St. Louis, MO) (Okrend et al., 1990). The sample was incubated at $37 \pm 2^{\circ}$ C for 6 h. The enriched broth was diluted appropriately and plated on $3M^{TM}$ Petrifilm *E. coli* plates (The 3M Corp., Healthcare Division). The *E. coli* plates were incubated at $35 \pm 2^{\circ}$ C for 18 h. Following 18 h of incubation the immunological tests were performed using the $3M^{TM}$ Petrifilm test kit HEC for hemorrhagic *E. coli* O157:H7 (The 3M Corp., Healthcare Division) (Okrend et al., 1990). The *E. coli* cell suspension provided in the kit was used as a control.

STATISTICAL DESIGN AND ANALYSES

The indicative bacterial quality of water was analyzed as split-plot with time treatment design with randomized complete blocks using SAS procedures (SAS, 1989). The samples were randomly collected for each diet (whole plot), while sub-plot was time (6, 12 and 18 weeks). Water samples from each tank represented the blocks and they were analyzed for aerobic, psychrotrophic, total coliform, fecal coliforms and *Escherichia coli*. The means of duplicate plate counts for all responses were averaged. Average counts for aerobic, psychrotrophic, total and fecal coliform counts were analyzed

following log transformation of the data. The means for *E. coli* were analyzed without the log transformation of the data.

The indicative bacterial quality (aerobic, psychrotrophic, total coliform, fecal coliform, and *E. coli* counts) of whole pacu was analyzed as randomized complete blocks with diet as the treatment (P32, P36 and zucchini) using SAS procedures (SAS, 1989). Five fish samples from each tank represented the sub-sample. The means of sub-samples were averaged. Average counts for aerobic, psychrotrophic, total and fecal coliform counts were analyzed following log transformation of the data. The means for*E. coli* were analyzed without the log transformation of the data.

RESULTS AND DISCUSSION

Microbiological Quality of Whole Pacu

Aerobic Plate Counts

The indicative microbiological quality of pacu and the water used for rearing pacu are given in tables 1 and 2 respectively. There were no significant differences in the aerobic plate counts among the diets (p < 0.05). The mean counts for aerobes were consistent among the diets ranging from 4.56 - 5.60 log CFU/g. The observations in the study compare well with the results obtained by other investigators. Nedoluha and Westhoff (1995) determined the microbiological quality of striped bass (*Morone saxatilis*) in flow through tanks and reported counts ranging from 3 - 6 log CFU/cm². Fernandes et al. (1996) analyzed the influence of size of processing operations and season on the bacterial quality of pond raised catfish (*Ictalurus punctatus*) and reported counts ranging from 3.0 - 6.0 log CFU/g. Enriquez et al. (1994) determined the microbial quality of fillets of hybrid striped bass obtained from a recirculating aquaculture system and reported counts ranging from 1.9 - 2.8 log CFU/g. McAdams et al.(1996) compared the effect of rearing aquaculture system on the microbiological quality of rainbow trout (*Onchorhynchus mykiss*) and reported counts ranging from 3.0 - 6.0 log CFU/g.

The results obtained in this study compare well with the counts reported earlier in the literature. Abeyta (1983) surveyed fresh seafood products from retail markets in Seattle, WA and noted that the counts for aerobes ranged from 4.0 - 7.0 log CFU/g. Okafor and Nzeako (1985) in a survey of freshwater fish in Nigeria obtained mean counts of 4×10^5 CFU/g. Nair and Nair (1988) determined the bacteriological quality of freshwater fish from a reservoir and found counts ranging from 6.4×10^4 to 4.8×10^5 CFU/g. However, these high numbers of bacteria are present mostly in the non - edible portions of the fish viz., surface slime, gills, and intestines which are separated during manual or mechanical processing of finfish and thus, have an insignificant influence on the quality of edible muscle.

The International Commission on Microbiological Specification for Foods (ICMSF, 1986) limits for freshwater fish and fish products for heterotrophic plate count are $m = 5 \times 10^5$ and $M = 10^7$. In this study, 4 of the 30 (13.3%) samples analyzed had

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counts greater 10^6 CFU/g and there were no samples with counts exceeding 10^7 CFU/g. Spoilage of most muscle foods is initiated when the APC exceeds 10^7 CFU/g and since none of the samples exceeded these levels, the whole pacu were concluded to be of good microbial quality.

Psychrotrophic Plate Counts

There were significant differences in the psychrotrophic counts for whole pacu (p < 0.05). Pacu sampled from one of the 36P trails had significantly lower psychrotrophic counts (3.52 log CFU/g) compared to other trials which had mean counts ranging from 4.87 - 5.63 log CFU/g. These results are consistent with the results reported earlier in the literature. Fernandes et al. (1996) analyzed the influence of size of processing operations and season on the bacterial quality of catfish and reported counts ranging from 3.0 - 6.5 log CFU/g. Enriquez et al. (1994) determined the microbial quality of hybrid striped bass fillets and reported counts ranging from 1.8 - 2.5 log CFU/g. McAdams (1996) compared the effect of rearing aquaculture system on the microbiological quality of rainbow trout and reported counts ranging from 3.0 - 6.0 log CFU/g. Fish are usually preserved on ice or frozen in retail seafood outlets. The presence of high numbers of psychrotrophic bacteria which have the ability to grow and multiply under such storage conditions and could potentially increase the spoilage resulting in a reduced shelf - life of the fish.

Total and Fecal Coliform Counts

There were significant differences in the counts for total and fecal coliforms (p < p0.05). Pacu grown in one of the P32 diet trials had significantly lower total coliform counts (p < 0.05) and pacu in one of the zucchini trials had significantly higher total coliform counts (p < 0.05). Fecal coliform counts were significantly lower (p < 0.05) in one zucchini trial (1.87 $\log CFU/g$). Abeyta (1983) reported average coliform counts of 296 MPN/g in seafood products from retail markets. Foster et al. (1977) surveyed fresh and frozen seafood products and reported mean coliform counts, as determined by the Most Probable Number (MPN) method, to be 7.8 - 4800 MPN/g. Fernandes et al. (1996) analyzed the influence of size of processing operations and season on the bacterial quality of catfish and reported counts ranging from 0.8 - 3.2 log CFU/g. Enriquez et al. (1994) determined the microbial quality of hybrid striped bass fillets and reported counts ranging from 0.6 - 2.0 log CFU/g. McAdams (1996) compared the effect of rearing aquaculture system on the microbiological quality of rainbow trout and reported counts ranging from 3.09 - 543 MPN/g. Nair and Nair (1988) reported coliform counts ranging form 120 -9200/g.

The ICMSF limits for freshwater fish products for fecal coliforms are m = 4 and M = 400. In this study, 21 of the 30 (70%) samples analyzed had fecal coliform counts higher than 400. Such, high numbers of fecal coliforms was attributed to fecal contamination by the fish and lack of filtration of water used for rearing. Pacu in the P32

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and P36 diet trials had counts exceeding the specified limits and were concluded to be of less than acceptable quality. Fecal coliforms in freshwater fish from warm waters is considered to be a low, indirect health hazard (case 4) and subsequent cooking before consumption reduces the degree of concern. The maximum allowable number of defectives (c) is 3. Pacu fed zucchini did not exceed these limits (c = 3) and were considered to be of acceptable quality.

Escherichia coli

Escherichia coli was isolated only from pacu grown on the 32% protein diet. *E. coli* was found in 3 of 30 (10%) of the samples analyzed and the counts were not significantly different among the treatments (p > 0.05). Foster et al (1977) isolated *E. coli* from 1.9 - 11.9 % of the fresh seafood products and the counts ranged from <3 - 1100 MPN/g. Abeyta (1983) reported *E. coli* counts of 3.6 - 210 MPN/g in fish products from retail seafood markets in Seattle, WA. Fernandes et al. (1997) reported *E. coli* counts ranging from 0.0 - 75.0 CFU/g depending on the season, with higher counts occurring during summer. McAdams (1996) reported counts ranging from 0.0 - 4.98 MPN/g. Nair and Nair (1988) detected *E. coli* in 50% of the freshwater fish sampled from a reservoir. Samadpour et al. (1994) surveyed the seafood grocery stores in Seattle, WA and isolated the organism from 10% (6/62) of fish samples.

ICMSF recommendations for *Escherichia coli* in freshwater fish products are m = 4 MPN/g and M = 400 MPN/g (Abeyta, 1983). None of the fresh whole pacu sampled

in this study had counts higher than "M" and hence were concluded to be of acceptable microbial quality.

Bacterial Quality of Water Used for Rearing Pacu

The general microbiological quality of water used for growing the pacu is given in table 2. Diets and time significantly influenced the indicative bacterial quality (p < 0.05). *Aerobic Plate Counts*

The counts for heterotrophes differed significantly among the treatments with time (p < 0.05). The P36 diet tanks had the lowest counts (2.91 - 2.66 log CFU/g) through out the growth period. The P32 diet tanks had significantly lower counts (3.07 log CFU/g) and zucchini had significantly higher counts (p < 0.05) at 18 weeks.

Psychrotrophic Plate Counts

The psychrotrophic counts were significantly different among the treatments (p < 0.05). Significantly lower counts were (2.61 - 3.41 log CFU/g) observed in the P36 tanks. In the other two treatments, the counts were higher at 12 weeks and lower at 18 weeks.

Total Coliforms and Fecal coliforms

Diet and time significantly influenced the total coliform counts (p < 0.05). The P32 diet tanks had significantly higher counts (2.18 log MPN/100 ml) at 6 weeks than at 12 and 18 weeks (1.50 and 1.55 log MPN/100 ml respectively). In the P36 tanks, the counts gradually increased with time. Significantly lower counts (1.39 log MPN/100 ml) were observed at 12 weeks in the zucchini tanks. Fecal coliform counts differed

significantly among the treatments and with time (p < 0.05). The counts remained consistent in the P32 tanks (1.84 - 1.86 log MPN/100 ml), whereas, they reduced significantly with time in P36 tanks (p < 0.05). In zucchini tanks, the counts were significantly lower (2.05 log MPN/100 ml) in the middle of the growth period.

Araujo et al. (1989) studied the correlation between the presence of mesophilic aeromonads and the number of fecal coliforms in fresh waters and reported numbers of fecal coliforms ranging from 9 - 10^7 CFU/ml. Larson et al. (1980) reported counts ranging from <200 - 5000 MPN/100 ml. Lopez - Torres et al. (1987) analyzed the bacterial quality of a tropical rain forest watershed in Puerto Rico and reported counts ranging from 5 ± 5 - 2196 ± 2748 CFU/100 ml. They reported *Klebsiella pneumoniae* counts ranging from 5 - 52 CFU/100 ml.

No bacterial human pathogens - *Listeria monocytogenes, Salmonella spp., Yersinia enterocolitica*, and *Clostridium botulinum* were isolated from any of the fish or water samples analyzed. It is possible that some of the *E. coli* isolated could have been a human pathogen. However, none were of the serotype O157:H7.

Listeria monocytogenes has been isolated from a variety of seafood products such as raw and frozen fish, shellfish, smoked, fermented and marinated fish (Dillon, 1994; Garland, 1995). Farber (1991) reported the incidence levels of the organism in fishery products at the wholesale and retail levels to be 9 - 50% and 20 - 25% respectively. Hartemink and Georgsson (1991) noted high incidence levels of *Listeria monocytogenes* in seafood salads and raw fish. McAdams (1996) compared the incidence levels of bacterial pathogens in aquacultured rainbow trout and reported incidence levels ranging from 20 - 90%. However, the *Listeria* counts reported were low ranging from 0.35 - 4.83 MPN/g.

The incidence of *Salmonella* has frequently been reported from natural and polluted waters. Arvanitidou et al. (1995) isolated *Salmonella* from 19.8% surface, 16.7% lake and 20.3% river water samples. Phelps (1991) isolated *Salmonella* from a wastewater aquaculture system. *Salmonella* was isolated from 21.3% (21/90) of the wastewater and 3.2% (2/61) of the fish samples. Nambiar and Iyer (1991) reported incidence levels of 5.76% and 8.66% for fresh and frozen fish.

Clostridium botulinum is widely distributed throughout the land and coastal waters of North America. Its incidence has been reported from numerous sources such as soil, marine and lake sediments, a variety of foods - fruits and vegetables, meats and poultry, fish, shellfish, and smoked fish. The organism could infect and produce disease symptoms in fish as well as humans. Eklund et al. (1991) reported two botulism outbreaks in juvenile salmon at the Washington State Elokomin Hatchery and Oregon State Klaskanine Hatchery which resulted in a loss of 1.25 million juvenile fish. Cann et al. (1975) isolated the organism from trout farms in Switzerland and reported incidence levels ranging from 2.9 - 100%. Venkateswaran et al. (1989) reported *Clostridium botulinum* from 28% (10/36) of the sediment samples and 9.4% of the fish samples from the Ohta River, Japan. McAdams (1996) isolated the organism from aquacultured

rainbow trout and the incidence levels ranged from 45 - 95%, however, very low counts (0.46 - 2.33 MPN/g) were reported.

Yersinia enterocolitica is frequently encountered in terrestrial and freshwater ecosystems, animals, foods, and water. Abeyta (1983) reported *Yerisinia enterocolitica* from 3.8% of the fresh seafood samples examined in retail markets in Seattle, WA. Arvanitidou (1995) reported *Yersinia intermedia* from river (10.8%) and lake (8.3%) water samples in Northern Greece. Aleksic et al. (1987) isolated *Yersinia rhodei* from surface waters in Germany. *Yersinia aldovae* has been reported in drinking and river water samples from Norway and Czechoslovakia. The incidence of *Yersinia spp*. has also been reported from smoked and fresh fish samples. Bruce and Drysdale (1988) isolated *Yersinia enterocolitica* O:14 and O:15 from 50% of fresh salmon, 47.5% of smoked salmon, and 55.6% of rainbow trout.

Escherichia coli O157:H7 has been isolated from a variety of dairy and meat food products, but fishery products have never been reported as carriers of the pathogen.

CONCLUSIONS

Though pacu in this study fed commercial feeds (P32 and P36) were found to have an unacceptable bacterial quality, it was felt that the increased fecal coliform counts were due to the pacu being maintained in an aquaculture system without an adequate filtration system. The fecal coliform counts were high because tanks used for raising pacu were not fitted with filters. High nutritive value of these feeds coupled with increasing mass of fish per unit area deteriorated the bacterial quality of water and hence the microbiological quality of fish. Since, the microbial quality of fish is a reflection of its environment, filtration and high water turnover would help alleviate the bacterial problem. However, fecal coliforms in fishery products is considered a low, indirect hazard and thermal processing of product before consumption would significantly reduce the microbial hazard.

REFERENCES

Abeyta, Jr., C. 1983. Bacteriological Quality of Fresh Seafood Products from Seattle Retail Markets. J. Food Prot. 46(10): 901 - 909.

Ahmed, F. E. 1992. Review: Assessing and Managing Risk Due to Consumption of Seafood Contaminated with Micro - organisms, Parasites, and Natural Toxins in the US. Intl. J. Food Sci. Tech. 27: 243 - 260.

Aleksic, S., A. G. Steigerwalt, J. Bockemuhl, G. P. Huntley - Carter, and D. J.
Brenner. 1987. *Yersinia rohdei spp*. Isolated from Human and Dog Feces and Surface
Water. Int. J. Syst. Bacteriol. 37(4):327 - 332.

Andrews, W. H., G. A. June, P. Sheered, T. S. Hammack, and R. M. Amaguana.
1995. *Salmonella*. In Food and Drug Administration Bacteriological Analytical Manual.
8th ed. Association of Official Analytical Chemists, Arlington, VA.

Anonymous. 1994. Cold Chain Could Yet Bring Warm Comfort to Roller Coaster Salmon Industry. Quick Frozen Foods International (October). 96 - 99.

Araujo, R. M., R. M. Arribas, F. Lucena, and R. Pares. 1989. Relation Between *Aeromonas* and Faecal Coliforms in Fresh Waters. J. Appl. Bacteriol. 67: 213 - 217.

Arvanitidou, M., G. A. Stathopoulos, T. C. Constantinidis, and V.
Katsouyannopoulos. 1995. The Occurrence of *Salmonella, Campylobacter* and *Yersinia spp.* in River and Lake Waters. Microbiol. Res. 150: 153 - 158.

Austin, B. and D. Allen - Austin. 1985. Microbial Quality of Water in Intensive Fish Rearing. J. Appl. Bacteriol. Symposium Supplement. 207S - 226S. Borghetti, J. R. and C. Canzi. 1993. The Effect of Water Temperature and Feeding Rate on the Growth Rate of Pacu (*Piaractus mesopotamicus*) Raised in Cages. Aquaculture. 61: 91 - 101.

Brown, L. D. and C. R. Dorn. 1977. Fish, Shellfish, and Human Health. J. Food Prot. 40(10): 712 - 717.

Bruce, J. and E. M. Drysdale. 1989. *Yersinia enterocolitica* in Fish. J. Appl. Bacteriol. 67(6): xviii - xix.

Cann, D. C., L. Y. Taylor, and G. Hobbs. 1975. The Incidence of *Clostridium botulinum* in Farmed Trout Raised in Great Britain. J. Appl. Bacteriol. 39: 331 - 336.

Carneiro, G. J., F. T. Rantin, T. C. R. Dias, and E. B. Malheiros. 1994a. Interaction Between Temperature and Dietary Levels of Protein and Energy in Pacu (*Piaractus mesopotamicus*).
I. The Effects on Growth and Body Composition.
Abstracts/Aquaculture. 124: 127 - 131.

Carneiro, G. J., F. T. Rantin, T. C. R. Dias, and E. B. Malheiros. 1994b. Interaction Between Temperature and Dietary Levels of Protein and Energy in Pacu (*Piaractus mesopotamicus*). II. Effects on Digestibility of Protein and Mean Transit Time Through the Gastrointestinal Tract. Abstracts/Aquaculture. 124: 127 - 131.

Davis, S. H. and R. Goulder. 1993. Deterioration in Bacteriological Quality of Water Through Fish Farms. J. Appl. Bacteriol. 74: 336 - 339.

Dillon, R., T. Patel, and S. Ratnam. 1994. Occurrence of *Listeria* in Hot and Cold Smoked Seafood Products. Int. J. Food Microbiol. 22: 73 - 77.

Eklund, M. W., M. E. Peterson, F. T. Poysky, L. W. Peck, and J. F. Conrad. 1982.Botulism in Juvenile Coho Salmon (*Oncorhynchus kisutch*) in the United States.Aquaculture. 27(1): 1 - 11.

Enriquez - Ibarra, L. The Use of Pulsed Energy (Flashbast[™]) Technology in the Shelf Life Extension of Selected Marine and Freshwater Fish Species Stored in Ice. 1994. Ph.D. Thesis. Virginia Polytechnic Institute and State University.

Farber, J. M. 1991. Listeria monocytogenes in Fish Products. J. Food Prot. 54(12): 922 - 924.

Fernandes, C. F., G. J. Flick, J. L. Silva, and T. A. McCaskey. 1997. Influence of Processing Schemes on Indicative Bacteria and Quality of Fresh Aquacultured Catfish Fillets. J. Food Prot. 60(1): 54 - 58.

Flick, G. J., G. P. Hong, and G. M. Knobl. 1991. Non - Traditional Methods of Seafood Preservation. Mar. Technol. Soc. J. 25(1): 35 - 43.

Foster, J. F., J. L. Fowler, and J. Dacey. 1977. A Microbial Survey of Various Fresh and Frozen Seafood Products. J. Food Prot. 40(5): 300 - 303.

Garland, C. D. 1995. Microbiological Quality of Aquaculture Products with Special Reference to *Listeria monocytogenes* in Atlantic Salmon. Food Australia. 47(12): 559 - 563.

Greenberg, A. E., L. S. Clesceri, and A. D. Eaton. 1992. Microbiological Examination In Standard Methods for the Examination of Water and Wastewater. pp 45 - 53. Hartemink, R. and F. Georgsson. 1991. Incidence of *Listeria* Species in Seafood and Seafood Salads. Int. J. Food Microbiol. 12: 189 - 196.

Hitchins, A. D. 1995. Listeria monocytogenes. In Food and Drug Administration
Bacteriological Analytical Manual. 8th ed. Association of Official Analytical Chemists,
Arlington, VA.

Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler. 1995. *Escherichia coli* and the Coliform Bacteria. In *Food and Drug Administration Bacteriological Analytical Manual.* 8th ed. Association of Official Analytical Chemists,
Arlington, VA.

International Commission on Microbiological Specifications for Foods of the International Union of Microbiological Societies. (ICMSF). 1986. Sampling Plans for Fish and Shellfish. pp 181 - 196. In *Micro - organisms in Foods, Volume 2. Sampling for Microbiological Analysis: Principles and Scientific Applications.* 2nd ed. University of Toronto Press, Toronto.

Larson, G. L., R. C. Mathews, and J. L. Klausmeyer. 1980. A Survey of Bacterial Water Quality in Abrams Creek, Great Smoky Mountains National Park. J. Tennessee Academy of Science. 55(1): 1 - 6.

Lopez - Torres A. J., T. C. Hazen, and G. A. Toranzos. 1988. Distribution and In Situ Survival and Activity of *Klebsiella pneumoniae* and *Escherichia coli* in a Tropical Rain Forest Watershed. Current Microbiol. 15: 213 - 218.

McAdams, T. J. 1996. The Determination of Microbial Quality and Presence of Pathogens and Chemical Contaminants in Aquacultured Rainbow Trout (*Onchorhynchus* *mykiss*) Fillets and Whole Fish from Different Aquaculture Production Systems. MS Thesis. Virginia Polytechnic Institute and State University.

Merola, N. 1988. Effects of Three Dietary Protein Levels on the Growth of Pacu, *Colossoma mitrei*, Berg, in Cages. Aquacult. Fish Manage. 19: 145 - 150.

Nair, K. K. S. and R. B. Nair. 1988. Bacteriological Quality of Fresh Water Fish from Krishnarajendra Sagar Reservoir. Fishery Technol. 25: 79 - 80.

Nambiar, V. N. and K. Mahadevaiyer. 1991. Distribution of *Salmonella* Serotypes in Fish in Retail Trade in Kochi. Fish. Technol. 28: 33 - 37.

Nedoluha, P. C. and D. Westhoff. 1995. Microbiological Analysis of Striped Bass (*Morone saxatilis*) Grown in Flow - Through Tanks. J. Food Prot. 58(12): 1363 - 1368.

Okafor, N. and B. C. Nzeako. 1985. Microbial Flora of Fresh and Smoked Fish from Nigerian Fresh - water. Food Microbiol. 2(1): 71 - 75.

Okrend, A. J. G., B. E. Rose, and C. P. Lattuada. 1990. Use of 5 - bromo - 4 - chloro - 3 - indoxyl β - D - glucuronide in MacConkey Sorbitol Agar to Aid in the Isolation of *Escherichia coli* O157:H7 from Ground Beef. J. Food Prot. 53: 941 - 943.

Otwell, W. S. 1989. Regulatory Status of Aquacultured Products. Food Technol. 43(11): 103 - 105.

Phelps, R. P. and C. L. Stiebel. 1991. *Salmonella* in a Waste - water Aquaculture System. Bioresource Technol. 37: 205 - 210.

Rawles, D. D., C. F. Fernandes, G. J. Flick, and G. R. Ammermann. 1997.Processing and Food Safety. Ch 13. In *Striped Bass Culture*. R. M. Harrel (Ed.),Elsevier Science Publishers. *In Press*.

Samadpour, M., J. E. Ongerth, J. Liston, N. Tran, D. Nguyen, T. S. Whittam, R. A. Wilson, and P. I. Tarr. 1994. Occurrence of Shiga - Like Toxin - Producing*Escherichia coli* in Retail Fresh Seafood, Beef, Lamb, Pork, and Poultry from Grocery Stores in Seattle, Washington. Appl. Environ. Microbiol. 60(3): 1038 - 1040.

SAS. 1989. SAS/STAT Guide for Personal Computers, Version 6.0 Ed. SAS Institute, Cary, NC.

Solomon, H. M., E. J. Rhodehamel, and D. A. Kautter. 1995. *Clostridium botulinum*. In *Food and Drug Administration Bacteriological Analytical Manual*. 8th ed. Association of Official Analytical Chemists, Arlington, VA.

Stickney, R. R. 1996. Aquaculture from World War II to 1970. In *Aquaculture in the United States: A Historical Survey.* pp 177 - 223. John Wiley and Sons Inc., New York, NY.

Swanson, K. M. J., F. F. Busta, E. H. Peterson, and M. G. Johnson. 1992. Colony Count Methods, pp. 75 - 95. In *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. C. Vanderzant and D. F. Splittoesser (eds.), American Public Health Association, Washington, D. C.

Venkateswaran, K., C. Kiiyukia, M. Takaki, H. Nakano, H. Matsuda, H. Kawakami, and H. Hashimoto. 1989. Characterization of Toxigenic *Vibrios* Isolated from the
Freshwater Environment of Hiroshima, Japan. Appl. Environ. Microbiol. 55(10): 2613 - 2618.

Ward, D. R. 1989. Microbiology of Aquaculture Products. Food Technol. 43(11): 82 - 85.

Weagant, S. D., P. Feng, P. and J. T. Stanfield. 1995. Yersinia enterocolitica and
Yersinia pseudotuberculosis. In Food and Drug Administration Bacteriological Analytical
Manual. 8th ed. Association of Official Analytical Chemists, Arlington, VA.

Diets	Replicat	Ae	erobes	Psych	rotrophes	Total Coliforms		Fecal Coliforms		Escherichia coli	
	e	(log CFU/g)		(log CFU/g)		(log CFU/g)		(log CFU/g)		(CFU/g)	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
32% Protein	1	5.11 ^{a,c}	4.84-5.60	5.45 ^a	4.80-6.81	2.00 ^a	1.68-2.36	3.11 ^a	2.60-3.64	0.20 ^a	0.00-1.00
	2	4.69 ^{a,c}	4.33-5.35	4.87 ^a	4.30-5.38	2.89 ^{a,c}	2.11-3.31	3.70 ^a	3.32-4.00	0.60 ^a	0.00-2.00
36% Protein	1	4.56 ^a	3.72-6.81	3.52 ^b	2.49-4.31	2.53 ^{a,c}	1.98-2.90	3.21 ^a	1.96-3.66	0.00 ^a	0.00-0.00
	2	5.47 ^{a,c}	4.97-6.81	5.63 ^a	5.04-6.81	2.49 ^{a,c}	1.58-3.84	2.92 ^a	1.88-3.92	0.00^{a}	0.00-0.00
Zucchini	1	4.91 a,c	4.49-5.17	5.29 ^a	4.93-5.87	3.58 ^{b,c}	1.79-4.21	2.74 ^{a,c}	1.48-3.90	0.00^{a}	0.00-0.00
	2	5.60 ^{b,c}	4.59-6.81	5.56 ^a	4.51-6.81	2.49 ^{a,c}	0.85-3.74	1.87 ^{b,c}	0.85-3.76	0.00^{a}	0.00-0.00

Table 1. Effect of Diet on the General Microbiological Quality of Pacu (Piaractus mesopotamicus)

Standard errors: aerobes ± 0.33 , psychrotrophes ± 0.33 , total coliforms ± 0.38 , fecal coliforms ± 0.32 , *E. coli* ± 0.21

Means followed by the same letter in columns are not significantly different (p > 0.05)

Diet	Weeks	Aerobes	Psychrotrophes	Colif	<i>E. coli</i> (MPN/100	
		(log CFU/g)	(log CFU/g)	(log MPN		
						ml)
				Total	Fecal	
P32	6	5.82 ^a	5.79 ^{a,c}	2.18 ^{a,e}	1.86 ^a	0.00 ^a
	12	5.36 ^a	6.28 ^c	1.50 ^{b,c}	1.86 ^a	0.00 ^a
	18	3.07 ^b	5.29 ^a	1.55 ^{b,c}	1.84 ^a	0.00 ^a
P36	6	2.91 ^b	2.61 ^b	1.77 ^{a,b,c}	2.69 ^b	0.00 ^a
	12	2.73 ^b	2.94 ^b	2.24 ^{a,e}	2.09 ^a	0.00 ^a
	18	2.66 ^b	3.41 ^b	2.64 ^{e,f}	1.46 °	0.00 ^a
Zucchini	6	4.84 ^a	5.04 ^{a,d}	2.83 ^{d,f}	2.60 ^b	0.00 ^a
	12	5.27 ^a	5.44 ^{a,c}	1.39 ^b	2.05 ^a	0.00 ^a
	18	6.88 ^c	4.31 ^d	3.35 ^d	2.52 ^b	0.00 ^a

Table 2. Effect of Diet on the Indicative Bacteria Quality of Water Used for Rearing Pacu(Piaractus mesopotamicus)

Means with same letter in columns are not significantly different (p > 0.05)

Standard errors: aerobes \pm 0.343, psychrotrophes \pm 0.296, total coliforms \pm 0.178, fecal coliforms \pm 0.128

Pathogen	Whole Pacu			Water Used for Rearing Pacu			
	Zucchini	P32	P36	Zucchini	P32	P36	
Listeria monocytogenes	0/20 ^a	0/20 ª	0/20 ª	0/3 ^a	0/3 ^a	0/3 ^a	
Salmonella spp.	0/20 ^a	0/20 ^a	0/20 ^a	0/3 ^a	0/3 ^a	0/3 ^a	
Clostridium botulinum	0/20 ^a	0/20 ^a	0/20 ^a	0/3 ^a	0/3 ^a	0/3 ^a	
Yersinia enterocolitica	0/20 ^a	0/20 ^a	0/20 ^a	0/3 ^a	0/3 ^a	0/3 ^a	
Escherichia coli O157:H7	0/20 ^a	0/20 ^a	0/20 ^a	0/3 ^a	0/3 ^a	0/3 ^a	

Table 3. Qualitative Levels of Bacterial Pathogens in Aquacultured Pacu (*Piaractus mesopotamicus*) and Water Used for Rearing Pacu.

Means with same letter in rows are not significantly different (p > 0.05).

SECTION IV.

Effect of Production System On The Indicative And Pathogenic Microbiological Quality Of Aquacultured Finfish

by

S. Pullela, C. F. Fernandes, G. J. Flick, G. S. Libey, S. A. Smith, and C. W. Coale.

ABSTRACT

The nature and number of indicator and pathogenic microbes in fish reared using pond and recirculating systems were compared. For each system, 20 samples of rainbow trout (Oncorhynchus mykiss), tilapia (Oreochromis spp.), hybrid striped bass (Morone saxatilis x M. chrysops), and pacu (Piaractus mesopotamicus) were randomly selected, gutted, and microbial analyses performed using AOAC procedures. Five fish were sub sampled and analyzed for indicative microbial quality using 3MTMPetrifilmTM. The general microbial quality differed significantly (p<0.05) among the treatments, except for total coliform counts. Rainbow trout cultured in pond and recirculating systems had lower counts for aerobes $(2.00 - 3.11 \log CFU/g)$ (p<0.05), whereas those reared in a recirculating system had significantly lower psychrotrophic numbers (0.86 - 1.85 log CFU/g). Pacu had the highest fecal coliform counts (2.74 - 3.70 log CFU/g), whereas hybrid striped bass and rainbow trout grown in ponds had lower fecal coliform counts (0.00 - 1.39 log CFU/g). Rainbow trout grown in ponds had significantly higher E. coli counts (0.00 - 2.11 log CFU/g). No human bacterial pathogens -Listeria monocytogenes,

Yerisinia enterocolitica, Escherichia coli O157:H7 and *Salmonella spp.* were isolated. The incidence of *Clostridium botulinum* ranged from 0 - 95%. However, the counts were low ranging from 0.0 - 2.3 MPN/g.

INTRODUCTION

The dietary intake of fish and fishery products has risen nearly 25%, from 5.6 Kg to 7.05 Kg, in the last decade and is expected to reach 9.0 Kg by the end of the century. Seafoods are considered healthy low caloric food sources being low in fat and high in protein. They are also rich sources of minerals and vitamins (Silva and White, 1994; Flick et al., 1991).

The natural fishery resources in the United States are declining and there is a need to considerably increase the seafood production through aquaculture and bridge the widening gap between demand and supply. Currently, aquaculture contributes to 15% of the seafood production in America and provides an year - round supply of safe and high quality fishery products to consumers (Ward, 1989; Fernandes et al., 1997).

Among the aquacultured fish species, channel catfish (*Ictalurus punctatus*) is produced and processed in the largest quantity (440 million pounds) followed by rainbow trout (*Onchorhynchus mykiss*)(53 million pounds) (FAO, 1995). Hybrid striped bass is gaining popularity among consumers for its organoleptic qualities and its present production level is approximately 3 million pounds (Rawles et al., 1997). Tilapia (*Oreochromis spp.*) and grass carp were introduced into the United States to control weeds and are currently being grown as a food source. Pacu (*Piaractus mesopotamicus*) is a freshwater fish inhabiting the Parana - Uruguay river system in South America. The fish is now being intensively aquacultured in south, south - east, and west - central

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regions of Brazil. Fed under intensive systems, pacu can take up to 2 years to attain 1.2 Kg in earthen ponds, but the time to attain the same yields could be reduced by feeding commercially formulated diets.

Fish and fish products have long been considered a vehicle of foodborne bacterial and parasitic infections leading to human illnesses (Brown and Dorn, 1977). The Center for Disease Control and Prevention (CDCP) reported that seafoods account for 5% of the individual cases and 10% of all foodborne illness outbreaks in the United States, with most of the outbreaks resulting from the consumption of raw molluscan shellfish (Otwell, 1989; Ahmed, 1992). In December 1997, the Food and Drug Administration (FDA) will require seafood processors to adopt a quality assurance program based on the hazard analysis and critical control point (HACCP) concept.

Fish are cultured in different systems and their microbiological quality may be significantly influenced by the production system employed. Further, the number and types of bacteria can be affected directly by the environment (water) in which they are grown (Nedoluha and Westhoff, 1995; Ward, 1989). Davis and Goulder (1993) reported that the bacteriological quality of growing water deteriorates when it flows through fish farms. They also noted enhanced microbial metabolism due to enzymatic breakdown of organic polymers to the oligomeric and monomeric molecules which are subsequently utilized by the bacteria. Recirculating (closed) systems have been gaining popularity in recent years with increasing land prices, waters shortages, and governmental regulations on the effluents from aquaculture facilities (Stickney, 1994). Constant and favorable environments for better fish growth can be achieved through these systems, but often have the disadvantage of deteriorating the microbiological quality of water. Such systems tend to accumulate inorganic materials dissolved in water, which eventually serve as an excellent substrate for microbial proliferation (Davis and Goulder, 1993; Austin and Austin, 1985). Hence, careful monitoring of water quality would help in the production of safe and high quality seafood products. In this study, four aquacultured finfish species - rainbow trout (*Onchorhynchus mykiss*), tilapia (*Oreochromis spp.*), hybrid striped bass (*Morone saxatilis* x *M. chrysops*) and pacu (*Piaractus mesopotamicus*) were procured from pond and recirculating systems and their indicative and pathogenic microbiological quality determined.

MATERIALS AND METHODS

Collecting and transporting fish samples

Pacu were fed three different diets - a) commercial 32% Protein (P32) diet (SSC 50822061, Southern States Co - Op Inc., Richmond, VA), b) commercial 36% Protein (P36) diet (SSC 338200, Southern States Co - Op Inc.), and zucchini diet (0.5% Protein) and grown for a period of 24 weeks in a pond aquaculture system. There were 2 tanks

(replicates) per treatment. At the end of 24 weeks, 10 fish per tank (20 per treatment) were randomly selected for microbiological analyses.

Other aquacultured fish were collected from pond and recirculating aquaculture systems located in Massachusetts, Virginia, and West Virginia. From each system, twenty whole fish (rainbow trout, tilapia, and hybrid striped bass) were randomly selected, individually wrapped in sterile polythene bags, placed on ice, and shipped to the laboratory at Department of Food Science and Technology, Virginia Tech, Blacksburg, VA.

Microbiological analyses of fish

Sample Preparation

Upon receipt of the fish, they were aseptically gutted, weighed and an equal volume of cold (0 to 1°C) sterile 15 mM Butterfield's phosphate buffer was added. Each fish was massaged by hand for 2 minutes, removed aseptically with sterile forceps, and the rinse solution was held on ice until microbiological analyses were performed. All microbial analyses were performed within 8 h of receiving the sample.

Aerobic and Psychrotrophic counts

The rinse from the fish was diluted in 15 mM Butterfield's phosphate buffer and appropriate dilutions plated on 3M[™] Petrifilm[™] Aerobic Count (PAC) plates (The 3M Corp., Healthcare Division, Minneapolis, MN) in duplicate. For aerobic and

psychrotrophic counts, the plates were incubated at $35 \pm 2^{\circ}$ C for 48 h and at $21 \pm 2^{\circ}$ C for 96 h, and all red colonies were enumerated (Swanson et al., 1992).

Escherichia coli, Total Coliform and Fecal Coliform counts

The wash from the whole gutted fish was diluted in 15 mM Butterfield's phosphate buffer and appropriate dilutions were enumerated on $3M^{TM}$ PetrifilmTM *E. coli* count plates and coliform count plates (The 3M Corp., Healthcare Division) in duplicate. Each dilution was plated onto four different plates - one set for the total coliforms and the other for fecal coliforms. *E. coli* was also plated in duplicate and incubated at $35 \pm 2^{\circ}C$ for 24 to 48 h (Swanson et al., 1992) and all the blue colonies with gas were counted as *E. coli* cells. The coliform count plates for enumerating total coliforms and fecal coliforms with gas were incubated for 48 h at $35 \pm 2^{\circ}C$ and $44 \pm 2^{\circ}C$ respectively. All the red colonies with gas were counted as coliforms.

Listeria monocytogenes

Twenty - five ml of the rinse solution and 225 ml of University of Vermont Medium (UVM) (Difco Laboratories, Detroit, MI) were added into a sterile stomacher 400 bag. Following mixing, the contents were pre-enriched at $30 \pm 2^{\circ}$ C for 24 h. Following pre-enrichment, 0.1 ml of the UVM culture was pipetted into 10 ml of Fraser's secondary enrichment broth (Difco Labs.), and incubated at $35 \pm 2^{\circ}$ C for 24 and 40 h. Enriched Fraser's broth was streaked onto Modified Oxford Agar (Difco Labs.) and incubated at $35 \pm 2^{\circ}$ C and examined for typical round *Listeria* colonies surrounded by a black zone. Presumptive *Listeria* colonies were confirmed using API *Listeria* strips (bioMe`rieux Vitek, Hazelwood, MO) (Hitchins, 1995). *Listeria monocytogenes* ATCC 7644 was used as a control during the isolating procedure to confirm the microbiological tests.

Salmonella spp.

Twenty - five ml of the rinse solution was added to 225 ml of Lactose Broth (Difco Labs.) and enriched primarily at $35 \pm 2^{\circ}$ C for 24 h. Secondary enrichment was carried out by transferring 1 ml of the incubated sample mixture to 10 ml of secondary enrichment (Selenite Cystine) Broth (Difco Labs.) and the mixture incubated at $35\pm 2^{\circ}$ C for 24 h. To isolate suspect colonies, the culture was streaked onto Xylose Lysine Desoxycholate Citrate (XLD) and Bismuth Sulfite (BS) Agar (Difco Labs.) and incubated at $35\pm 2^{\circ}$ C for 24 h. Suspect colonies were subjected to preliminary biochemical tests using Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) (Difco Labs.). To determine the genus and species, Rapid 20E test kits (bioMe`rieux Vitek) were used (Andrews et al., 1995). *Salmonella typhimurium* ATCC 19585 was carried through the experiments to ensure the accuracy of media reactions.

Yersinia enterocolitica

An enrichment was carried out using 25 ml of the sample and 225 ml of Peptone Sorbitol Bile Broth (PSBB) and incubated at 4°C for 10 days. Following enrichment, the

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enriched broth was transferred to 1.0 ml of 0.5% KOH in 0.5% saline and immediately streaked onto MacConkey's Agar (Difco Labs.) and incubated at $35 \pm 2^{\circ}$ C for 48 h. Pink suspect colonies were transferred to Lysine Arginine Agar, Esculin Bile Agar, and Christensen's Urea Agar (Difco Labs.) slants for preliminary biochemical testing. To determine the genus and species API Rapid 20E test kits were used (bioMe`rieux Vitek) (Weagant et al., 1995). *Yersinia enterocolitica* ATCC 9610 was used as a control to confirm the microbiological tests.

Clostridium botulinum

Enrichment was performed using 25 g equivalents of the sample and 225 g of Cooked Meat Medium and incubated at $35 \pm 2^{\circ}$ C for 7 days. Following preenrichment, 1.0 ml of the enriched medium was treated with 1.0 ml of absolute ethanol for 1 h and subsequently streaked onto Anaerobic Egg Yolk Agar and incubated under anaerobic conditions using anaerobe jars and BBL® GasPak PlusTM anaerobic system envelopes with a paladium catalyst (Becton Dickinson, Cockeysville, MD) at $35 \pm 2^{\circ}$ C for 48 h. The plates were then checked for typical *C. botulinum* colonies and the positive cultures were checked for toxin production using the mouse bioassay. For each sample tested, three mice were used: one receiving a heat treated sample, one receiving a trypsinized sample, and one receiving an untreated sample (Solomon et al., 1995). *Clostridium botulinum* Beluga E was used as a control during toxicity testing.

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E. coli O157: H7

The fish sample (25.0 g) was enriched in modified EC Broth (225 ml) (Difco Labs.) containing 20 µg/ml of filtered sterilized (0.2 µm: Nalgene Co., Rochester, NY) sodium novobiocin (Sigma Chemical Co., St. Louis, MO) (Okrend et al., 1990). The sample was incubated at $37 \pm 2^{\circ}$ C for 6 h. The enriched broth was diluted appropriately and plated on $3M^{TM}$ PetrifilmTM *E. coli* plates (The 3M Corp. Minneapolis, MN). The *E. coli* plates were incubated at $35 \pm 2^{\circ}$ C for 18 h. Following 18 h of incubation the immunological tests were performed using the $3M^{TM}$ PetrifilmTM test kit HEC for hemorrhagic *E. coli* O157:H7 (The 3M Corp., Health science group) (Okrend et al., 1990). The *E. coli* cell suspension provided in the kit was used as a control.

STATISTICAL DESIGN AND ANALYSES

The indicative bacterial quality of the aquacultured finfish was analyzed as complete randomized design with the production system as the treatment (fish from a system type - four for ponds and three for recirculating). Five fish samples from each system represented the replicates and were analyzed using SAS procedures (SAS, 1989) for aerobic, psychrotrophic, total coliform, fecal coliform and *Escherichia coli* counts. The means of duplicate plate counts for all responses were averaged. Average counts for aerobic, psychrotrophic, total and fecal coliform counts were analyzed following log transformation of the data. The means for *E. coli* were analyzed without the log transformation of the data. The incidence levels of *Clostridium botulinum* was analyzed using a chi-square test. If a pathogen was isolated, the sample was scored as "one" or "1". On the other hand, if a pathogen was not identified the sample was scored "zero" or "0". Twenty samples were examined for each production system. For analyzing the quantitative levels, counts in all 20 fish were averaged and subjected to analysis of variance.

RESULTS AND DISCUSSION

The indicative microbiological quality of all the aquacultured fish sampled is given in table 1. The aquaculture system employed to grow fish significantly influenced the indicative microbiological quality (p < 0.05).

Aerobic Plate Counts

The aquaculture system employed significantly influenced the aerobic plate counts (p < 0.05). Pacu had the highest mean counts for aerobes (4.95 log CFU/g) whereas the lowest counts were observed in rainbow trout grown in a recirculating systems (2.25 log CFU/g). Pond and recirculating systems significantly influenced the counts for aerobes in tilapia, but no differences were observed in samples of hybrid striped bass and rainbow trout. Lower counts were observed in tilapia grown in a recirculating system. Nedoluha and Westhoff (1995) determined the microbiological quality of striped bass (*Morone saxatilis*) in flow - through tanks and found counts ranging from 3 - 6 log CFU/cm². Fernandes et al. (1997) analyzed the influence of size of processing operations and season on the bacterial quality of pond cultured catfish and
reported counts ranging from 3.0 - 6.0 log CFU/g. Enriquez et al. (1994) determined the microbial quality of fillets obtained from hybrid striped bass grown in a recirculating aquaculture system and reported counts ranging from 1.9 - 2.8 log CFU/g. McAdams (1996) compared the effect of rearing aquaculture system on the microbiological quality of rainbow trout and reported counts ranging from 3.0 - 6.0 log CFU/g.

The aerobic counts in this study compare well with the numbers previously reported in the literature. Abeyta (1983) in a survey of fresh seafood products from retail markets in Seattle, WA noted that the counts for aerobes ranged from 4.0 - 7.0 log CFU/g. Okafor and Nzeako (1985) in a survey of freshwater fish in Nigeria obtained mean counts of 4×10^5 /g. Nair and Nair (1988) determined the bacteriological quality of freshwater fish from a reservoir and found counts ranging from 6.4×10^4 to 4.8×10^5 CFU/g. However, these high numbers of bacteria are present mostly in the non - edible portions of the fish viz., surface slime, gills, and intestines which are separated during manual or mechanical processing of finfish and thus, have an insignificant influence on the quality of edible muscle.

The International Commission on Microbiological Specification for Foods (ICMSF, 1986) limits for freshwater fish products for standard plate count are $m = 5 \times 10^5$ and $M = 10^7$. Since the counts for all the fish sampled were within these limits, the fish were concluded to be of acceptable microbial quality.

Psychrotrophic Plate Counts

Aquaculture rearing system significantly influenced the psychrotrophic plate counts (p < 0.05). As in the case of aerobes, highest counts for psychrotrophes were found in pacu and the lowest counts (1.42 log CFU/g) in rainbow trout grown in a recirculating system. Pond and recirculating systems significantly influenced the psychrotrophic counts in rainbow trout (p < 0.05), but had no influence in hybrid striped bass and tilapia (p > 0.05). These results are consistent with the results reported earlier in the literature. Fernandes et al. (1997) analyzed the influence of size of processing operations and season on the bacterial quality of pond cultured catfish and reported counts ranging from 3.0 - 6.5 log CFU/g. Enriquez et al. (1994) determined the microbial quality of hybrid striped bass fillets obtained from a recirculating aquaculture system and reported counts ranging from 1.8 - 2.5 log CFU/g. McAdams (1996) compared the effect of rearing aquaculture system on the microbiological quality of rainbow trout and reported counts ranging from 3.0 - 6.0 log CFU/g.

Total Coliforms

The aquaculture system used did not influence the total coliform counts (p > 0.05). The lowest total coliform counts were observed in rainbow trout grown in ponds (1.61 log CFU/g) and the highest counts were seen in tilapia from ponds (2.84 log CFU/g). Fernandes et al. (1997) analyzed the influence of size of processing operations and season on the bacterial quality of catfish and reported counts ranging from 0.8 - 3.2 log CFU/g.

Enriquez et al. (1994) determined the microbial quality of hybrid striped bass fillets and reported counts ranging from 0.6 - 2.0 log CFU/g. McAdams (1996) compared the effect of rearing aquaculture system on the microbiological quality of rainbow trout. The total coliform counts, as determined by the most probable number (MPN) method, ranged from 3.09 - 543 MPN/g. Abeyta (1983) reported average coliform counts of 296 MPN/g in seafood products from retail markets. Foster et al. (1977) analyzed the microbial quality of fresh and frozen seafood products and reported mean coliform counts ranging from 7.8 - 4.8×10^3 MPN/g in fresh seafood products. Nair and Nair (1988) reported coliform counts ranging form 120 - 9.2×10^3 /g.

Fecal Coliforms

The aquaculture system employed significantly influenced the fecal coliform counts (p < 0.05). The lowest counts were observed in hybrid striped bass grown in ponds (0.36 log CFU/g) and the highest counts (3.14 log CFU/g) in pacu. Hybrid striped bass grown in ponds had significantly lower counts than their counterparts in recirculating systems, whereas the reverse trend was observed in tilapia. Tilapia grown in a recirculating system had a lower fecal coliform count whereas pond cultured tilapia had a higher fecal coliform count.

The ICMSF fecal coliform counts limits for freshwater fish products are m = 4 and M = 400. All the pacu samples analyzed had counts higher than 400/g and hence, were concluded to be of unacceptable quality. Tilapia grown in both recirculating and

pond culture systems had 40% (2 of 5) of the samples above the specified limits and 20% (1 of 5) of hybrid striped bass grown in a recirculating system had counts higher than 400/g. Fecal coliforms are considered to be a low, indirect health hazard, though cooking the product before consumption significantly reduces the hazard. The maximum allowable number of defectives (c) is 3. None of the fish samples, except pacu, exceeded the limit (c=3) and were concluded to be of acceptable quality.

Escherichia coli

Pond and recirculating aquaculture systems significantly influenced the *Escherichia coli* counts. The highest counts (1.00 CFU/g) were observed in pond cultured rainbow trout (p < 0.05). Fernandes et al. (1997) reported *E. coli* counts ranging from 0.0 - 75.0 CFU/g depending on the season, with higher counts during summer. McAdams (1996) reported counts ranging from 0.0 - 4.98 MPN/g. Foster et al. (1977) isolated *E. coli* from 1.9 - 11.9 % of the fresh seafood products and the counts ranged from < 3 - 1100 MPN/g. Abeyta (1983) reported *E. coli* counts ranging from 3.6 - 210.0 MPN/g in fish products from retail seafood markets in Seattle, WA. Nair and Nair (1988) detected *E. coli* in 50% of the freshwater fish sampled from a reservoir.

Pathogenic Microbial Quality Of Aquacultured Fish

During the study, *Listeria monocytogenes*, *Salmonella spp.*, *Yerisinia enterocolitica*, and *Escherichia coli* O157:H7 were not isolated from any of the aquacultured fish sampled.

Listeria monocytogenes has been isolated from a variety of seafood products such as raw and frozen fish, shellfish, smoked, fermented and marinated fish (Dillon, 1994; Garland, 1995). Farber (1991) reported the incidence levels of the organism in fishery products at the wholesale and retail levels to be 9 - 50% and 20 - 25% respectively. Hartemink and Georgsson (1991) noted high incidence levels of *Listeria monocytogenes* in seafood salads and raw fish. McAdams (1996) compared the incidence levels of bacterial pathogens in aquacultured rainbow trout and reported incidence levels ranging from 20 -90%, however, the *Listeria* counts reported were low, ranging from 0.35 - 4.83 MPN/g.

Incidence of *Salmonella* has frequently been reported from natural and polluted waters. Arvanitidou et al. (1995) isolated *Salmonella* from 19.8% surface water, 16.7% lake ,and 20.3% river water samples. Phelps (1991) isolated *Salmonella* from a wastewater aquaculture system. *Salmonella* was isolated from 21.3% (21/90) of the wastewater and 3.2% (2/61) of the fish samples. Nambiar and Iyer (1991) reported incidence levels of 5.76% and 8.66% for fresh and frozen fish.

Yersinia enterocolitica is frequently encountered in terrestrial and freshwater ecosystems, animals, foods, and water. Abeyta (1983) reported *Yersinia enterocolitica* from 3.8% of the fresh seafood samples examined in retail markets in Seattle, WA. Arvanitidou (1995) reported the presence of *Yersinia intermedia* in river (10.8%) and lake (8.3%) water samples in Northern Greece. Aleksic et al. (1987) isolated *Yersinia rhodei* from surface waters in Germany. *Yersinia aldovae* was reported from drinking and river water samples from Norway and Czechoslovakia. The incidence of *Yersinia spp.* has been reported from smoked and fresh fish samples. Bruce and Drysdale (1988) isolated *Yersinia enterocolitica* O:14 and O:15 from 50% of fresh salmon, 47.5% of smoked salmon, and 55.6% of rainbow trout samples. *Escherichia coli* O157:H7 has been isolated from a variety of dairy and meat food products, but fishery products have never been reported as carriers of the pathogen.

Clostridium botulinum was isolated from all the aquacultured fish sampled, except for pacu and tilapia grown in a recirculating system. The highest incidence (95%) of the organism was found in pond raised tilapia. However, the numbers of the pathogen were very low, with the mean counts ranging from 1.31 - 2.30 MPN/g. Clostridium botulinum is widely distributed throughout the land and coastal waters of North America. Its incidence has been reported from numerous sources such as soil, marine and lake sediments, a variety of foods - fruits and vegetables, meats and poultry, fish, shellfish, and smoked fish. The organism could infect and produce disease symptoms in fish as well as humans. Eklund et al. (1991) reported two botulism outbreaks in juvenile salmon at the Washington State Elokomin Hatchery and Oregon State Klaskanine Hatchery which resulted in a loss of 1.25 million juvenile fish. Cann et al.(1975) isolated the organism from trout farms in Switzerland and reported incidence levels ranging from 2.9 - 100%. Venkateswaran et al. (1989) reported *Clostridium botulinum* from 28% (10/36) of the sediment samples and 9.4% of the fish samples from Ohta River, Japan. McAdams

(1996) isolated the organism from aquacultured rainbow trout and the incidence levels ranged from 45 - 95%, however, very low counts (0.46 - 2.33 MPN/g) were reported.

CONCLUSIONS

Although the production systems significantly influenced the microbiological quality of aquacultured fish, no conclusions could be drawn as to which system was preferred since the responses were species specific. However, it should be noted that fish aquacultured in all the systems, with the single exception of pacu, met the ICMSF criterion and were concluded to be of good microbial quality. Pacu were found to have an unacceptable bacterial quality as the fecal coliform counts were in excess of the limits specified by the ICMSF. However, this was probably due to the aquaculture system maintaining the pacu not being fitted with an adequate filtration system. Since, the microbial quality of fish is a reflection of its environment, filtration and high water turnover would help alleviate the bacterial problem. However, fecal coliforms in fishery products is considered a low, indirect hazard and thermal processing of product before consumption would significantly reduce the microbial hazard.

REFERENCES

Abeyta, Jr., C. 1983. Bacteriological Quality of Fresh Seafood Products from Seattle Retail Markets. J. Food Prot. 46(10): 901 - 909.

Ahmed, F. E. 1992. Review: Assessing and Managing Risk Due to Consumption of Seafood Contaminated with Micro - organisms, Parasites, and Natural Toxins in the US. Intl. J. Food Sci. Tech. 27: 243 - 260.

Aleksic, S., A. G. Steigerwalt, J. Bockemuhl, G. P. Huntley - Carter, and D. J.Brenner. 1987. *Yersinia rohdei spp*. Isolated from Human and Dog Feces and SurfaceWater. Int. J. Syst. Bacteriol. 37(4): 327 - 332.

Andrews, W. H., G. A. June, P. Sheered, T. S. Hammack, and R. M. Amaguana.
1995. Salmonella. In Food and Drug Administration Bacteriological Analytical Manual.
8th ed. Association of Official Analytical Chemists, Arlington, VA.

Austin, B. and D. Allen - Austin. 1985. Microbial Quality of Water in Intensive Fish Rearing. J. Appl. Bacteriol. Symposium Supplement. 2078 - 226S.

Boyd, L. C., D. P. Green, and L. A. LePors. 1992. Quality Changes of Pond - raised Hybrid Striped Bass During Chillpack and Refrigerated Storage. J. Food Sci. 57(1): 59 -62.

Brown, L. D. and C. R. Dorn. 1977. Fish, Shellfish, and Human Health. J. Food Prot. 40(10): 712 - 717.

Bruce, J. and E. M. Drysdale. 1989. *Yersinia enterocolitica* in Fish. J. Appl. Bacteriol. 67(6): xviii - xix.

Cann, D. C., L. Y. Taylor, and G. Hobbs. 1975. The Incidence of *Clostridium botulinum* in Farmed Trout Raised in Great Britain. J. Appl. Bacteriol. 39: 331 - 336.

Davis, S. H. and R. Goulder. 1993. Deterioration in Bacteriological Quality of Water Through Fish Farms. J. Appl. Bacteriol. 74: 336 - 339.

Dillon, R., T. Patel, and S. Ratnam. 1994. Occurrence of *Listeria* in Hot and Cold Smoked Seafood Products. Int. J. Food Microbiol. 22: 73 - 77.

Eklund, M. W., M. E. Peterson, F. T. Poysky, L. W. Peck, and J. F. Conrad. 1982.Botulism in Juvenile Coho Salmon (*Oncorhynchus kisutch*) in the United States.Aquaculture. 27(1): 1 - 11.

Enriquez - Ibarra, L. The Use of Pulsed Energy (Flashbast[™]) Technology in the Shelf Life Extension of Selected Marine and Freshwater Fish Species Stored in Ice. 1994. Ph.D. Thesis. Virginia Polytechnic Institute and State University.

Farber, J. M. 1991. *Listeria monocytogenes* in Fish Products. J. Food Prot. 54(12):922 - 924.

Fernandes, C. F., G. J. Flick, J. L. Silva, and T. A. McCaskey. 1997. Influence of Processing Schemes on Indicative Bacteria and Quality of Fresh Aquacultured Catfish Fillets. J. Food Prot. 60(1): 54 - 58.

Flick, G. J., G. P. Hong, and G. M. Knobl. 1991. Non - Traditional Methods of Seafood Preservation. Mar. Technol. Soc. J., 25(1): 35 - 43.

Food and Agriculture Organization. 1995. Aquaculture Production Statistics. Fishery Information, Data and Statistics Service; Food and Agriculture Organization of the United Nations, Rome.

Foster, J. F., J. L. Fowler, and J. Dacey. 1977. A Microbial Survey of Various Fresh and Frozen Seafood Products. J. Food Prot. 40(5): 300 - 303.

Garland, C. D. 1995. Microbiological Quality of Aquaculture Products with Special Reference to *Listeria monocytogenes* in Atlantic Salmon. Food Australia. 47(12): 559 - 563.

Hartemink, R. and F. Georgsson. 1991. Incidence of *Listeria* Species in Seafood and Seafood Salads. Int. J. Food Microbiol. 12: 189 - 196.

Hitchins, A. D. 1995. Listeria monocytogenes. In Food and Drug AdministrationBacteriological Analytical Manual. 8th ed. Association of Official Analytical Chemists,Arlington, VA.

Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler. 1995. *Escherichia coli* and the Coliform Bacteria. In *Food and Drug Administration Bacteriological Analytical Manual.* 8th ed. Association of Official Analytical Chemists,
Arlington, VA.

International Commission on Microbiological Specifications for Foods of the International Union of Microbiological Societies. (ICMSF). 1986. Sampling Plans for Fish and Shellfish. pp 181 - 196. In *Micro - organisms in Foods, Volume 2. Sampling for Microbiological Analysis: Principles and Scientific Applications.* 2nd ed. University of Toronto Press, Toronto. Larson, G. L., R. C. Mathews, and J. L. Klausmeyer. 1980. A Survey of Bacterial Water Quality in Abrams Creek, Great Smoky Mountains National Park. J. Tennessee Academy of Science. 55(1): 1 - 6.

Lopez - Torres, T. C. Hazen, and G. A. Toranzos. 1988. Distribution and In Situ Survival and Activity of *Klebsiella pneumoniae* and *Escherichia coli* in a Tropical Rain Forest Watershed. Current Microbiol. 15: 213 - 218.

McAdams, T. J. 1996. The Determination of Microbial Quality and Presence of Pathogens and Chemical Contaminants in Aquacultured Rainbow Trout (*Onchorhynchus mykiss*) Fillets and Whole Fish from Different Aquaculture Production Systems. MS Thesis. Virginia Polytechnic Institute and State University.

Nair, K. K. S. and R. B. Nair. 1988. Bacteriological Quality of Fresh Water Fish from Krishnarajendra Sagar Reservoir. Fishery Technol. 25: 79 - 80.

Nambiar, V. N. and K. Mahadevaiyer. 1991. Distribution of *Salmonella* Serotypes in Fish in Retail Trade in Kochi. Fish. Technol. 28: 33 - 37.

Nedoluha, P. C. and D. Westhoff. 1995. Microbiological Analysis of Striped Bass (*Morone saxatilis*) Grown in Flow - Through Tanks. J. Food Prot. 58(12): 1363 - 1368.

Okafor, N. and B. C. Nzeako. 1985. Microbial Flora of Fresh and Smoked Fish from Nigerian Fresh - water. Food Microbiol. 2(1): 71 - 75.

Okrend, A. J. G., B. E. Rose, and C. P. Lattuada. 1990. Use of 5 - bromo - 4 - chloro - 3 - indoxyl β - D - glucuronide in MacConkey Sorbitol Agar to Aid in the Isolation of *Escherichia coli* O157:H7 from Ground Beef. J. Food Prot. 53: 941 - 943.

Otwell, W. S. 1989. Regulatory Status of Aquacultured Products. Food Technol. 43(11): 103 - 105.

Phelps, R. P. and C. L. Stiebel. 1991. *Salmonella* in a Waste - water Aquaculture System. Bioresource Technol. 37: 205 - 210.

Rawles, D. D., C. F. Fernandes, G. J. Flick, and G. R. Ammermann. 1997.Processing and Food Safety. Ch 13. In *Striped Bass Culture*. R. M. Harrel (Ed.),Elsevier Science Publishers. *In Press*.

SAS. 1989. SAS/STAT Guide for Personal Computers, Version 6.0 Ed. SAS Institute, Cary, NC.

Silva, J. L. and T. D. White. 1994. Bacteriological and Color Changes in Modified Atmosphere - packaged Refrigerated Channel Catfish. J. Food Prot. 57(8): 715 - 719.

Solomon, H. M., E. J. Rhodehamel., and D. A. Kautter. 1995. *Clostridium botulinum*. In *Food and Drug Administration Bacteriological Analytical Manual*. 8th ed. Association of Official Analytical Chemists, Arlington, VA.

Stickney, R. R. 1994. Introduction. pp 1 - 44. In *Principles Of Aquaculture*. John Wiley and Sons Inc., New York, NY.

Stickney, R. R. 1996. Aquaculture from World War II to 1970. In *Aquaculture in the United States: A Historical Survey*. pp 177 - 223. John Wiley and Sons Inc., New York, NY.

Swanson, K. M. J., F. F. Busta, E. H. Peterson, and M. G. Johnson. 1992. Colony Count methods, pp. 75 - 95. In *Compendium of Methods for the Microbiological*

Examination of Foods. 3rd ed. C. Vanderzant and D. F. Splittoesser (eds.), American Public Health Association, Washington, D. C.

Venkateswaran, K., C. Kiiyukia, M. Takaki, H. Nakano, H. Matsuda, H. Kawakami, and H. Hashimoto. 1989. Characterization of Toxigenic *Vibrios* Isolated from the Freshwater Environment of Hiroshima, Japan. Appl. Environ. Microbiol. 55(10): 2613 - 2618.

Ward, D. R. 1989. Microbiology of Aquaculture Products. Food Technol. 43(11): 82 - 85.

Weagant, S. D., P. Feng, P., and J. T. Stanfield. 1995. Yersinia enterocolitica and Yersinia pseudotuberculosis. In Food and Drug Administration Bacteriological Analytical Manual. 8th ed. Association of Official Analytical Chemists, Arlington, VA.

Table 1. Effect of Aquaculture Production System on the Indicative Microbiological Quality of Different Fish Species - Hybrid Striped Bass (*Morone saxatilis* \times *M. chrysops*), Rainbow Trout (*Onchorhynchus mykiss*), Tilapia (*Oreochromis spp.*) and Pacu (*Piaractus mesopotamicus*)

Fish Species	Aquaculture System	Aerobes	Psychrotrophes	Total Coliforms	Fecal Coliforms	Escherichia	
		log CFU/g	log CFU/g	log CFU/g	log CFU/g	<i>coli</i> CFU/g	
Hybrid Striped Bass	Non - recirculating	3.48 ^a	3.51 ^a	2.41 ^a	0.36 ^a	0.00 ^a	
Hybrid Striped Bass	Recirculating	3.62 ^a	3.92 ^{a, c}	1.71 ^a	2.19 ^{b, f}	0.00 ^a	
Rainbow Trout	Non - recirculating	2.76 ^{c, d, f}	3.56 ^a	1.61 ^a	0.77 ^{a, d, e}	1.00 ^b	
Rainbow Trout	Recirculating	2.25 ^{b, d}	1.42 ^b	2.08 ^a	1.41 ^{b, d}	0.00 ^a	
Tilapia	Non - recirculating	4.41 ^e	4.42 ^{a, c}	2.84 ^a	2.81 ^{c, f}	0.00 ^a	
Tilapia	Recirculating	3.09 ^{a, f}	3.45 ^a	2.33 ^a	1.14 ^{b, e}	0.45 ^a	
Pacu	Non - recirculating	4.95 ^e	4.95 ^c	2.67 ^a	3.14 ^c	0.16 ^a	

Means with same letter in columns are not significantly different (p > 0.05)

Standard Errors: aerobes \pm 0.189, psychrotrophes \pm 0.418, total coliforms \pm 0.432, fecal coliforms \pm 0.324, and *E. coli* \pm 0.162

Fish Species	Aquaculture System	Listeria	Salmonella	Clostridium	Yersinia	Escherichia
		monocytogenes	spp.	botulinum	enterocolitica	coli 0157:H7
Hybrid Striped Bass	Non - recirculating	0/20 (0%) ^a	0/20 (0%) ^a	3/20 (15%) ^b	0/20 (0%) ^a	0/20 (0%) ^a
Hybrid Striped Bass	Recirculating	0/20 (0%) ^a	0/20 (0%) ^a	13/20 (65%) ^c	0/20 (0%) ^a	$0/20~(0\%)^{a}$
Rainbow Trout	Non - recirculating	0/20 (0%) ^a	$0/20~(0\%)^{a}$	10/20 (50%) ^c	0/20 (0%) ^a	0/20 (0%) ^a
Rainbow Trout	Recirculating	0/20 (0%) ^a	$0/20~(0\%)^{a}$	6/20 (30%) ^b	0/20 (0%) ^a	0/20 (0%) ^a
Tilapia	Non - recirculating	0/20 (0%) ^a	$0/20~(0\%)^{a}$	19/20 (95%) ^d	0/20 (0%) ^a	0/20 (0%) ^a
Tilapia	Recirculating	0/20 (0%) ^a	$0/20~(0\%)^{a}$	$0/20~(0\%)^{a}$	0/20 (0%) ^a	0/20 (0%) ^a
Pacu	Non - recirculating	0/20 (0%) ^a	0/20 (0%) ^a	$0/20~(0\%)^{a}$	0/20 (0%) ^a	0/20 (0%) ^a

Table 2. Effect of Production System on the Incidence Levels of Bacterial Human Pathogens in Aquacultured Finfish

Means with same letter in columns are not significantly different (p>0.05)

Fish Species	Production System	Mean (MPN/g)	Range (MPN/g)
Hybrid Striped Bass	Non - recirculating	0.35 ^a	0.0 - 2.3
Hybrid Striped Bass	Recirculating	1.12 ^{b, c}	0.0 - 2.3
Rainbow Trout	Non - recirculating	1.01 ^{b, d}	0.0 - 2.3
Rainbow Trout	Recirculating	0.62 ^{a, c, d}	0.0 - 2.3
Tilapia	Non - recirculating	1.25 ^b	0.0 - 2.3
Tilapia	Recirculating	0.00 ^e	0.0 - 0.0
Pacu	Non - recirculating	0.00 ^e	0.0 - 0.0

Table 3. Quantitative Levels of Clostridium botulinum in Aquacultured Finfish

Means with same letter in columns are not significantly different (p > 0.05)

SECTION V.

Comparison of Aquacultured Pacu Fillets with other Aquacultured Fish Fillets Using Subjective and Objective Sensorial Traits

by

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ABSTRACT

Pacu (Piaractus mesopotamicus) were raised in 1165L fiberglass tanks for 24 weeks, processed into skinless fillets and their sensory qualities compared. At the end of 24 weeks, the pacu were purged for a period of 0, 2, 4, and 6 days and the effect of depuration procedures on preference was determined using a simple ranking test. Pacu with the highest preference was compared with other aquacultured fish - channel catfish (Ictalurus punctatus), rainbow trout (Onchorhynchus mykiss), tilapia (Oreochromis spp.), and hybrid striped bass (Morone saxatilis x M. chrysops) for differences and preferences using triangle and simple ranking test respectively. The samples were cooked using the National Marine Fisheries Service (NMFS) procedure (350°F for 20 minutes) and served under red lighting to mask the color differences among fish fillets. The influence of cooking on the Hunter color readings (L, a, and b values) of ground fish fillets was measured using a Minolta chromometer. Purging significantly influenced the preference for pacu (p < 0.05). Pacu purged for 6 days had the highest preference, however, there was statistically no significant difference in preference for pacu purged for 2, 4, and 6

days. Pacu was not significantly different in flavor from hybrid striped bass, tilapia and rainbow trout (p > 0.05). However, there were flavor differences between pacu and catfish (p < 0.05). There were no significant differences in preferences for all the fish (p > 0.05). Cooking increased the "L" value and decreased "a" and "b" values.

Keywords: sensory quality, aquaculture, fish, sensory traits

INTRODUCTION

Color and flavor are important sensory attributes which influence the consumer's decision to purchase or consume food products. Color is a phenomenon which involves physical and psychological components: the perception by the eye of light of wavelengths 400 - 500 nm (blue), 500 - 600 nm (green and yellow), and 600 - 800 nm (red). Flavor is defined as the impressions perceived via the chemical senses from a product in the mouth. Flavor includes olfactory perceptions (aromatics), gustatory perceptions (taste), and chemical feeling factors (Meilgaard et al., 1991).

Among the aquacultured finfish species in the United States, channel catfish (*Ictalurus punctatus*) is produced and processed in the largest quantities (Fernandes et al., 1997). However, other species such as rainbow trout (*Onchorhynchus mykiss*), tilapia (*Oreochromis spp.*), and hybrid striped bass (*Morone saxatilis* x *M. chrysops*) are gaining popularity among consumers (Nedoluha and Westhoff, 1995; Tave et al., 1990; No and Storebakken, 1991).

Most consumers prefer fresh fish over frozen fish. Freshness in seafood products is assessed in terms of appearance and odor. Further, consumers often equate freshness with color (Peavey et al., 1994). There is a considerable variability in color within different parts of the fish and the intensity of the color is dependent on the type and quantity of the pigment present in their body. Additionally, the color of the fish is influenced by the diet, storage, and thermal processing procedures which indirectly affect

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the desirability and overall consumer acceptance as well as the price of the product (No and Storebakken, 1991).

Consumers generally have a preference for white - fleshed fish (Tave et al., 1990). However, this cannot be generalized for all fish species, because consumers have a preference for brightly colored fish in some geographical areas. Aquaculturists respond to these demands by inhibiting or promoting the pigment required for the appealing color by manipulating cultural and genetic practices. In rainbow trout and other salmonids, red flesh color is an important quality attribute and hence, synthetic pigments such as astaxanthin and canthaxanthin (carotenoid) are incorporated into various feed formulations to give effective coloration to the fish muscle. In the case of tilapia and channel catfish, where normal gray pigmentation (dark color) due to melanin is a liability to the producer, the color is suppressed by manipulation of genetic factors, so that a lighter, more desirable flesh color is obtained (Tave et al., 1990).

The color of fish flesh is also affected by the storage and processing conditions, because most of the pigments are labile to heat, light, and oxygen (No and Storebakken, 1991; Skrede et al., 1989). Heating usually alters the red color and increases the lightness in food products. No and Storebakken (1991) determined the storage stability of carotenoid pigments (astaxanthin and canthaxanthin) during frozen storage of rainbow trout fillets and noted significant increases in lightness and redness. Scott et al. (1994) studied the stability of the carotenoid pigments in cultured rainbow trout during frozen

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storage and cooking and reported no differences in color values during storage. Cooking resulted in increased redness of the sample, inspite of a pigment loss.

The color of fish flesh could be analyzed by several analytical methods; sensory analysis using trained panelists, comparing the color with standard color charts, or instrumental analysis based on light reflected from flesh samples (Skrede et al., 1989). Instrumentally measured color correlates highly with sensory values (Skrede et al., 1990b).

Consumers have a divided opinion on the characteristic "fishy" odor in seafood products. It is considered as an undesirable flavor attribute in the mid - West and often equated to spoilage odors. On the coast, the "fishy" flavor is a desirable flavor attribute affecting customer's purchasing decision (Johnsen, 1989; Peavey et al., 1994).

The objectives of the study were (a) to determine the effect of purging (depuration) on the preference for pacu, (b) to evaluate sensory flavor differences between pacu and four other aquacultured fish species - channel catfish (*lctalurus punctatus*), tilapia (*Oreochromis spp.*), rainbow trout (*Onchorhynchus mykiss*), pacu (*Piaractus mesopotamicus*) and hybrid striped bass (*Morone saxatilis* x *M. chrysops*), and (c) to determine the pigment stability in the five aquacultured fish species before and after cooking.

MATERIALS AND METHODS

Growing Pacu

Pacu (*Piaractus mesopotamicus*) were grown in 1165L fiberglass tanks for a period of 24 weeks. They were fed three different diets - a) commercial 32% protein aquaculture diet (P32) (SSC 50822061, Southern States Co - Op. Inc., Richmond, VA), b) commercial 36% protein aquaculture diet (P36) (SSC 338200, Southern States Co - Op Inc.), and c) zucchini (0.5% protein).

Purging Pacu

At the end of the growth period, the fish from each of the three treatments were pooled, purged using a trickle filtration system for a period of 0, 2, 4, and 6 days and their sensory qualities compared to other aquacultured finfish. Pacu was analyzed for preferences using a simple ranking test following purging. The panelists were briefed, but not trained to evaluate the samples.

Source of Other Fish Samples

Fillets of channel catfish, rainbow trout, tilapia and d hybrid striped bass were obtained from retail seafood markets The samples were evaluated under red lighting to mask any color differences that might exist within the samples.

Sample preparation

Purged pacu was manually processed into skinless fillets. The fillets of all the fish were approximately cut into 1 - 2 inch pieces, individually wrapped in aluminum foil, and

baked according to procedures specified by the National Marine Fisheries Service (350°F for 20 min.).

Simple Ranking test

The samples were presented to panelists in a balanced, random order simultaneously. The subjects were asked to evaluate the samples for preference and assign rank 1 to the fillet with the highest preference, rank 2 to the next preferred fillet, 3 for the next and so on. The rank sums for each samples were calculated and the value of the test statistic T was calculated using the equation:

 $T = ([12/bt(t+1)] (X_i^2) - 3b(t+1))$

where b = the number of panelists, t = the number of samples, and $X_j =$ the rank sum of the sample j.

Significant difference was concluded between the samples if the value of T exceeded the upper - (critical value of a χ^2 random variable with (t - 1) degrees of freedom. A multiple comparison procedure was performed to determine which of the samples differed significantly by computing Fisher's least significant difference (LSD) for rank sums as follows:

LSD _{rank} = $z_{\alpha/2} (bt(t + 1)/6)^{1/2}$

Triangle test

The sample sets containing 3 samples (2 are identical, one is odd) were presented randomly to the panel. The subjects were instructed to taste each sample from left to

right and select the odd sample. The number of correct responses and the total number of responses were counted. The results were then interpreted using the table for the critical number of correct answers (Meilgaard et al., 1991).

Instrumental analyses

The fish fillets were ground using a waring blender and the color (L* a* b*) values measured using a Minolta color meter (Minolta Corp., Ramsey, NJ) before and after cooking the samples. The color were measured at three different locations and the values averaged.

RESULTS AND DISCUSSION

Subjective measurements

Purging Study

The results of the depuration study for pacu are given in tables 1 and 2. Purging resulted in a weight loss of the pacu. The highest decrease (-12.6%) was observed during the first two days of purging, followed by a loss of 3.63% and 0.3% at the 4th and 6th day of purging respectively.

The preference for pacu was significantly influenced by the length of purging (p < 0.05). Fillets of pacu purged for 6 days had a significantly higher preference compared to unpurged pacu fillets (p < 0.05). However, there were no significant differences in preference from the pacu purged for 2, 4, and 6 days.

The preference for pacu had a direct relationship with the length of purging. More than one - third (35.7%) of the panel preferred pacu purged for 6 days whereas only 10.7% of the subjects preferred unpurged pacu. This could be due to the panel's misconception of "fishy flavor" being a spoilage odor or not fresh fish.

The main purpose of depuration is to eliminate the fish of earthy flavors. Since, there were no statistical differences in preferences at 2, 4, and 6 days, pacu could be purged for 2 days and marketed without any additional weight losses associated with purging.

Pacu vs. Other Aquacultured Fish

Triangle Test for Differences in Flavor

The results of the triangle test for differences in flavor between pacu and other fish are illustrated in table 3. Pacu flavor was different from that of catfish (p < 0.05). However, the panelists were unable to distinguish the differences in flavor of other fish and pacu was concluded to be indifferent from tilapia, rainbow trout, and hybrid striped bass (p > 0.05).

The preferences for all the aquacultured fish analyzed are given in table 4. There were no differences in preferences for all the fish fillets analyzed (p > 0.05). Although, pacu had the highest overall preference, the preference was not significantly different from other fish (p > 0.05). Tilapia had the next highest preference followed by catfish, rainbow trout, and hybrid striped bass.

Channel catfish was ranked one by more than 1/3 (34.6%) of the panelists. However, more than 40% of the subjects ranked catfish 4 or 5, which significantly reduced its overall performance. On the other hand, pacu was ranked one by 23.1% of the panel, but only a small proportion of the panelists ranked it 4 or 5, which significantly improved its overall sensory performance. Tilapia was ranked one by a meager 7.7% of the subjects, but was ranked two by more than 20% of the panelists, which placed it behind tilapia in its overall performance.

Objective measurements

The effect of cooking on the L, a, b color values for all the fish fillets examined is shown in figure 1. The Hunter color reading 'L" represents lightness if positive and darkness if negative, "a" represents red character if positive and green character if negative, and "b" represents yellowness if positive and blueness if negative (Hung et al., 1995). Cooking significantly increased the lightness (L value) and decreased the "a" (redness) and "b" (yellowness) values.

All the fish fillets analyzed had positive L values before and after cooking. Before cooking, rainbow trout had the highest "L" value, followed by hybrid striped bass, catfish, pacu and tilapia (62.8), and hence would have the highest consumer acceptance. Cooking significantly increased the "L" (lightness) value. Rainbow trout had the highest "L" value (95.54) followed by catfish, tilapia, pacu, and hybrid striped bass (82.97). The

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highest amount of change in lightness was observed in catfish (25.78) and the least change in hybrid striped bass (16.04).

All the ground fillets had a red color prior to cooking. Hybrid striped bass had the highest "a" value (6.62) followed by pacu, tilapia, catfish, and rainbow trout which had the least redness (2.25). The redness in all the ground fish fillets was reduced by cooking. After cooking, hybrid striped bass had the highest "a" value (1.54) followed by pacu, catfish, tilapia, and rainbow trout. However, tilapia and rainbow trout had negative "a" values indicating slight greenness in the samples which could be attributed to the loss of carotenoid pigments.

In the case of rainbow trout, red flesh color is a dominant quality attribute affecting consumer acceptance. Hence, cooking trout reduces the red color and makes the product less desirable for the consumer. Skrede et al. (1989) analyzed the color in raw, baked, and smoked flesh of rainbow trout fed astaxanthin or canthaxanthin. They noted an increase in lightness and a slight decrease in redness and yellowness in baked trout. Smoked trout, however, showed a slight decrease in L, a, and b values. The reduction in redness was attributed to the loss of carotenoids. Scott et al. (1994) studied the stability of carotenoid pigments in cultured rainbow trout during cooking and noted an increase in lightness during baking and a decrease in lightness during smoking. In contrast to the results obtained in this study, Scott et al. (1994) obtained increased "a" values, despite a net loss of the pigments. Increment in "L" values, and decreases in redness and yellowness were also reported for Pacific chum Salmon (*Oncorhynchus keta*) following thermal processing (Bhattacharya et al., 1994).

All the ground fillets had a yellow color before cooking and exhibited a decrease in yellowness with cooking. However, catfish had negative "b" value indicating slight blueness in the sample after cooking. Catfish had the lowest "b" value before (4.81) and after cooking (- 0.97). The highest "b" value was observed for uncooked rainbow trout (15.6) and hybrid striped bass after cooking (- 5.06).

Since yellow color in fish fillets is an undesirable trait, all the samples would be unacceptable to the consumers before and after cooking, except for channel catfish. Channel catfish had a slightly negative "b" value indicating blueness in the sample after cooking.

CONCLUSIONS

The panelists were unable to detect differences between the flavors of different cultured fish, which indicates that either the differences in flavor was minimal or flavor is not a major criterion for determining fish acceptability. Cooking significantly influenced the appearance of the fillets resulting in unfavorable changes in rainbow trout by reducing the redness of the flesh and favorable changes in the rest of the fish by increasing the lightness (whiteness), which is a desirable quality trait for seafood.

REFERENCES

Bhattacharya, S., G. S. Choudhury, and S. Studebaker. 1994. Color Changes During
Thermal Processing of Pacific Chum Salmon. J. Aquatic Food Product Technol. 3(1): 39
- 48.

Fernandes, C. F., G. J. Flick, J. L. Silva, and T. A. McCaskey. 1996. Influence of Processing Schemes on Indicative Bacteria and Quality of Fresh Aquacultured Catfish Fillets. J. Food Prot. 60(1): 54 - 58.

Hung, Y. C., K. Morita, R. Shewfelt, A. V. A. Resurreccion, and S. Prussia. 1995. Sensory and Instrumental Evaluation of Apple Color. J. Sensory Studies. 10: 15 - 23.

Johnsen, P. B. 1989. Factors Influencing the Flavor Quality of Farm - Raised Catfish. Food Technol. 43(11): 94 - 97.

Meilgaard, M., G. V. Civille, and B. T. Carr. 1991. Attribute Difference Tests: How Does Attribute X Differ Between Samples. In *Sensory Evaluation Techniques*. 2nd ed. pp 99 - 122. CRC Press Inc., Boca Raton, FL.

Meilgaard, M., G. V. Civille, and B. T. Carr. 1991. Overall Difference Tests: Does a Sensory Difference Exist Between Samples. In *Sensory Evaluation Techniques*. 2nd ed. pp 59 - 98. CRC Press Inc., Boca Raton, FL.

Nedoluha, P. C. and D. Westhoff. 1995. Microbiological Analysis of Striped Bass (*Morone saxatilis*) Grown in Flow - through Tanks. J. Food Prot. 58(12): 1363 - 1368.

No, H. K. and T. Storebakken. 1991. Color Stability of Rainbow Trout Fillets during Frozen Storage. J. Food Sci. 56(4): 969 - 972,984.

Peavey, S., T. Work, and J. Riley. 1994. Consumer Attitudes Toward Fresh and Frozen Fish. J. Aquatic Food Product Technol. 3(2): 71 - 87.

Scott, T. M., B. A. Rasco, and R. W. Hardy. 1994. Stability of Krill Meal,
Astaxanthin, and Canthaxanthin Color in Cultured Rainbow Trout (*Onchorhynchus mykiss*) Fillets During Frozen Storage and Cooking. J. Aquatic Food Product Technol. 3(4): 53 - 63.

Skrede, G., T. Storebakken, and T. Naes. 1989. Color Evaluation in Raw, Baked and Smoked Flesh of Rainbow Trout (*Onchorhynchus mykiss*) Fed Astaxanthin or Canthaxanthin. J. Food Sci. 55(6): 1574 - 1578.

Tave, D., R. T. Lovell, R. O. Smitherman, and M. Rezk. 1990. Flesh and Peritoneal Lining Color of Gold, Bronze, and Black *Tilapia mossambica*. J. Food Sci. 55(1): 255 - 256.

		Subjects (Percent)					
Days Purged	Rank Sum	Like Extremely	ke Extremely Like Slightly		Dislike Extremely		
		(Rank 1)	(Rank 2)	(Rank 3)	(Rank 4)		
0	92	10.7 ^a	10.7	17.9	60.7		
2	66	25.0 ^b	25.0	39.2	10.7		
4	63	28.6 ^b	35.7	17.9	17.9		
6	59	35.7 ^b	28.6	25.0	10.7		

Table 1. Effect of Purging Time on the Percent of Subjects Showing Preference of Pacu

Percentages with the same letter in columns are not significantly different (p > 0.05)

Test Statistic (T) = 14.35

LSD _{rank} = 9.66

Length of Purging (days)	Initial Weight (g)	Final Weight (g)	Weight Loss (%)
0	511.8	511.8	0.0 ^a
2	511.8	499.2	12.6 ^b
4	479.5	475.8	3.6 ^a
6	490.1	489.8	0.3 ^a

 Table 2. Effect of Purging on the Weight Loss of Pacu (Piaractus mesopotamicus)

Mean weight losses with same letter in a column are not significantly different (p > 0.05)

Aquacultured Fish	Number of Correct	Number of Incorrect	Critical Number of	Difference
	Responses	Responses	Correct Responses	(p < 0.05)
Pacu vs. Channel Catfish	11	7	10	YES
Pacu vs. Tilapia	5	13	10	NO
Pacu vs. Rainbow Trout	7	11	10	NO
Pacu vs. Hybrid Striped Bass	8	10	10	NO

 Table 3. Triangle Test for Difference Between Various Aquacultured Finfish

		Subjects (percent)							
Aquacultured Fish	Rank Sum	Like	Like Slightly	Neither Like Nor	Dislike	Dislike			
		Extremely		Dislike	Slightly	Extremely			
		(Rank 1)	(Rank 2)	(Rank 3)	(Rank 4)	(Rank 5)			
Hybrid Striped Bass	89	11.6 ^a	19.2	15.4	23.1	30.8			
Catfish	75	34.6 ^a	7.7	15.4	19.2	23.0			
Pacu	70	23.1 ^a	19.2	34.6	11.5	11.5			
Rainbow Trout	82	23.1 ^a	11.5	11.5	19.2	30.8			
Tilapia	71	7.7 ^a	42.3	23.0	23.1	3.8			

Table 4. Percentage of Subjects Showing Preference for Different Aquacultured Fish Species

Percentages with the same letter in a column are not significantly different (p > 0.05)

Test Statistic (T) = -3.35



Figure 1. Effect of Cooking on the L, a, and b values of Aquacultured Finfish

APPENDIX 1

WATER CHEMISTRY DURING GROWTH PERIOD

TEMP = Temperature, DO = Dissolved Oxygen (mg/ml), $pH = H^+$ Ion Concentration,

TAN = Total Ammonical Nitrogen (mg/ml), ABS = Absorbance, NO2 = Nitrites (mg/ml),

NO3 = Nitrates (mg/ml), ALK = Alkalinity (mg/ml), and HAR = Hardness (mg/ml)

32% Protein Diet

DAYS	TANK 1]	ΓΑΝΚ	2	
	TEMP	DO	pН	TAN	ABS	,	ТЕМР	DO	pН	TAN	ABS
1	25.80	5.90	8.20	0.28	ND*		28.60	5.50	8.30	0.53	ND
2	26.50	6.50	8.80	0.71	ND		28.40	5.40	8.50	1.73	ND
3	25.90	5.00	8.30	1.34	ND		28.20	5.20	8.20	3.70	ND
4	25.90	5.50	7.90	2.05	ND		28.40	5.60	7.80	4.30	ND
5	23.70	6.30	8.00	2.48	ND		25.50	5.50	7.90	4.05	ND
6	26.60	4.50	7.50	2.17	ND		27.70	4.10	7.50	8.70	ND
7	24.50	6.30	7.50	2.59	ND		26.20	5.90	7.60	7.60	ND
8	24.60	7.10	8.10	2.24	ND		26.40	6.60	7.60	9.70	ND
9	27.10	6.10	9.00	0.81	ND		29.10	5.60	8.60	0.18	ND
10	26.50	5.60	7.70	1.38	ND		28.50	6.30	8.00	1.32	ND
11	26.90	5.90	7.60	1.55	ND		28.90	6.20	8.00	0.98	ND
12	26.30	5.80	7.60	1.37	ND		28.50	6.30	8.00	2.56	ND
13	26.00	6.20	7.80	1.20	ND		27.70	6.70	7.90	2.20	ND
14	25.60	6.00	7.80	1.34	ND		27.60	6.30	7.90	2.66	ND
15	27.10	6.30	7.90	0.62	ND		28.90	6.20	7.90	0.44	ND
16	27.50	6.00	7.90	0.51	ND		29.50	6.00	7.70	0.51	ND
17	27.00	6.10	7.50	0.61	ND	28.90	6.60	7.70	0.57	ND	
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18	25.70	5.50	7.10	0.53	ND	27.40	5.90	7.20	0.57	ND	
19	26.70	6.30	7.20	0.43	ND	27.80	6.30	7.30	0.63	ND	
20	27.70	6.70	8.00	0.56	0.06	29.20	6.10	7.90	0.74	0.15	
21	27.00	7.00	7.90	0.41	0.10	29.00	6.10	8.00	0.64	0.16	
22	26.20	7.00	8.00	0.59	0.09	27.80	6.30	7.90	0.92	0.16	
23	26.90	7.10	8.30	0.46	0.07	28.80	6.50	8.10	0.74	0.17	
24	25.90	7.20	7.70	0.63	0.06	27.90	6.00	7.60	1.12	0.11	
25	26.00	6.60	7.30	0.52	0.11	27.20	6.40	7.30	0.91	0.13	
26	25.80	7.10	7.00	0.73	0.16	27.00	6.40	7.10	1.06	0.16	
27	26.30	7.00	7.70	0.46	0.19	28.00	6.50	7.50	0.26	0.15	
28	26.70	5.20	7.40	0.49	0.19	28.00	5.50	7.40	0.36	0.10	
29	26.80	5.10	7.40	0.30	0.26	28.60	5.30	7.40	0.25	0.20	
30	28.60	5.30	8.00	0.20	0.26	30.20	5.20	7.70	0.14	0.20	
31	27.00	6.40	7.30	0.49	0.21	28.90	5.80	7.20	0.16	0.38	
32	26.20	6.70	7.70	0.23	0.22	28.50	6.30	7.50	0.22	0.44	
33	25.90	6.50	7.10	0.53	0.25	27.80	5.90	6.90	0.39	0.43	
34	25.60	4.90	7.60	0.72	0.25	27.40	4.70	7.30	0.43	0.45	
35	26.10	4.70	7.10	0.64	0.23	28.10	4.80	7.00	0.43	0.51	
36	27.50	6.70	7.50	0.45	0.26	29.70	5.70	7.10	0.30	0.55	
37	27.20	4.50	7.70	0.21	0.27	29.30	4.60	7.70	0.29	0.55	
38	26.90	4.60	7.20	0.18	0.32	28.80	4.40	7.10	0.43	0.53	
39	26.90	4.60	7.60	0.21	0.12	29.00	4.50	7.50	0.25	0.21	
40	26.50	7.10	7.50	0.47	0.14	26.50	6.00	7.50	0.28	0.24	
41	26.70	6.80	7.40	0.53	0.18	28.60	5.60	7.40	0.66	0.26	
42	26.80	6.50	7.30	0.55	0.27	28.70	4.90	7.30	0.88	0.26	

43	26.70	7.30	7.30	0.20	0.33	28	8.70	6.20	7.10	0.25	0.26
44	27.00	7.30	7.80	0.45	0.31	29	ə.10	6.70	7.70	0.73	0.27
45	27.20	6.70	7.00	0.26	0.35	29	9.20	5.30	7.00	0.65	0.35
46	27.20	6.30	6.90	0.17	0.35	29	9.10	5.30	6.80	1.36	0.27
47	26.20	6.70	6.90	0.62	0.45	28	8.20	5.90	7.00	1.61	0.26
48	26.80	6.40	6.70	0.62	0.38	28	8.60	5.70	6.90	1.88	0.26
49	26.70	3.60	6.80	0.90	0.41	28	8.40	3.30	6.70	2.01	0.32
50	27.30	4.70	6.70	1.17	0.40	29	9.10	4.70	7.10	1.68	0.32
51	28.00	6.20	7.20	1.43	0.43	29	9.60	6.30	7.50	1.64	0.38
52	27.00	6.10	6.80	1.32	0.41	28	8.90	5.70	6.80	1.71	0.41
53	27.10	6.20	7.60	1.04	0.30	28	8.50	6.00	7.30	1.57	0.48
54	26.80	6.30	6.40	0.94	0.31	28	8.70	5.80	6.80	1.19	0.48
55	30.50	6.40	6.50	1.58	0.41	31	1.30	5.70	6.90	1.42	0.58
56	27.10	6.70	6.40	1.68	0.35	29	9.10	6.00	6.20	1.16	0.53
57	23.70	7.70	6.30	1.55	0.35	24	4.50	7.00	6.10	1.03	0.61
58	24.40	8.10	7.00	1.30	0.43	24	4.30	7.40	6.40	1.12	0.64
59	22.50	5.70	6.80	1.53	0.27	22	2.60	5.70	6.60	1.24	0.55
60	22.80	6.10	6.50	1.29	0.28	22	2.70	6.10	6.30	1.02	0.56
61	23.60	6.70	6.50	0.92	0.29	23	3.40	6.50	6.50	0.92	0.54
62	22.90	6.80	7.10	1.75	0.34	23	3.00	6.60	6.90	1.01	0.66
63	26.90	6.40	7.20	2.04	0.31	25	5.90	6.00	6.80	0.91	0.63
64	27.20	6.80	7.40	2.27	0.35	26	5.20	6.80	6.70	1.81	0.63
65	29.50	6.70	7.40	1.72	0.27	27	7.90	6.60	6.60	0.70	0.63
66	26.60	6.80	7.20	2.36	0.27	25	5.80	6.80	5.80	1.32	0.63
67	26.60	6.50	6.60	1.75	0.27	25	5.80	6.90	5.30	1.47	0.67
68	26.00	6.40	7.60	1.60	0.33	24	4.90	6.80	7.60	1.70	0.13

69	25.60	6.60	6.90	1.68	0.48	24.20	7.00	6.90	1.76	0.45
70	26.10	6.20	6.90	1.45	0.38	24.70	6.00	6.80	1.17	0.41
71	26.40	6.60	7.50	1.22	0.49	25.00	7.00	7.00	1.02	0.48
72	26.20	6.40	5.60	1.17	0.50	24.90	7.00	5.40	0.84	0.54
73	29.20	6.70	7.00	1.45	0.42	27.80	6.80	6.90	0.95	0.58
74	26.40	6.30	7.10	1.36	0.49	25.00	6.70	6.70	1.33	0.59
75	26.20	6.20	6.80	1.49	0.60	24.70	6.60	5.80	1.02	0.65
76	26.40	6.10	6.10	1.82	0.45	24.90	6.80	5.50	0.97	0.50
77	26.20	5.90	5.70	1.73	0.31	24.80	6.30	5.60	1.14	0.56
78	26.00	6.10	5.70	1.69	0.51	24.70	6.50	5.60	1.17	0.59
79	27.10	6.50	5.70	1.23	0.64	25.60	6.80	5.50	1.29	0.72
80	26.30	5.50	7.20	1.28	0.63	24.90	6.10	7.40	1.16	0.77
81	26.40	5.70	7.80	0.75	0.65	25.00	6.10	8.00	1.04	0.75
82	26.40	5.40	8.00	1.07	0.69	24.90	6.20	8.10	1.19	0.85
83	26.40	5.50	8.20	0.88	0.67	25.00	6.60	8.20	1.13	0.91
84	26.70	5.60	7.70	1.06	0.69	25.60	6.30	7.90	1.13	0.90
85	24.80	7.50	8.40	0.21	0.32	26.60	6.60	8.10	1.20	0.81
86	23.60	6.80	7.50	0.47	0.28	25.80	6.10	7.60	1.05	0.85
87	23.40	7.40	7.10	0.57	0.36	26.00	6.20	7.20	1.03	0.75
88	23.50	6.00	7.80	0.43	0.41	26.00	5.60	7.60	1.13	0.79
89	23.10	6.60	7.40	1.01	0.31	25.20	5.30	7.30	1.31	0.64
90	26.30	5.60	7.70	0.65	0.36	25.60	5.40	7.40	0.76	0.59
91	26.30	5.90	7.40	1.26	0.29	25.50	4.80	7.40	1.22	0.43
92	28.10	8.10	7.30	0.93	0.43	27.10	7.20	7.10	0.68	0.63
93	27.50	8.70	7.30	0.77	0.47	26.70	7.70	7.20	0.77	0.70
94	27.20	8.40	6.90	0.72	0.47	26.30	7.10	7.30	0.81	0.63

95	27.60	8.30	6.50	1.03	0.52	26.70	7.40	6.50	0.85	0.68
96	27.70	6.20	6.90	0.80	0.34	26.80	5.40	7.00	0.93	0.61
97	27.50	6.10	7.10	0.98	0.35	26.60	6.10	7.10	0.98	0.37
98	27.30	5.50	6.30	1.95	0.50	26.30	6.00	6.30	1.15	0.81
99	27.80	5.30	6.90	1.57	0.42	26.80	6.00	6.90	1.15	0.66
100	27.70	5.60	7.50	1.96	0.42	26.80	5.60	7.50	1.16	0.71
101	27.90	6.00	5.90	1.82	0.42	26.80	6.40	6.00	1.23	0.71
102	27.60	5.90	7.20	1.45	0.49	26.70	6.30	7.20	1.23	0.80
103	26.90	6.10	7.30	1.24	0.49	25.80	6.40	7.20	1.25	0.64
104	26.60	5.00	7.20	1.28	0.51	25.50	5.00	7.10	1.20	0.85
105	26.70	4.30	6.80	1.32	0.56	25.70	4.20	6.70	1.30	0.85
106	28.60	5.60	6.80	1.30	0.58	25.70	5.40	6.70	1.24	0.89
107	28.00	5.90	7.10	1.35	0.30	26.90	5.90	7.10	1.29	0.84
108	27.80	4.00	7.50	1.36	0.60	26.80	3.80	7.50	1.40	0.72
109	28.00	2.80	6.60	1.27	0.57	26.90	3.00	6.60	1.25	0.86
110	28.00	4.80	7.20	1.32	0.69	26.80	4.50	7.20	1.29	0.97
111	25.80	6.10	5.40	1.42	0.70	24.90	5.90	6.20	1.29	0.78
112	25.90	6.30	5.30	1.29	0.75	25.30	5.10	5.40	1.26	0.87
113	25.70	3.70	6.20	1.35	0.47	25.80	5.40	6.50	1.32	0.68
114	27.30	5.00	5.40	1.63	0.52	27.20	5.60	5.80	1.27	0.72
115	26.50	4.30	5.30	1.57	0.48	26.80	5.20	5.60	1.38	0.58
116	26.10	4.10	5.50	1.49	0.54	26.40	5.10	5.60	1.26	0.68
117	26.20	4.50	5.50	1.28	0.55	26.40	5.80	5.60	1.30	0.74
118	25.30	4.70	5.90	1.32	0.56	25.50	5.20	5.70	1.28	0.79
119	25.50	3.70	5.50	1.35	0.64	25.70	4.10	5.70	1.22	0.77
120	27.20	4.20	5.50	1.33	0.60	27.20	5.50	5.80	1.13	0.85

121	27.80	3.70	5.70	1.38	0.64	27.80	5.10	5.80	1.38	0.81
122	26.30	4.00	6.20	1.42	0.48	26.70	4.90	5.70	1.39	0.80
123	25.80	4.30	5.60	1.26	0.60	26.00	5.10	5.70	1.27	0.85
124	25.70	3.80	6.30	1.30	0.66	25.60	5.30	7.20	1.27	0.82
125	26.30	3.60	5.70	1.26	0.58	26.50	5.30	5.80	1.25	0.89
126	26.80	4.00	6.60	1.70	0.63	27.20	5.40	6.20	1.48	0.68
127	28.80	4.20	6.10	1.43	0.67	28.30	5.50	6.70	1.37	0.83
128	25.20	5.50	6.20	1.96	0.69	25.40	5.40	6.60	1.23	0.84
129	25.50	4.90	6.00	1.28	0.73	26.50	5.30	5.80	1.26	0.76
130	25.80	4.30	6.40	1.34	0.72	26.70	5.50	5.90	1.37	0.79
131	24.70	4.40	5.70	1.31	0.71	25.80	5.60	6.10	1.32	0.79
132	25.00	3.10	6.20	1.23	0.66	25.80	5.00	5.70	1.27	0.84
133	23.70	5.80	7.80	2.06	0.25	25.70	5.70	6.10	1.08	0.41
134	24.50	5.60	6.70	2.04	0.27	25.80	5.50	6.00	1.57	0.36
135	24.60	5.80	6.40	2.08	0.28	26.10	5.50	5.80	1.69	0.41
136	24.90	5.00	6.50	1.94	0.32	26.10	4.80	6.10	1.42	0.45
137	25.40	5.50	6.10	1.71	0.43	26.50	5.50	5.60	1.39	0.46
138	24.40	5.10	6.30	1.68	0.39	23.60	5.30	5.80	1.42	0.51
139	24.50	4.60	7.20	1.58	0.38	25.60	4.30	7.10	1.37	0.45
140	24.70	5.90	6.00	1.66	0.39	28.60	5.10	5.90	1.36	0.49
141	25.40	6.10	5.80	1.68	0.37	29.40	5.30	5.80	1.57	0.41
142	25.10	5.80	5.90	1.68	0.39	29.10	5.40	5.60	1.49	0.47
143	27.60	6.80	8.00	0.59	0.40	30.00	5.80	7.70	0.55	0.41
144	26.70	5.80	7.80	0.36	0.33	29.20	4.80	7.70	0.49	0.47
145	26.70	5.90	7.70	0.42	0.40	29.40	5.20	7.40	0.63	0.49
146	26.80	5.00	7.50	0.54	0.41	29.10	4.50	7.30	0.45	0.56

147	26.80	5.90	7.40	0.55	0.45	29.40	4.80	6.70	0.80	0.53
148	26.50	4.90	7.20	0.41	0.43	29.00	4.40	6.40	1.26	0.60
149	25.40	4.50	7.00	0.42	0.47	28.50	4.60	6.50	1.30	0.60
150	26.30	7.90	6.90	0.54	0.46	29.20	7.90	6.20	1.30	0.60
151	25.50	8.60	6.80	0.42	0.49	28.60	8.30	7.00	1.34	0.69
152	29.50	5.00	6.90	0.50	0.55	31.40	4.60	6.30	1.34	0.73
153	28.60	5.50	7.20	0.46	0.50	30.80	5.40	6.30	1.39	0.69
154	27.20	5.60	7.80	0.21	0.52	30.00	5.10	6.20	1.40	0.72
155	27.90	6.40	5.90	0.73	0.60	30.30	5.80	5.30	1.30	0.73
156	26.80	5.80	6.30	1.22	0.55	29.30	5.50	6.10	1.26	0.75
157	27.10	5.10	5.90	1.27	0.60	29.40	5.10	6.00	1.31	0.78
158	27.40	5.80	6.10	1.30	0.61	30.00	5.20	6.20	1.31	0.79
159	27.10	5.90	5.80	1.30	0.64	29.80	5.10	5.80	1.30	0.63
160	27.10	5.70	5.60	1.29	0.62	29.40	5.70	5.80	1.31	0.56
161	27.10	5.30	5.30	1.42	0.70	29.10	4.90	5.40	1.47	0.61
162	26.40	5.20	5.20	1.22	0.68	29.30	4.90	5.30	1.28	0.66
163	24.40	4.50	8.10	1.30	0.58	27.80	3.90	7.10	1.32	0.59
164	26.90	5.00	7.60	0.97	0.66	29.40	4.70	6.50	1.30	0.62
165	26.80	5.30	7.90	0.93	0.35	29.30	4.40	7.90	1.27	0.56
166	26.40	5.30	7.00	1.00	0.73	29.30	3.70	6.90	1.22	0.67
167	27.80	3.50	7.20	1.34	0.55	28.30	4.20	7.60	1.89	0.36
168	26.40	5.80	6.97	1.12	0.43	27.28	5.69	6.84	1.30	0.57

36% Protein Diet

TANK

TANK4

	3									
DAYS	TEMP	DO	pН	TAN	ABS	TEMP	DO	рН	TAN	ABS
1	26.80	5.80	8.30	1.11	ND	29.50	6.00	8.50	0.38	ND
2	27.20	6.10	8.50	0.75	ND	29.30	6.30	8.90	0.74	ND
3	26.30	5.70	8.30	0.54	ND	28.80	5.40	8.20	1.44	ND
4	26.30	6.40	7.80	0.86	ND	29.00	5.50	7.80	1.85	ND
5	24.50	5.90	8.00	1.01	ND	26.40	5.30	8.30	2.62	ND
6	27.10	4.70	7.50	0.39	ND	29.30	3.70	7.50	4.80	ND
7	24.60	6.30	7.70	0.89	ND	27.10	5.70	7.70	3.70	ND
8	25.30	6.60	7.70	1.39	ND	27.60	6.10	8.00	3.30	ND
9	28.10	5.40	8.20	0.51	ND	31.00	5.50	8.40	6.85	ND
10	26.60	6.00	7.70	1.34	ND	28.60	6.10	8.10	4.45	ND
11	26.90	6.20	7.80	2.32	ND	28.00	6.30	8.00	2.63	ND
12	26.50	6.20	7.70	0.60	ND	28.30	6.30	8.10	3.70	ND
13	26.10	6.40	8.00	0.76	ND	28.10	6.20	9.20	4.30	ND
14	25.70	6.70	8.50	0.62	ND	27.50	5.70	8.50	2.90	ND
15	27.30	6.20	7.90	0.76	ND	28.70	5.90	8.00	3.40	ND
16	28.00	6.00	7.60	0.83	ND	29.30	5.70	7.70	1.99	ND
17	26.40	6.10	7.40	0.78	ND	29.20	5.50	7.60	1.46	ND
18	26.00	5.40	6.90	0.48	ND	27.80	5.50	7.00	0.45	ND
19	27.30	5.90	7.20	0.62	ND	29.00	6.00	7.30	0.42	ND
20	28.00	6.30	7.90	0.87	0.20	29.60	6.10	8.00	0.51	0.115
21	27.20	6.70	7.90	0.31	0.19	28.90	6.20	7.90	0.26	0.076
22	26.00	6.70	7.90	1.10	0.20	27.90	6.50	8.10	0.55	0.081
23	26.30	7.10	8.00	0.69	0.20	28.50	6.60	8.30	0.37	0.075
24	26.00	6.70	7.40	1.81	0.22	28.00	6.20	7.50	1.40	0.071

25	25.00	6.90	7.20	1.58	0.16	27.4	40 6.	.20	7.20	0.90	0.119
26	25.00	6.80	7.00	0.67	0.23	27.	10 6.	.60	7.20	0.13	0.184
27	25.90	6.60	7.40	0.86	0.26	27.	60 6.	.70	7.70	2.28	0.245
28	26.30	5.40	6.80	0.56	0.22	28.	30 5.	.30	7.20	0.31	0.226
29	26.80	6.20	7.30	0.14	0.24	25.	60 5.	.50	7.00	0.95	0.329
30	28.90	5.50	8.20	0.30	0.13	31.	20 5.	.30	7.30	0.66	0.332
31	27.20	6.40	7.40	0.21	0.07	29.	60 5.	.60	7.10	0.85	0.113
32	26.50	6.60	7.80	0.13	0.13	28.	20 5.	.90	7.60	0.63	0.13
33	26.20	6.50	7.10	0.38	0.09	27.	70 5.	.90	7.00	1.36	0.101
34	26.00	5.20	7.80	0.62	0.11	27.	40 4.	.80	7.50	0.84	0.122
35	26.60	5.30	7.30	0.53	0.13	29.	00 4.	.70	7.00	0.41	0.127
36	27.80	6.30	7.70	0.91	0.13	30.4	40 5.	.70	7.10	1.17	0.101
37	27.30	4.90	7.80	0.15	0.12	29.	30 4.	.70	8.00	0.40	0.078
38	27.10	5.40	7.20	0.12	0.15	29.1	20 5.	.00	7.50	0.11	0.151
39	27.30	5.20	7.60	0.04	0.08	29.4	40 4.	.50	7.80	0.37	0.071
40	26.80	6.70	7.60	0.94	0.08	28.	90 6.	.00	7.70	1.47	0.088
41	27.10	6.60	7.70	0.08	0.12	29.	10 6.	.00	8.20	0.43	0.117
42	27.30	6.20	7.60	0.48	0.12	29.4	40 5.	.40	7.80	0.54	0.159
43	27.10	7.50	7.60	0.21	0.16	29.	10 6	.90	7.80	0.18	0.202
44	27.40	7.20	8.30	0.47	0.17	29.	80 7.	.40	8.90	0.44	0.221
45	27.60	6.20	7.40	0.87	0.14	29.	20 5.	.70	7.40	0.15	0.272
46	27.60	5.90	7.30	0.53	0.21	29.4	40 5.	.70	9.20	0.55	0.27
47	26.60	6.70	7.70	0.69	0.25	24.	80 6.	.50	7.90	0.86	0.335
48	26.90	6.50	7.30	0.68	0.22	25.	10 6.	.10	7.40	0.44	0.269
49	26.40	4.30	7.20	0.56	0.27	25.	50 4.	.50	7.20	0.37	0.339
50	27.40	5.20	7.50	0.66	0.22	26.	20 5.	.20	7.60	0.64	0.313

51	27.80	6.50	8.30	0.54	0.26	26.7	0 6.80	8.40	0.67	0.391
52	27.50	6.50	7.30	0.67	0.24	25.8	0 6.50	7.80	0.55	0.302
53	27.10	6.50	7.70	0.61	0.16	25.7	0 5.90	7.60	0.68	0.336
54	27.00	6.30	7.20	0.28	0.28	25.1	0 5.90	7.20	0.36	0.309
55	30.60	6.20	7.80	0.43	0.31	30.1	0 6.10	8.80	1.31	0.408
56	27.30	6.50	6.50	0.68	0.29	26.1	0 6.20	6.70	0.80	0.393
57	24.80	7.20	7.20	1.51	0.36	24.9	0 7.10	8.10	0.59	0.355
58	27.70	7.00	6.30	1.42	0.45	25.7	0 7.00	6.70	0.76	0.377
59	25.80	5.30	7.00	1.54	0.44	24.2	0 5.30	7.30	0.51	0.304
60	25.90	5.70	6.60	1.59	0.37	24.3	0 5.40	7.50	0.69	0.264
61	27.10	6.50	6.60	1.56	0.37	25.4	0 6.00	7.20	1.37	0.248
62	26.60	6.30	7.00	1.77	0.45	24.5	0 6.20	6.90	1.38	0.239
63	27.80	6.80	7.10	1.97	0.45	25.9	0 6.50	7.30	1.94	0.343
64	27.90	6.90	7.00	1.91	0.36	26.6	0 6.70	7.00	2.03	0.259
65	30.20	6.30	7.20	1.76	0.23	28.7	0 6.20	7.50	2.26	0.361
66	27.20	7.00	6.50	2.06	0.38	25.8	0 7.10	6.50	1.93	0.135
67	27.30	6.70	6.80	1.80	0.33	25.1	0 6.30	6.50	1.86	0.081
68	26.80	6.60	7.60	1.68	0.37	25.0	0 6.20	7.10	1.88	0.271
69	26.30	6.70	6.90	1.85	0.53	24.3	0 6.70	6.20	2.16	0.421
70	26.90	7.00	6.80	1.81	0.43	24.9	0 6.30	6.50	2.12	0.285
71	27.30	6.70	7.10	1.74	0.36	25.2	0 6.50	7.10	2.08	0.308
72	27.00	7.10	5.40	1.44	0.40	24.5	0 6.40	6.50	1.89	0.338
73	30.10	6.40	6.50	1.54	0.48	28.3	0 6.70	7.20	1.81	0.31
74	27.30	7.00	6.40	1.47	0.44	25.2	0 6.10	7.20	1.76	0.333
75	27.20	6.70	5.20	1.59	0.59	24.9	0 6.70	6.40	1.67	0.454
76	27.10	6.90	5.10	1.40	0.44	25.0	0 6.20	6.80	2.05	0.391

77	26.90	6.80	5.20	1.55	0.47	25.40	6.00	6.90	2.32	0.207
78	26.70	6.80	5.20	1.59	0.52	25.30	7.00	7.10	2.09	0.394
79	28.10	7.10	5.30	1.33	0.58	26.10) 7.20	7.50	2.05	0.318
80	27.20	6.40	7.60	1.47	0.61	25.60	6.00	7.10	2.02	0.302
81	27.10	6.30	8.20	0.86	0.64	25.30	5.80	7.40	2.14	0.303
82	27.10	6.70	8.10	0.97	0.75	25.60) 5.50	7.60	1.76	0.304
83	27.30	6.50	8.10	1.06	0.44	25.90) 5.10	7.80	1.91	0.262
84	27.80	6.70	8.00	1.05	0.69	26.40) 5.90	7.60	1.96	0.327
85	27.70	7.00	8.50	1.19	0.90	27.60	6.60	8.10	1.06	0.738
86	26.50	6.30	8.00	0.92	0.91	26.60) 5.90	7.80	1.09	0.698
87	26.50	6.50	7.60	1.24	0.90	26.60	6.20	7.40	1.02	0.716
88	26.60	5.90	7.50	1.01	0.86	26.90	5.80	7.80	1.04	0.732
89	26.00	5.90	7.60	1.29	0.93	26.00) 5.70	7.50	1.24	0.621
90	25.80	5.70	7.70	1.16	0.98	26.30) 5.60	7.50	0.92	0.777
91	25.90	5.90	7.80	1.26	0.88	26.40	5.10	7.60	1.49	0.421
92	28.30	8.00	7.80	1.27	1.05	28.30) 7.40	7.70	1.10	0.898
93	27.40	8.70	8.10	1.18	0.85	27.90	8.90	7.60	0.86	0.584
94	27.50	8.30	7.60	1.07	0.82	27.30) 7.60	7.20	0.52	0.534
95	27.90	7.90	7.20	1.02	0.85	27.80) 7.60	7.10	0.62	0.595
96	28.00	5.80	7.90	1.08	0.75	27.80	5.80	7.60	0.95	0.555
97	27.70	5.70	7.60	0.93	0.68	27.60	6.50	7.50	0.83	0.586
98	27.50	6.00	6.90	0.76	0.54	27.30) 5.60	6.70	1.11	0.745
99	28.10	5.90	7.00	0.64	0.57	28.10	6.00	7.00	0.89	0.611
100	27.90	6.40	8.00	0.81	0.60	27.90) 5.70	7.90	0.85	0.697
101	28.20	6.60	7.60	0.83	0.58	28.10	6.20	7.10	0.94	0.698
102	27.80	6.50	7.40	0.87	0.65	27.70) 5.90	7.30	0.95	0.666

103	25.20	6.90	7.30	0.83	0.49	26.80	5.70	7.30	1.08	0.72
104	24.10	5.80	7.40	0.57	0.58	26.40	4.10	7.30	0.89	0.796
105	24.20	4.40	6.90	0.54	0.55	26.50	3.70	6.90	0.95	0.648
106	25.10	4.80	6.80	0.59	0.56	26.80	4.50	6.80	0.93	0.756
107	27.00	6.30	7.20	1.05	0.50	28.30	5.50	7.10	1.09	0.822
108	27.00	3.40	7.50	0.78	0.58	28.10	3.40	7.40	1.30	0.796
109	27.30	2.50	6.50	0.75	0.55	28.30	2.80	6.70	1.31	0.83
110	26.80	4.70	7.50	1.01	0.58	28.70	4.80	7.50	1.22	0.891
111	23.60	6.10	5.70	1.19	0.48	25.70	6.10	5.70	1.19	0.906
112	23.80	6.80	5.80	1.13	0.65	25.90	6.20	5.80	1.29	0.92
113	23.60	7.10	7.70	1.26	0.38	26.10	5.80	7.70	1.32	0.53
114	25.90	7.30	6.60	0.78	0.38	28.00	4.90	7.10	1.34	0.549
115	25.30	6.70	6.00	0.68	0.38	27.40	5.10	5.80	1.00	0.569
116	24.50	6.30	6.10	1.29	0.44	26.60	5.40	5.90	1.23	0.617
117	24.70	6.80	5.90	1.52	0.48	26.80	6.30	5.90	1.24	0.642
118	23.50	6.40	6.20	1.48	0.44	25.80	4.90	5.90	1.27	0.652
119	23.30	6.50	6.00	1.28	0.55	25.90	4.40	6.20	1.21	0.659
120	25.40	6.90	6.10	1.19	0.58	27.80	5.60	6.10	1.31	0.673
121	26.70	6.60	6.10	1.33	0.57	28.80	5.00	6.20	1.34	0.682
122	24.80	6.30	5.80	1.27	0.53	27.10	4.10	5.60	1.48	0.603
123	23.90	6.30	6.00	1.17	0.56	26.20	4.40	5.90	1.17	0.627
124	24.50	5.90	7.10	1.28	0.61	26.10	3.80	7.10	1.31	0.696
125	25.60	5.60	6.00	1.19	0.59	27.20	4.30	6.00	1.23	0.759
126	26.20	5.60	6.00	1.67	0.50	28.10	4.20	5.80	1.75	0.446
127	28.20	5.70	6.10	1.35	0.63	30.20	4.80	6.10	1.32	0.699
128	23.80	6.60	5.70	0.86	0.61	25.80	5.30	5.80	1.32	0.8

129	25.90	5.20	6.10	1.27	0.62	26.90	3.40	6.30	1.19	0.688
130	26.20	5.50	6.10	1.29	0.65	27.20	3.70	6.50	1.35	0.752
131	25.10	5.50	6.10	1.30	0.65	26.10	3.40	6.40	1.22	0.631
132	25.20	5.80	5.70	1.21	0.73	26.30	3.40	6.60	1.53	0.428
133	25.20	4.90	6.80	1.95	0.33	26.20	4.60	6.00	1.73	0.414
134	25.50	6.10	6.20	1.87	0.32	26.30	4.30	6.40	1.55	0.393
135	25.30	5.90	6.00	1.88	0.35	25.90	4.70	6.20	1.79	0.376
136	25.60	5.80	6.10	1.68	0.42	26.30	3.50	6.40	1.60	0.543
137	25.90	6.20	6.00	1.63	0.42	26.60	4.90	5.80	1.58	0.508
138	25.80	5.50	5.80	1.64	0.39	24.80	5.40	5.60	1.58	0.533
139	25.20	4.70	7.20	1.47	0.45	26.00	2.90	7.20	1.38	0.51
140	24.50	2.60	6.10	0.59	0.47	26.40	5.60	6.20	1.23	0.587
141	24.30	6.40	5.90	0.93	0.45	27.30	6.10	5.80	1.26	0.606
142	24.20	6.50	5.80	1.17	0.49	27.00	2.60	5.80	1.31	0.674
143	28.80	7.20	8.30	0.42	0.57	28.50	6.10	7.80	0.81	0.663
144	27.50	5.40	8.00	0.52	0.57	27.60	5.30	7.80	0.84	0.687
145	27.70	6.20	7.70	0.73	0.63	27.60	5.80	7.30	0.90	0.77
146	27.40	5.20	7.10	0.65	0.66	27.50	4.90	7.20	0.97	0.777
147	27.20	6.60	7.60	0.40	0.49	27.70	5.50	6.80	0.43	0.668
148	27.40	5.60	7.60	0.48	0.54	27.30	5.50	7.30	0.42	0.529
149	26.60	4.40	7.20	0.50	0.53	26.60	4.60	6.80	0.43	0.53
150	27.40	8.70	7.20	0.40	0.58	27.00	8.90	6.30	1.12	0.545
151	26.90	8.30	7.10	0.46	0.56	26.50	8.70	6.50	1.18	0.603
152	30.70	5.40	7.10	0.53	0.64	30.70	5.00	6.20	1.17	0.618
153	29.50	5.30	7.20	0.62	0.65	29.40	5.40	6.20	1.24	0.619
154	28.10	6.20	6.90	0.88	0.54	27.60	5.90	5.80	1.38	0.577

155	28.50	6.30	6.60	0.69	0.63	28.20	6.00	5.40	1.30	0.607
156	27.10	5.60	6.60	0.73	0.63	27.40	5.40	5.80	1.24	0.62
157	27.70	6.60	6.10	0.83	0.65	27.50	6.90	6.10	1.27	0.635
158	27.60	5.30	6.10	1.14	0.50	27.70	5.60	5.60	1.31	0.362
159	27.50	5.40	5.70	1.15	0.65	27.40	5.50	5.30	1.25	0.708
160	27.30	6.30	5.90	1.29	0.66	27.60	6.30	5.60	1.28	0.659
161	27.00	5.50	5.20	1.28	0.68	27.20	5.80	5.40	1.49	0.729
162	26.90	5.70	5.20	1.20	0.59	27.30	5.70	5.20	1.27	0.716
163	26.30	4.50	8.20	1.11	0.62	27.00	4.50	8.40	0.02	0.551
164	26.30	5.40	7.60	0.94	0.72	27.70	5.00	5.70	1.26	0.75
165	26.90	5.10	7.90	0.88	0.64	27.80	5.40	8.00	0.98	0.694
166	26.70	5.30	7.60	1.02	0.66	27.70	5.50	7.70	1.06	0.744
167	26.90	5.80	7.80	1.92	0.33	27.30	3.20	7.70	2.13	0.276
168	26.66	6.15	7.08	1.01	0.47	27.25	5.64	7.11	1.33	0.470
179	1.08	0.64	0.50	0.55	0.11	1.77	0.68	0.61	1.33	0.109

0.5% Vegetable Diet (zucchini)

DAYS	TANK					TANK6
	5					
	TEMP	DO	pН	TAN	ABS	TEMP DO pH TAN ABS
1	27.20	6.10	8.40	0.28	ND	22.50 7.40 8.50 0.16 ND
2	27.50	5.60	8.50	0.05	ND	23.40 7.30 8.80 0.07 ND
3	26.70	5.20	8.00	0.43	ND	22.50 6.20 8.00 0.19 ND
4	26.70	5.70	7.70	0.35	ND	22.80 6.40 7.80 0.11 ND
5	24.10	5.30	8.10	0.68	ND	24.10 5.50 8.40 0.29 ND

6	26.90	4.70	7.80	0.13	ND	26.6	0 5.20	8.90	0.12	ND
7	25.10	6.10	7.70	0.33	ND	24.9	0 6.30	7.80	0.49	ND
8	25.30	6.90	7.80	0.08	ND	25.1	0 7.60	7.80	0.08	ND
9	28.90	5.90	8.80	0.03	ND	28.4	0 7.00	9.30	0.10	ND
10	27.40	5.60	7.50	0.53	ND	26.0	0 6.10	8.60	0.00	ND
11	27.50	5.80	7.80	0.45	ND	25.1	0 1.40	7.80	0.69	ND
12	26.50	6.40	7.60	0.65	ND	25.2	0 5.90	7.60	0.30	ND
13	26.30	6.10	7.70	0.56	ND	24.6	0 6.10	7.50	0.56	ND
14	26.20	6.20	8.40	0.62	ND	24.7	0 6.50	8.40	1.33	ND
15	27.70	5.80	8.10	0.75	ND	26.7	0 6.40	8.00	1.78	ND
16	28.20	5.90	7.80	0.92	ND	26.8	0 6.40	7.50	2.18	ND
17	27.60	5.70	7.60	1.15	ND	27.5	0 5.80	7.50	1.60	ND
18	26.20	5.70	7.00	0.94	ND	26.5	0 5.70	6.80	1.84	ND
19	26.90	5.40	7.30	0.79	ND	27.8	0 5.60	7.30	1.01	ND
20	28.50	5.10	7.90	0.43	0.13	28.4	0 6.50	8.30	0.55	0.205
21	27.80	5.70	8.00	0.62	0.08	27.9	0 6.50	8.10	0.32	0.174
22	27.00	4.80	7.80	0.47	0.07	26.9	0 6.60	8.20	0.45	0.085
23	27.50	6.40	8.60	0.45	0.07	28.6	0 6.80	8.80	0.44	0.101
24	26.80	5.90	7.60	0.51	0.02	27.4	0 6.20	7.60	0.50	0.052
25	26.20	6.10	7.20	0.44	0.08	27.0	0 6.20	7.10	0.51	0.126
26	26.00	6.50	7.40	0.26	0.11	26.7	0 6.60	7.30	0.17	0.158
27	26.60	6.30	7.80	0.30	0.08	27.3	0 6.80	8.00	0.37	0.154
28	27.10	5.50	7.50	0.14	0.11	27.7	0 5.40	7.40	0.17	0.011
29	26.80	5.50	7.20	0.38	0.09	28.7	0 5.20	7.00	0.20	0.173
30	29.50	6.30	8.20	0.11	0.07	30.2	0 5.60	8.00	0.20	0.153
31	27.50	5.50	7.30	0.17	0.14	28.8	0 5.70	7.10	0.29	0.163

32	26.70	6.10	7.90	0.13	0.13	28.00	6.10	7.80	0.19	0.16
33	26.40	6.20	7.20	0.05	0.11	27.70	6.20	7.10	0.60	0.134
34	26.40	5.50	8.00	0.20	0.15	27.60	4.60	8.00	0.20	0.155
35	27.10	5.20	7.50	0.77	0.13	28.40	5.10	7.30	0.51	0.164
36	28.20	6.30	8.20	0.91	0.2	29.50	6.30	7.90	0.84	0.171
37	28.00	5.10	7.80	0.27	0.1	29.20	5.00	7.20	0.30	0.153
38	27.30	4.70	7.20	0.56	0.16	28.90	5.00	7.30	0.18	0.173
39	27.30	5.20	7.70	0.43	0.11	28.60	4.90	7.50	0.15	0.185
40	26.80	6.40	7.70	0.09	0.13	28.60	6.10	7.50	0.51	0.163
41	26.90	6.20	7.70	0.20	0.18	28.40	6.00	7.60	0.58	0.135
42	27.00	5.60	7.70	0.75	0.11	28.90	5.50	7.70	0.78	0.117
43	27.00	6.40	7.60	1.49	0.14	28.70	6.20	7.70	1.08	0.11
44	27.30	6.60	8.10	0.69	0.15	29.40	6.50	8.20	1.65	0.169
45	26.90	5.60	7.60	0.73	0.32	29.00	5.30	7.40	0.30	0.186
46	27.60	4.80	7.30	0.60	0.03	28.90	5.80	7.30	0.45	0.091
47	26.60	5.60	7.90	0.69	0.06	28.10	5.60	8.00	0.88	0.102
48	27.10	3.80	7.40	0.44	0.07	28.80	5.70	7.50	0.37	0.077
49	27.30	3.50	7.40	0.57	0.05	28.80	3.90	7.70	0.64	0.09
50	27.50	5.30	7.80	0.57	0.05	29.30	4.90	7.90	0.85	0.054
51	28.00	6.60	8.20	0.43	0.15	29.60	6.30	8.30	0.40	0.158
52	27.90	4.20	7.60	0.50	0.08	29.40	5.40	7.40	0.64	0.095
53	27.70	4.50	7.60	0.64	0.14	28.80	5.40	7.90	1.01	0.081
54	27.50	5.00	7.50	0.62	0.09	29.10	6.60	7.50	0.84	0.069
55	30.80	7.70	9.10	0.82	0.15	32.70	5.30	8.70	1.11	0.084
56	27.70	5.40	7.30	0.87	0.13	29.30	5.60	7.90	0.67	0.064
57	24.80	6.70	8.20	1.25	0.14	24.40	7.10	8.00	0.98	0.043

58	24.00	8.50	8.30	2.06	0.16	25.10	7.80	8.00	1.07	0.445
59	22.50	2.10	7.40	0.73	0.14	23.30	5.70	7.80	1.06	0.37
60	22.60	6.00	7.80	0.66	0.11	23.30	5.70	7.10	0.96	0.359
61	23.50	6.40	8.10	0.22	0.1	24.40	6.10	7.80	0.61	0.238
62	23.00	6.00	7.50	0.84	0.36	23.90	6.20	7.30	0.80	0.167
63	25.50	6.40	7.80	0.90	0.1	27.60	6.10	7.70	1.55	0.332
64	25.90	8.50	7.90	0.61	0.09	27.80	6.70	7.90	1.00	0.234
65	28.40	8.50	8.40	0.71	0.04	29.90	6.80	7.90	0.52	0.696
66	25.20	6.70	7.40	0.35	0.07	26.60	6.30	7.60	0.79	0.127
67	25.40	5.80	7.60	0.69	0.03	26.50	6.10	7.10	0.41	0.021
68	24.70	5.70	7.70	0.45	0.13	25.70	5.80	7.40	0.03	0.095
69	24.20	6.30	7.40	0.70	0.21	25.30	6.30	7.10	0.81	0.129
70	24.70	5.60	7.50	0.79	0.16	25.90	6.00	7.00	0.47	0.087
71	24.90	7.00	7.70	0.39	0.3	26.30	5.70	7.80	0.91	0.195
72	24.40	6.40	7.50	0.59	0.24	25.40	6.30	6.90	0.56	0.157
73	27.80	10.00	8.20	0.45	0.25	29.20	6.60	7.90	0.15	0.147
74	24.90	4.90	7.60	0.80	0.23	25.90	5.50	7.50	1.07	0.162
75	24.70	5.80	7.90	0.22	0.36	26.40	6.10	6.10	1.82	0.222
76	24.60	6.30	8.10	1.12	0.31	26.10	5.80	8.00	0.72	0.294
77	24.40	6.40	8.10	1.98	0.25	26.00	6.20	8.00	1.35	0.164
78	24.30	6.50	8.20	1.07	0.88	25.90	6.40	8.00	1.08	0.48
79	25.60	7.90	8.60	0.93	0.66	27.10	6.70	8.30	0.35	0.46
80	24.60	6.00	7.70	1.07	0.58	26.20	5.80	7.60	0.38	0.653
81	24.40	6.50	8.30	0.69	0.43	26.60	5.40	8.10	0.63	0.388
82	24.60	6.30	8.20	0.86	0.41	26.90	5.40	8.00	1.08	0.666
83	24.70	6.30	8.20	0.27	0.46	27.10	5.50	8.00	0.25	0.233

84	25.10	6.50	8.10	1.62	0.55	27.50	5.50	7.90	0.72	0.607
85	27.00	7.60	8.50	0.61	0.52	28.40	6.70	8.30	1.62	0.523
86	25.50	6.10	7.80	0.50	0.19	27.40	5.80	7.50	1.26	0.591
87	25.60	6.50	7.60	0.60	0.22	27.40	6.10	7.30	0.67	0.556
88	25.80	5.70	7.70	1.11	0.71	27.70	5.40	7.30	0.25	0.508
89	25.00	5.20	7.40	1.53	0.41	26.80	5.40	7.10	1.16	0.496
90	25.10	6.00	7.50	1.04	0.61	27.00	5.80	7.30	1.08	0.646
91	25.10	5.40	7.60	0.86	0.78	26.70	5.40	7.50	0.82	0.575
92	27.10	8.40	8.00	1.20	0.88	28.80	7.50	7.70	1.16	0.7
93	26.20	9.40	7.80	1.26	0.18	27.90	8.10	7.50	0.45	0.417
94	26.20	7.90	7.50	0.47	0.19	27.80	7.50	7.20	0.53	0.081
95	26.60	9.00	7.30	0.32	0.33	28.30	7.90	6.80	0.57	0.361
96	26.50	6.80	8.10	0.31	0.37	28.10	5.70	7.90	0.31	0.252
97	26.20	7.30	8.10	0.25	0.38	28.00	6.80	8.00	0.26	0.2
98	25.70	6.40	7.10	0.33	0.43	27.70	5.90	6.90	0.86	0.342
99	26.70	7.10	7.00	0.23	0.49	28.60	6.50	7.00	0.55	0.337
100	26.60	6.90	7.90	0.40	0.46	28.30	6.30	7.90	0.29	0.221
101	26.80	5.80	8.00	0.63	0.3	28.50	5.90	7.70	0.65	0.284
102	25.80	6.00	7.40	1.24	0.16	28.10	6.00	7.40	0.36	0.291
103	25.70	2.90	7.30	1.91	0.15	27.10	5.80	7.40	0.34	0.443
104	25.30	4.80	7.40	2.25	0.16	26.50	4.50	7.40	0.26	0.523
105	25.50	4.10	6.90	1.91	0.12	26.80	3.90	6.90	0.39	0.309
106	25.70	4.60	6.80	0.93	0.13	26.90	4.20	6.80	0.41	0.138
107	27.10	6.10	7.30	0.41	0.14	28.60	5.50	7.30	0.62	0.376
108	26.90	4.00	7.50	0.39	0.12	28.50	3.10	7.60	0.32	0.122
109	27.20	3.40	7.00	0.53	0.12	29.00	2.60	7.00	0.38	0.192

110	27.10	5.40	7.60	0.45	0.12	25.80	6.10	5.40	1.42	0.439
111	24.50	6.10	8.10	0.40	0.13	26.40	5.20	7.50	0.09	0.493
112	24.80	6.30	8.30	0.43	0.2	26.80	5.10	8.10	0.55	0.512
113	25.00	5.80	8.30	2.60	0.11	26.40	5.10	7.80	1.71	0.468
114	27.00	5.90	8.30	2.63	0.13	28.30	4.10	8.20	0.76	0.538
115	26.30	5.90	8.20	3.56	0.09	27.90	3.30	8.20	0.54	0.486
116	25.60	5.90	8.00	2.56	0.16	27.10	3.50	7.90	0.45	0.618
117	25.80	6.00	7.60	0.92	0.16	27.70	5.40	7.50	0.48	0.634
118	24.90	6.10	7.90	0.40	0.17	26.50	4.00	7.70	0.63	0.654
119	24.90	6.20	7.90	0.41	0.17	26.40	3.00	7.70	0.10	0.593
120	26.60	6.50	8.30	0.35	0.17	27.80	4.20	7.60	0.56	0.669
121	27.40	6.00	8.30	0.31	0.2	28.70	5.90	8.20	2.43	0.249
122	25.80	6.70	7.30	0.36	0.15	27.30	5.40	7.60	2.47	0.195
123	25.20	6.10	8.10	0.38	0.17	26.60	5.80	8.00	2.45	0.217
124	25.30	6.00	7.00	0.32	0.19	26.80	4.60	7.10	0.28	0.376
125	26.10	6.00	7.90	0.33	0.21	27.50	4.90	7.80	0.40	0.365
126	26.50	5.60	7.30	0.32	0.14	28.40	4.70	7.30	0.02	0.252
127	28.40	5.80	8.90	0.49	0.19	30.30	6.60	9.00	0.50	0.37
128	24.50	6.60	7.80	0.44	0.21	26.10	5.60	7.50	0.37	0.41
129	25.70	5.40	7.90	0.47	0.22	27.20	5.00	7.70	0.16	0.418
130	25.80	6.30	8.00	0.33	0.26	27.50	5.10	7.90	0.24	0.467
131	24.80	6.20	8.20	0.51	0.25	26.30	4.40	8.10	0.44	0.552
132	25.10	6.50	7.50	0.43	0.33	26.50	4.60	7.50	0.16	0.587
133	24.70	5.80	7.30	0.49	0.33	26.10	5.00	7.40	0.08	0.614
134	24.90	6.00	8.10	0.71	0.3	26.20	5.50	8.10	0.09	0.541
135	24.90	6.20	8.00	0.48	0.36	26.10	5.30	8.00	0.63	0.593

136	25.10	5.40	8.00	0.53	0.36	26.2	0 5.10	8.00	0.17	0.615
137	25.30	6.60	7.90	0.58	0.3	26.6	0 5.90	7.70	0.30	0.631
138	24.60	6.20	7.50	0.56	0.38	26.1	0 5.20	7.50	1.48	0.693
139	24.50	6.70	8.00	0.58	0.34	26.1	0 5.90	7.90	0.26	0.515
140	24.60	5.90	8.00	2.65	0.13	26.2	0 4.80	7.70	9.70	0.109
141	25.40	6.10	8.10	2.66	0.09	27.1	0 5.10	7.60	9.60	0.084
142	25.20	6.50	7.80	5.65	0.15	27.1	0 4.90	7.60	6.50	0.141
143	26.50	6.70	8.10	2.67	0.13	27.7	0 7.60	7.80	0.64	0.11
144	25.60	5.50	7.70	2.67	0.12	27.4	0 3.80	7.60	0.34	0.228
145	25.60	5.80	7.60	0.56	0.11	27.3	0 6.80	7.70	0.25	0.208
146	25.50	5.10	7.70	0.33	0.12	27.4	0 4.20	7.90	0.33	0.271
147	25.50	5.70	7.80	0.31	0.07	27.3	0 6.50	7.70	0.31	0.257
148	25.10	6.10	7.90	0.29	0.12	26.9	0 5.00	7.90	0.38	0.337
149	24.40	5.50	7.60	0.30	0.12	25.9	0 4.90	7.60	0.26	0.356
150	25.00	5.80	7.00	0.31	0.1	26.9	0 9.30	7.40	0.15	0.378
151	24.10	10.20	7.70	0.24	0.14	26.4	0 8.70	7.50	0.51	0.391
152	27.90	5.70	7.10	0.25	0.19	30.0	0 5.50	7.80	0.06	0.418
153	27.00	6.00	7.20	0.23	0.16	28.9	0 5.60	7.80	0.21	0.43
154	25.50	6.70	6.90	0.40	0.11	27.3	0 6.30	7.20	0.28	0.391
155	26.20	6.80	6.40	0.23	0.14	27.7	0 6.50	6.80	0.10	0.456
156	25.20	6.50	6.90	0.27	0.11	27.0	0 5.90	7.20	0.07	0.491
157	25.50	7.90	8.00	0.32	0.17	27.0	0 7.60	7.90	0.15	0.468
158	25.70	6.00	7.20	0.33	0.21	27.4	0 5.90	7.40	0.12	0.622
159	25.50	6.10	6.80	0.40	0.2	27.1	0 5.20	7.10	0.17	0.531
160	25.60	6.80	7.80	0.17	0.15	27.1	0 6.50	7.70	0.10	0.539
161	25.20	6.20	7.50	0.27	0.1	26.8	0 5.80	7.30	0.13	0.476

162	24.40	6.10	7.70	0.17	0.15	26	5.80	6.20	7.40	0.36	0.511
163	26.20	5.80	8.20	0.24	0.18	26	5.90	5.00	7.60	0.97	0.426
164	25.40	5.60	7.80	0.21	0.08	26	5.90	5.10	7.50	0.15	0.509
165	25.30	6.40	8.10	0.19	0.16	27	00.	4.70	7.80	0.10	0.579
166	25.30	6.40	7.90	0.44	0.12	26	5.90	5.40	7.70	0.02	0.564
167	26.60	6.20	8.70	2.41	0.15	27	.70	5.80	8.60	2.39	0.156
168	26.05	6.05	7.76	0.72	0.207	27	.18	5.74	7.67	0.75	0.321

ND* = NOT DETERMINED

32% Protein Diet

DAYS TANK

TANK2

	1								
	NO2	NO3	ALK	HAR	NO2	NO3	ALK	HAR	
1	6.5	29	98	132	5.32	7	88	119	
2	1.2	ND	ND	191	0.29	ND	ND	152	
3	0.045	ND	ND	ND	0.1	ND	ND	ND	
4	0.019	ND	66	124	0.06	ND	62	118	
5	0.028	ND	47	115	0.03	ND	43	120	
6	0.04	13.8	57	149	0.05	15.5	55	162	
7	0.104	35.5	76	119	0.03	5.5	52	147	
8	0.143	16	67	151	0.02	5.6	25	93	
9	0.03	24.2	53	159	0.02	19.2	38	113	
10	0.029	3.1	176	68	0.1	9.1	112.1	112	
11	0.05	17.5	93	119	0.04	10.4	64	94	
12	0.192	18.5	24	84	0.01	8.2	24	106	
13	0.163	15.2	79	137	0.04	14.4	63	149	

14	0.111	13.3	78	166	0.04	9.6	17	135
15	0.041	9	23	133	0.02	13.3	13	119
16	0.013	11.6	6	156	0.18	4.6	25	142
17	0.322	9.6	144	94	0.3	9.9	98	151
18	0.001	0.6	96	203	0.01	0.2	104	118
19	0	0.3	132	143	0.01	0	162	187
	36% Pro	otein						
	TANK				TA	NK4		
	3							
DAYS	NO2	NO3	ALK	HAR	NO2	NO3	ALK	HAR
1	0.321	18.4	75	171	0.19	15.9	134	131
2	0.059	ND	ND	160	1.03	ND	ND	155
3	0.089	ND	ND	ND	0.14	ND	ND	ND
4	0.04	ND	76	128	0.03	ND	45	94
5	0.024	ND	44	107	0.03	ND	46	164
6	0.082	15	41	140	0.11	13.9	50	135
7	0.037	4.7	47	160	0.02	1.7	67	169
8	0.142	21.5	29	149	0.03	17.4	20	146
9	0.057	21.6	49	148	0.02	11.1	52	135
10	0.1	4.1	160	99	0.04	5.6	117	118
11	0.023	3.8	141	109	0.02	8	109	105
12	0.032	6.7	75	99	0.01	13.2	48	109
13	0.036	17.8	103	122	0.02	27	117	197
14	0.013	14.5	12	93	0.1	12.3	15	104
15	0.016	11.5	9	92	0.02	6.8	14	119

16	0.036	11	13	118	0.0)6 6.9	39	138
17	0.298	8.6	92	92	0.3	31 10.4	98	112
18	0.003	0.3	93	78	C	0.2	127	77
19	0.002	0.1	101	132	C	0.3	133	154

0.5% Vegetable Diet (zucchini)

DAYS	TANK	TANK6						
	5							
	NO2	NO3	ALK	HAR	NO2	NO3	ALK	HAR
1	0.06	10.7	88	123	0.05	9.3	85	160
2	0.042	ND	ND	266	1.08	ND	ND	128
3	0.053	ND	ND	ND	0.07	ND	ND	ND
4	0.029	ND	108	116	0.04	ND	122	154
5	0.024	ND	136	131	0.05	ND	149	142
6	0.09	17.3	258	170	0.09	24.3	69	156
7	0.031	1.3	288	176	0.01	3	62	119
8	0.021	2.4	244	158	0.06	10.2	116	160
9	0.012	12.5	309	195	0.04	16.1	139	145
10	0.035	15.4	156	104	0.1	12.7	61	118
11	0.48	4.5	159	62	0.1	3.1	52	82
12	0.03	2.6	172	79	0.03	4.8	163	135
13	1.28	8.9	131	73	0.02	3.7	216	119
14	0.6	5.5	121	70	0.1	2.5	154	102
15	0.021	2.8	63	49	0.26	6.3	169	69
16	0.006	4.3	64	68	0.01	0.9	78	132
17	0.002	6.9	86	70	0	5.5	98	65

18	0.001	0.2	110	98	0	0.1	111	72
19	0.001	0.2	95	111	0	0.2	82	84

VITAE

Sharma V. S. Pullela was born on June 23, 1973 in Hyderabad, India. In June 1990, he graduated from Little Flower Junior College. He entered the Andhra Pradesh Agricultural University in the Fall of 1990 to pursue his B. S. (Agriculture) and graduated in Fall of 1994. In the Spring of 1995, Sharma entered the Master's program of the Department of Food Science and Technology, Virginia Polytechnic Institute and State University (Virginia Tech) under the direction of Dr. George J. Flick, Jr. As a student, he has been a member of the Institute of Food Technologists.

PRESENTATIONS AT PROFESSIONAL MEETINGS:

Institute of Food Technologists, Orlando, Florida. June 14 - 18, 1997.

• Effect of Dietary Protein Content on Growth Performance and Proximate Composition of Aquacultured Pacu (*Piaractus mesopotamicus*)

International Association of Milk, Food and Environmental Sanitarians

Orlando, Florida. July 6 - 9, 1997.

- Effect of Diet on the Indicative and Pathogenic Bacterial Quality of Aquacultured Pacu (*Piaractus mesopotamicus*).
- Effect of Production System on the Indicative and Pathogenic Microbiological Quality of Aquacultured Finfish