FATE OF *ESCHERICHIA COLI* O157:H7 ON

CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

AS AFFECTED BY HARVESTING AND PROCESSING SCHEMES

by

RICO R. WAWORUNTU SUHALIM

(Under the Direction of YAO-WEN HUANG)

ABSTRACT

Survival of *Escherichia coli* O157:H7 in channel catfish (*Ictalurus punctatus*), pond, holding tank water, on live catfish and catfish fillet packaged under modified atmosphere (MA) and overwrapped inoculated at 10^5, 10^6, and 10^7 CFU/ml was investigated. *E. coli* O157:H7 were detected by direct plating for 33 d and 69 d in pond and holding tank water, respectively. A rapid decrease of the pathogen was observed in the first two weeks to reach 2 log CFU/ml. The pathogen was isolated by enrichment procedure for approximately another 30 d from pond and holding tank water. The internal organs and skin scrape had 5.5 log and 2.5 log CFU *E. coli* O157:H7/ml, respectively.

E. coli O157:H7 survived in overwrapped and MA packaged fillet for 20 d. There was a significant difference on the survival of the organism between MA and overwrapped packaging at 0ºC and between MA packaged fillets stored at 0º and 10ºC. A 0.5 and 1 log reduction of *E. coli* O157:H7 in MA packaged fillet was observed after 5 d storage on fillets stored at 10 and 0ºC, respectively. There was no significant difference between the survival of *E. coli* O157:H7 on fillets with and without skin either in MA or overwrapped packages. Aerobic bacterial populations at the end of the 20 d storage increased by 1 and 3 log CFU/g while psychrotropic populations increased by 2 and 3 log CFU/g for the 0 and 10ºC storage, respectively.

E. coli O157:H7 attached to catfish skin mucus as confirmed by confocal scanning laser microscopy (CSLM) images. *E. coli* O157:H7 were seen at 15 μm into the mucus layer. Plate count revealed a half a log decrease in *E. coli* O157:H7 population/cm^2 on skin without mucus than on skin with mucus, while a 1-log decrease in population/cm^2 was observed on skin without mucus than on skin with mucus by microscopic count. Approximately 18 and 16% of all the cells observed under CSLM appeared to be dead cells on skin with or without mucus, respectively.

INDEX WORDS: Channel catfish, *Escherichia coli* O157:H7, Pond water, Holding tank water, Modified atmosphere packaging, Overwrapped packaging, Confocal scanning laser microscopy

FATE OF *ESCHERICHIA COLI* O157:H7 ON CHANNEL CATFISH (*ICTALURUS PUNCTATUS*) AS AFFECTED BY HARVESTING AND PROCESSING SCHEMES

by

RICO R. WAWORUNTU SUHALIM

B. S. HUMBOLDT STATE UNIVERSITY, 1993

M. S. AUBURN UNIVERSITY, 1997

A Dissertation Submitted to the Graduate Faculty of The University of Georgia

in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2003

FATE OF *ESCHERICHIA COLI* O157:H7 ON

CHANNEL CATFISH (*ICTALURUS PUNCTATUS*)

AS AFFECTED BY HARVESTING AND PROCESSING SCHEMES

by

RICO R. WAWORUNTU SUHALIM

Major Professor: Yao-wen Huang

Committee: Gary J. Burtle
Jinru Chen
Mark A. Harrison
Robert L. Shewfelt

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
August 2003

DEDICATION

To

My Parents

Lecky and Yustina Kurniadi

and

Sisters

Windija and Mona

for their infinite support, encouragement and love

"My mother had a great deal of trouble with me, but I think she enjoyed it"

-Mark Twain-

ACKNOWLEDGEMENTS

"Kind words can be short and easy to speak, but their echoes are truly endless"

-Mother Teresa-

I would like to express my appreciation to Dr. Yao-wen Huang, my major advisor, for having me as his student and for his guidance and support throughout my doctorate program. I am grateful to Drs. Jinru Chen, Mark Harrison, Robert Shewfelt, and Gary Burtle, my committee members for their valuable time and suggestions. Also, my gratitude to those people who helped me in the work of this dissertation as mentioned in the acknowledgement section of each chapter.

Thanks to all my colleagues for your laughs and fun times we spent in food science department and downtown Athens especially Isabel Blackman, Hsiao-yun Chu, Elaine D'sa, Brenda Jennings, Ruth Ann Rose-Morrow, Chaeho Park, Yusuf Yilmaz, and Antonio Zenere, and to all my friends and relatives far and near especially in Indonesia, Netherlands, Japan, Korea, Germany, California and Alabama for cheering me up with your e-jokes. Lastly, my deepest gratitude to Jeongyi Lee for believing in me.
It's been real !

"To accomplish great things we must not only act but also dream,

not only plan but also believe"

-Anatole France-

TABLE OF CONTENTS

INTRODUCTION

Escherichia coli O157:H7 has been isolated from outbreaks involving lake water and improperly chlorinated drinking water (Dev et al., 1991; Keene et al., 1994). The pathogen has also been recovered in cattle farm environments through irrigation water and manure-fertilized soil and has caused contamination to produce (CDC, 1997). Several studies have demonstrated the survival of *E. coli* O157:H7 in water. *E. coli* O157:H7 survived in drinking water for weeks depending on temperature (Rice et al., 1992; Warburton et al., 1998). In an environment similar to a fish pond, Wang and Doyle (1998) observed the survival of the pathogen in lake water. It was also demonstrated that temperature played a role in the survival of the pathogen in the water.

The culture of channel catfish consists of earthen ponds fed by nearby streams and/or rainfall and run off from adjacent farms. As an open culture system, catfish ponds are subject to contamination from animals both domestic and wild. The risk of contamination of water supplies is increased by the shedding of pathogens in feces of farm animals (Porter, 1997). Contamination by farm animals is apparent in developing countries where fish culture practices usually involve land animals such as pigs and cows as part of an integrated farming system. In some countries, night soil is also introduced into the fish pond as fertilizer. These cultural practices may contribute to foodborne illnesses upon consumption of aquaculture products.

Foodborne outbreaks involving the consumption of seafood, both finfish and shellfish, have been reported. Certain viruses including hepatitis and Norwalk, and microbial pathogens such as *Salmonella* spp*., Clostridium botulinum* and *C. perfringens, Staphylococcus aureus, Streptococus, Shigella spp., Yersinia* spp*., Campylobacter* spp*., Listeria monocytogenes,* and various vibrios are the microbes of concern associated with seafood (Anonymous, 1976; Garrett et al., 1997). Outbreaks of *E. coli* O157:H7 in seafood have not been reported. However, since the first identified *E. coli* O157:H7 outbreak was reported in 1982 involving the consumption of undercooked beef products, this pathogen has been implicated with a variety of foods and water such as apple cider, raw milk, leaf lettuce, salami, drinking water (Ackers et al., 1998; Besser et al., 1993; Keene et al. 1994; Rice et al., 1992). Fish products can become contaminated by pathogenic microorganisms through exposure to feces, pond water, wildlife, domestic animals, and post-harvest handling. Catfish is available commercially in many forms from dressed to fillets and consumers in the southeastern states can also purchase live catfish where they are available at farmers markets.

The effect of *E. coli* O157:H7 on modified atmosphere (MA) packaged catfish fillets is investigated in this study. Modified atmosphere packaging has been used to extend shelf life of fresh perishable food products (Genigeorgis, 1985). The increasing demand for fresh food prompted the extensive use of MAP. MAP systems can maintain the quality and safety of the food by retarding the microbial growth.

In order to study the distribution and penetration of *E. coli* O157:H7 on live catfish, confocal scanning laser microscope (CSLM) technique was used. CSLM is a nondestructive microscopy method to observe fluorescent specimens which allows the

observation of hydrated samples in situ without disruption of growth and metabolism (Blonk and Aalst, 1993). This study was designed to determine the survival of *E. coli* O157:H7 in catfish pond water. Its survival prompted us to examine the possibility of transmission of the pathogen from the water to the fish and potential cross-contamination of the pathogen from fish to other muscle food, and vice versa at the retail level. The potential of mucus to serve as a substrate for *E. coli* O157:H7 contamination is also investigated.

REFERENCES

Ackers, M. L., B. E. Mahon, E. Leahy, B. Goode, T. Damrow, P. S. Hayes, W. F. Bibb, D. H. Rice, T. J. Barrett, L. Hutwagner, P. M. Griffin, and L. Slutsker. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. J. Infec. Dis. 177:1588-1593.

Anonymous. 1976. Foodborne and waterborne disease outbreaks, annual summary 1973 and 1974. CDC, Atlanta, GA.

Besser, R. E., S. M. Lett, J. T. Webber, M. P. Doyle, T. J. Barrett., J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA 269:2217-2220.

Blonk, J. C., and H. V. Aalst. 1993. Confocal scanning light microscopy in food research. Food Res. Int. 26:297-311.

Garrett, E. S., M. L. Jahncke, J. M. Tennyson. 1997. Microbiological hazards and emerging food safety issues associated with seafoods. J. Food Prot. 60:1409-1415.

Genigeorgis, C. A. 1985. Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. Int. J. Food Microbiol. 1:237-251.

Keene, W. E., J. M. McAnulty, F. C. Hoesly, L. P. Williams, K. Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. W. Fleming. 1994. A swimming associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and Shigella *sonnei*. N. Engl. J. Med. 331-579-584.

National Fisheries Institute. 2002. About our industry. Available at http//www.nfi.org. Accessed 16 September 2002.

Porter, J., K. Mobbs, C. A. Hart, J. R. Saunders, R. W. Pickup, C. Edwards. 1997. Detection, distribution and probable fate of *Escherichia coli* O157 around an asymptomatic dairy farm. J. Appl. Microbiol. 83:297-306.

Rice, E. W., C. H. Johnson, D. K. Wild, and D. J. Reasoner. 1992. Survival of *Escherichia coli* O157:H7in drinking water associated with a waterborne disease outbreak of hemorrhagic colitis. Lett. Appl. Microbiol. 15:38-40.

LITERATURE REVIEW

Foodborne Pathogens Associated with Seafood

Channel catfish (*Ictalurus punctatus*)

In the U.S. more than $50 billion is spent annually on a wide variety of seafood products. Health and nutritional benefits from seafood consumption reflects in rising sales. Today, Americans consume approximately 15 lb of seafood each year, a 2.5 lb increase since 1980 or 5 lb increase since 1960. This strong consumer demand will continue as Americans become increasingly aware of the benefits (low fat, low calorie, easily digestible, and a good source of protein, important minerals and vitamins) (NFI, 2002; Silva and White, 1994). It was also noted that demographic trends within the United States indicated growing number of Asian and Hispanic consumers whose diets have traditionally favored relatively high levels of fish and seafood (Heil and Peck, 1998).

As the demand for fish as healthy food continues to increase and the natural fishery resources decline, there is a need to increase the seafood production through aquaculture. Aquaculture is estimated to supply 29% of global food fish production of 121 million t in 1996 (FAO, 1999). In the U. S., aquaculture is the fastest growing segment of agriculture. It was estimated that between 1980 and 1998, the value of U.S. production rose more than 400 percent to nearly $1 billion (NFI, 2002). The trend towards aquaculture was a significant one, not only because of the growing proportion of

the U.S. supply of fish and seafood that it contributed, but also because it brought harvesting and processing into closer conjunction and thereby assured a higher and more consistent quality of product (NFI, 2002).

Channel catfish is one of the important aquaculture species and is processed in the largest quantity in the U.S. The per capita consumption of channel catfish increased from 0.7 in 1990 to 1.13 lb in 2000. The production of catfish is increasing due to the higher demand of alternative protein source. In addition to providing white flesh, it supplies a high-quality source of protein and nutrition. Almost half of all U.S. aquaculture production comes from catfish farming with the total area encompassing about 180,000 acres. Aquaculture production is concentrated in Mississippi, Alabama, Arkansas, and Louisiana. Other major species grown in the United States are trout, salmon, tilapia, hybrid striped bass, sturgeon, walleye, and yellow perch, all of which are freshwater except salmon, a saltwater species (NFI, 2002).

As farm-raised fish, catfish is consistently high-quality and is available all-year long with less impact from adverse external environmental conditions. The following is a glance at the 1995 production and sales figures of the top five U.S. aquacultured species (Catfish Institute, 2002):

SPECIES	POUNDS PRODUCED	ESTIMATED SALES
Catfish	447 million pounds	$ 351 million
Trout	55 million pounds	$ 61 million
Salmon	34 million pounds	$ 80-84 million
Tilapia	20 million pounds	$ 24-27 million

Fresh catfish are sold as steaks, fillet, skinless and bone-in, whole or head-on and gutted. Individually quick frozen (IQF) catfish is sold in the same manner, except no whole fish. Sixty percent of the harvest is frozen. In addition, breaded, smoked, minced and marinated items are available (Catfish Institute, 2002). Live catfish are also available in retail farmers markets especially in the southeast of the United States. Catfish are transported live from the grow-out facility to the market. Consumers then have the options of purchasing live or dressed catfish.

Safety hazard in seafood

In general food safety hazards of aquaculture products, though vary according to the culture system, may include foodborne parasitic infections, foodborne disease due to pathogenic bacteria and viruses, veterinary drug residues, and contamination by agrochemicals and toxic metals. The sources of human pathogenic bacteria in finfish (and crustaceans) in aquaculture can be classified into two groups: indigenous bacteria those naturally present in the aquatic environment including *Vibrio cholerae, V. parahaemolyticus, V. vulnificus, Listeria monocytogenes, Clostridium botulinum*, and *Aeromonas hydrophyla*, and bacteria that are introduced into the aquatic environment through contamination by human or animal feces or otherwise such as *Salmonella* spp., pathogenic *Escherichia coli*, *Shigella* spp., *Campylobacter* spp., and *Yersinia enterocolitica*. Additional bacterial hazard can also be introduced during processing through handling *Bacillus cereus*, *L. monocytogenes*, and *S. aureus* (Reilly and Kaferstein, 1997). The presence of indigenous pathogenic bacteria is usually found at

fairly low levels and food safety hazard is not significant when these products are properly cooked.

Although most seafood products (with the exception of certain shellfishes) will undergo some type of heat treatment prior to consumption, improper cooking of fish may pose health hazards to the consumers as in the case of undercooked ground beef. In addition, fish and shellfish may be cooked lightly or consumed raw (Dalsgaard, 1998). The USDA guideline on cooking temperatures states that fin fish is considered cooked when the fish muscle turns opaque and flakes easily with fork (USDA, 1999).

Bacterial seafoodborne diseases

Foodborne illness is predicted to cause 5,000 deaths and 76 million illnesses per year (CDC, 2001). Between 1990 and 2001 it was estimated that contaminated seafood was the vehicle in 340 outbreaks with 5,133 cases of food poisoning (CSPI, 2001). Seafood was taunted as the leading known cause of foodborne illness outbreaks followed by eggs, fruits and vegetables, beef and poultry. In comparison, fruits and vegetables caused 148 outbreaks with 9,413 cases of food poisoning, while beef caused 134 outbreaks with 6,089 cases of foodborne illness and contaminated poultry caused 79 outbreaks with 4,279 cases.

Approximately 20% of the reported seafood outbreaks from 1990-2001 were bacterial in nature. Food poisoning caused by toxins produced in fish especially fin-fish prior to consumption, such as scombrotoxin and ciguatoxin, comprised the large portion of the outbreaks. In contrast, outbreaks involving shellfishes were predominantly caused

by *Vibrio* spp., followed by Norwalk virus (and/or Norwalk-like viruses) and paralytic shelfish poisoning (PSP) in equal proportion.

The etiological agents of seafoodborne disease in the U.S. between 1988-1992 are summarized as follows:

Table 1. Etiology of seafoodborne disease outbreaks in the United States from 1988-1992 (Bean et al. 1996)

Etiology	Number of outbreaks (% of total) associated with	
	Shellfish	Fish
Bacterial	12 (35%)	18 (12%)
Biotoxins	5 (5%)	41 (28%)
Scombrotoxin	-	74 (51%)
Parasitic	0	0
Viral	1 (3%)	0
Confirmed	18 (53%)	134 (92%)
Unknown	16 (47%)	12 (8%)
Total	34 (100%)	146 (100%)

The bacterial outbreaks shown in the table were attributed to *C. botulinum, E. coli, Salmonella* spp.*, Staphylococcus* spp., *Vibrio* spp.*,* and *Bacillus cereus*.

Fish and fish products have been associated with several human illnesses (Ghittino, 1972). A number of reports documented that fish and fish products have been considered a vehicle of foodborne bacterial infections in human (Brown and Dorn, 1977**,** Huss, 1997). *Edwardsiella tarda*, a human enteric pathogen, has been isolated from the

lesions of diseased channel catfish (Meyer and Bullock, 1973), while Troast (1975) observed a high serum titers to *E. coli* and *Enterobacter cloacae* from brown bullhead catfish. These catfish were found to inhabit contaminated waters. *Salmonella* have been reported at low number in farmed channel catfish (Andrews et al., 1977). *Salmonella* and *E. coli* were observed in tilapia (Youseff et al., 1992), a fresh water species cultured in the Southeastern U. S., and shrimp (D'Aout et al., 1980). Other human enteric pathogens were isolated in carp (Lee, 1972), a bottom-feeder scavenger commonly cohabit with channel catfish.

Contamination with certain viruses including hepatitis, and Norwalk, and microbial pathogens such as *Salmonella* spp*., C. botulinum* and *perfringens, S. aureus, Streptococus, Shigella spp., Yersinia* spp*., Campylobacter* spp*., L. monocytogenes,* and various vibrios are the primary microbial concern associated with seafood (Garrett et al., 1997). *E. coli* has been isolated in seafood products such as tuna pot pies (Wentz et al., 1984), dried squids (Bui and Yen, 1992), and ready-to-eat fried fish (Himelbloom, 1991). It is believed that fish may become carriers of these human pathogens in water environments polluted by human sewage or diseased animals (Ghittino, 1972). *Salmonella* and *Shigella* food poisoning were usually associated with fish from polluted water or contaminated during processing (Dalsgaard, 1998). *Staphylococcus* and *Streptococcus* foodborne illnesses are usually due to contamination of fish on a fishing vessel or in a processing plant (Brown and Dorn, 1977). Increasing evidence suggested that certain *Salmonella* types may be part of the microbial flora in tropical aquaculture (Huss et al., 2000). *Salmonella* spp. and other human pathogens are believed to be spread by aquatic birds in the environment (Beveridge, 1988). Several studies have indicated the

presence of *Salmonella* spp. in 21 % of Japanese eel culture ponds (Saheki, et al. 1989), 5% of North American catfish ponds (Wyatt et al., 1979) and 22% shrimp ponds (Lobrerra et al.. 1990). Although *Salmonella* tend to be linked with intestinal tracts of warm blooded animals, this pathogen have also been found from the gut of tilapia and carps raised in waste-fed and non-waste-fed fresh water ponds (Buras, 1993; Iyer et al., 1989).

Of equal importance in causing foodborne illnesses is *E. coli* O157:H7, a bacterial species pathogenic to human. *E. coli* O157:H7 has been detected in variety of foods from unpasteurized apple cider, meat product (Doyle, 1991), to salad vegetables (Abdul-Raouf, 1993). Studies showed that *E. coli* O157:H7 is the third or fourth most common enteric pathogen recovered from human stool samples (MacDonald et al., 1988). Hemorrhagic colitis caused by *E. coli* O157:H7 can be fatal to young children and immunocompromised individuals.

Channel catfish has never been associated with *E. coli* O157:H7 outbreak. However, this pathogenic bacteria may be introduced into a channel catfish rearing system via animals around the fish ponds. A previous study showed that the pathogen can survive in the channel catfish pond water for two weeks under controlled conditions (Suhalim et al., 2000). Live catfish contaminated with *E. coli* O157:H7 may further spread the pathogen at the point of sale in the market during processing. Similarly, raw and processed fish products may also be cross-contaminated by meat and poultry products processed and prepared at farmers markets.

Source of contamination

Foods can become contaminated during production either on the farms or in watercourses. It was believed that *E. coli* present on raw vegetables may originate from agronomic systems employing irrigation with contaminated water (Merson et al., 1976). The point at which contamination occurs will depend on the natural sources of a pathogen and on the opportunities for transfer at each stage of the food chain. Contamination of foods with *E. coli* also is likely to occur during assembling and preparation of the food. These foods can serve as carriers of the organism. The presence of human enteric organisms on fish implies that cross contamination from terrestrial source has occurred. Thus the presence of these bacteria suggests the need for strict hygiene during handling and processing of fish to prevent transfer of potentially pathogenic bacteria to humans.

Similarly, fish cultured in a system that receives waste/contaminated water supply may harbor organisms that are present in the water (Dalsgaard, 1998; Fapohunda et al., 1994; Silvakami et al., 1996). Foodborne illnesses due to *E. coli* O157:H7 has not been implicated with the consumption of channel catfish or other freshwater species. However, enterotoxigenic (ETEC) *E. coli*, enterophatoghenic (EPEC) *E. coli*, and shiga-like toxin-producing *E. coli* (SLTEC) have been isolated from seafood (Ayulo, et al. 1994; Samadpour et al., 1994). Studies on the microbiology of channel catfish indicated that bacteria such as *E. coli*, total coliform, and *S. aureus* were observed on the fillets from several processing plants and their counts vary with seasons (Fernandez et al., 1997a). *Salmonella* and *Listeria* have been isolated from a variety of fish and shellfish products at processing plant and retail level (Andrews et al., 1977; Hatha and Lakshmanaperumalsamy, 1997; McCaskey et al. 1999). In addition, Fernandez et al.

(1997b) also screened fresh channel catfish fillets for *E. coli* 0157:H7, *Campylobacter, Klebsiella, Vibrio cholerae,* and *Plesiomonas shigelloides* from catfish processors in the southeastern U. S. during four annual seasons. Only the latter two pathogens were isolated during the warm weather. A separate study compared the growth of *E. coli* on beef and (speckled trout) fish cubes and incubated them at 35ºC. It was found that *E. coli* grew more slowly on fish Fapohunda et al. (1994). In 1995, 3 cases of *E. coli* O157:H7 outbreak in Washington state were associated with fish product. However, fish was not the primary vehicle of the outbreak. *E. coli* O157:H7 was believed to have been transferred by food handlers from ground beef to fish (CSPI, 2001).

The highest bacterial concentration in tilapia after experimental exposure of live fish with *E. coli* in fish tank water was recovered from the digestive tract at the level similar to that of the inoculated water, followed by the skin, spleen and liver. Most of the muscle samples were not contaminated (Fattal et al. 1992). Silvakami et al. (1996) also reported that bacterial species isolated from fish pond water were similar to those isolated from the intestinal tract of some fish species. More than 50% of the total bacterial population were *E. coli* and *P. aeruginosa*. Similarly, Leung et al. (1992) observed the presence of fecal streptococci, and fecal coliform in water and sediments of fish ponds and also in fish viscera at higher counts. *Campylobacter* has been isolated from surface water samples. The most isolated species is *C. jejuni* although its presence is seasonal with autumn and winter months as being the most prolific (Koenraad et al. 1994). In integrated livestock-fish farming system *E. coli, Salmonella* spp. and *Klebsiella* spp. were detected in fish where pig manure was used as fertilizer (Quines, 1988). This type of farming system has been practiced and is common in developing countries around the

world, and may pose a potential risk to public health. Shedding of feces containing pathogen from farm animals will increase the risk of contamination of water supplies (Porter et al., 1997).

The outbreak of *E. coli* O157:H7 contamination in seafood product has yet to be explored. Subsequent reports on *E. coli* O157:H7 outbreak associated with food and other media, such as well water (Anonymous, 1992; McGowan et al, 1989), and improperly chlorinated swimming pool water (Blake, 1998; Brewster et al., 1994; Friedman et al. 1999; Moore et al., 1993), have prompted us to investigate the possibility of freshwater fish as carriers of *E. coli* O157:H7. *E. coli* O157:H7 has also been reported to survive in reservoir water for 10 weeks (Wang and Doyle, 1998).

Many of the freshwater fish culture system in the southern U. S. are subject to contamination by warm blooded animals such as birds, cows, and deer, carriers of *E. coli* O157:H7. These aquaculture facilities are usually fed by run off or other water supplies that may be contaminated by pathogenic organisms. Sources of fecal contamination can come from farmed and wild animals grazing in water as well as accidental ingress of raw sewage into water system (Chalmers et al., 2000). Risk of contamination by enteric organisms is higher for onshore establishments than off shore installations because fecal material from animals, birds, and human can enter into water bodies directly or from run off from the land (Howgate, 1998). Additional concern is related to the use of human and animal excreta as fertilizers in pond aquaculture. Often these systems receive water from agriculture and wastewater (Howgate, 1998).

E. coli O157:H7 characteristics

E. coli are gram-negative bacteria commonly found in the intestinal tract of warm blooded animals and it is used as an indicator of fecal contamination of food and water (Jay, 1996). There are several groups of *E. coli* strains that cause diarrheal illness based on virulence properties, clinical syndromes, mechanisms of pathogenicity, and distinct O:H serogroups. The categories are enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), diffuse-adhering strains (DAEC), enteroaggregative strains (EaggEC), and enterohemorrhagic strains (EHEC). *E. coli* serotype O157:H7 was classified as EHEC and has become an important human pathogen since its first reported outbreak in 1982 in Oregon and Michigan involving the consumption of undercook ground beef (Riley et al., 1983). Other stains and serogroups of *E. coli* including O26:H11, O103, O104, O11, and sorbitol fermenting O157:H^{-} have been classified as EHEC (CDC, 1995a; CDC, 1995b).

Although *E. coli* O157:H7 shares most characteristics of non-pathogenic *E. coli*, this pathogenic strain is unable to grow at temperature above 44ºC, unable to produce β-glucuronidase (lack of 4-methylumbelliferone glucuronide [MUG] hydrolysis) (Doyle and Schoeni, 1984). *E. coli* O157:H7 also lacks of sorbitol fermentation within 24 h (Farmer and Davis 1985), possesses an attaching and effacing gene (eae) (Donnenberg et al. 1993), produces Shiga toxins (Stxs) that inhibit protein synthesis (O'Brien and Holmes, 1987), and carries a 60-MDa plasmid (Karch et al. 1987).

E. coli O157:H7 is highly acid resistant in comparison to the non-pathogenic *E. coli* and can survive up to 17 d in tryptic soy broth (TSB) acidified with HCl to pH 4 and has a minimal growth pH of 4.0-4.5 (Glass et al., 1992). In general, *E. coli* O157:H7

strains are antibiotic sensitive against antibiotics to gram-negative bacteria (Ratnam et al., 1988) with the exception of a strain resistant to streptomycin, sulfisoxazole, and tetracycline isolated from a waterborne outbreak in Missouri (Swerdlow et al., 1992). *E. coli* O157:H7 infectious dose is estimated to be of less than 100 organisms. Although infants, younger children, and elderly individuals are at higher risk of developing illness, all age groups are prone to *E. coli* O157:H7 infection (Griffin and Tauxe, 1991).

Prevalence of *E. coli* O157:H7 in foods and environment

A variety of foods have been implicated as vehicles of *E. coli* O157:H7 infection. In addition to raw or undercooked foods of bovine origin, the most common vehicle, (Nataro and Kaper, 1998), apple cider, lettuce, alfalfa sprouts, salami, turkey sandwiches, yogurt, and mayonnaise have also been linked to *E. coli* O157:H7 infection (CDC, 1995c; Morgan et al., 1993; Feng, 1995; Carter et al., 1987; Besser, 1993). Cattle are the most important reservoir of *E. coli* O157:H7 (Park, et al. 1999). Subsequent studies indicated that sheep (Kudva et al, 1996), birds (Wallace et al., 1997, Dell'Omo, 1998), deer (Keene et al., 1997) and domestic animals including pigs, cats and dogs (Beutin et al., 1993) have also been identified as carriers. Numerous studies reported that *E. coli* O157:H7 are frequently isolated from feces of healthy cattle (Montenegro et al. 1990, Rahn et al., 1997), and young animals tend to carry the organism more frequently than adults (Zhao et al., 1995). Transmission of *E. coli* O157:H7 has been reported to occur from direct cattle-to-human (Chapman et al., 1997) and person-to-person in day-care centers (Belongia et al. 1993), nursing homes (Carter et al., 1987), and in households (Ostroff et al., 1989).

E. coli O157:H7 in water

Several major human pathogenic bacteria that have been associated with water include : *Aeromonas* spp., *Campylobacter* spp., *Listeria monocytogenes, Plesiomonas shigelloides, E. coli* (including O157:H7 strain), *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Clostridium perfringens*, *Helicobacter pylori*, *Legionella* spp., *Pseudomonas aeruginosa*, and *Yersinia enterocolitica* (Black and Finch 1993; Rice et al., 1992).

Consumption of contaminated municipal drinking water has in the past led to outbreaks of *E. coli* O157:H7. In most cases the municipal water was improperly or not chlorinated (Dev et al., 1991, Swerdlow et al. 1992). Recently, an outbreak occurred in Walkerton, Ontario, Canada due to contamination by *E. coli* O157:H7 of the municipal water supply resulted in 6 deaths (Anonymous, 2000). In 1991, lake bathers near Portland, OR, were thought to ingest fecally contaminated lake water and became infected with *E. coli* O157:H7. Further investigations suggested that the source of contamination might include cattle manure slurry and fecal excrement from toddlers who were not yet toilet trained (Keene et al., 1994). A similar outbreak occurred in a semi-rural area of southeast Scotland in which a children's paddling pool served as the focal point in the transmission of infection causing the outbreak (Brewster et al., 1994).

In cattle farm environment, the water trough and feed may play an important role in the transmission of the *E. coli* O157:H7. Furthermore, a study indicated that *E. coli* O157:H7 can survive in water trough sediments for at least 4 months and multiply (Lynn, et al. 1998). Hancock et al. (1998) were able to isolate *E. coli* O157:H7 from feedlot, trough-water and biofilm, feedlot, and even flies from cattle farms in northwestern states.

In recent years irrigating pastures with manure slurries have increased. This agricultural practice has been suggested to create an environment that sustains the survival of *E. coli* O157:H7 (Hancock et al., 1994). An association between manure slurry application to pastures and *E. coli* O157:H7-carrier herds have been speculated, in which *E. coli* O157:H7 can be transmitted from manure to cattle on farms and produce grown on manure-fertilized soil (Wang et al., 1996). This postulate was previously confirmed by an outbreak in Montana (Ackers et al., 1995). *E. coli* O157:H7-contaminated water was used to irrigate the field where lettuce was grown. Subsequent investigations have also been documented related to *E. coli* O157:H7 contamination to produce (CDC, 1997; Hilborn et al., 1999; Solomon et al., 2002a, 2002b,).

E. coli O157:H7 has been demonstrated to survive in drinking water for several weeks depending on the temperature (Rice et al., 1992, Warburton et al. 1998), in lake water (Wang and Doyle, 1998), in river water and soil cores containing rooted grass (Maule, 2000). In 1992, a large outbreak caused by *E. coli* O157 occurred in Africa. It was speculated that drought, carriage of *E. coli* O157 by cattle, and heavy rains with contamination of surface water appeared to be important factors contributing to this outbreak (Effler et al., 2001).

Modified Atmosphere Packaging

"Modified atmosphere" is defined as the storage conditions where the atmospheric carbon dioxide and oxygen concentrations are altered before storage and allowed to vary as a function of time, temperature and package transmission (Brody, 1989). Modified atmosphere packaging (MAP) has been recognized to permit storage of fresh meats for 4-5 weeks since 1882 (Holland, 1980). In over a period of a century later,

MAP has become an appealing approach to extend shelf life of fresh perishable food products for fresh meat shipments from continental USA to a variety of places (Genigeorgis, 1985). Today, MAP has been applied widely for variety of food from bakery to vegetable products. The increasing demand for fresh food prompted the extensive use of MAP. MAP system can maintain the quality and safety of the food by retarding the microbial growth.

The three main parameters of the MAP system are gas mixture, packaging film, and storage temperature (Reddy et al., 1992). These parameters are regulated by the type of fish products to be packaged. MAP system employs the use of gasses usually O_2, CO_2 and N_2 ratio in the package to maintain the quality and safety of the food. The role of CO_2, O_2, and the effects of MAP on foods has been elucidated elsewhere (Brody, 1989; Robertson, 1993). Barrier films for O_2, CO_2 and N_2 are usually used to prevent product moisture loss and to maintain gas composition.

Although MAP of seafoods are widely practiced in European countries, regulatory agencies, thus seafood processors, in the U.S. are shying away from using MAP. MAP helps retain seafood quality, however, temperature abuse during storage is a serious problem. Advantages and disadvantages of this packaging system especially of fresh fishery products have been identified (Cann, 1988; Yambrach, 1987). One of the main disadvantages of MAP is that it offers conditions for pathogenic microorganisms such as the *Clostridium botulinum* type E to grow and produce toxin.

The use of MAP to extend shelf-life of fishery products started in 1930s (Smith et al., 1988). The efficiency of MAP system is apparent when coupled with optimum refrigeration. Inhibition of bacterial growth and extension of shelf-life by MAP have been

investigated (Brody, 1989; Genigeorgis, 1985; Reddy et al., 1992, Robertson, 1993). Carbon dioxide at levels above 20% is commonly used in MAP due to its ability to retard growth of spoilage microorganisms by extending the lag phase. At these levels carbon dioxide can also reduce oxidate rancidity of fats (Brody, 1989). Stammen et al. (1990) reported that elevated CO_2 in MAP has been shown to inhibit the natural spoilage flora of seafood and double or triple the shelf life. Elevated level of CO_2 pack also resulted in longer shelf life and limited the level of lactic acid bacteria in cold-smoked salmon (Nilsson et al. 1997; Paludan et al., 1998). The effectiveness of gas composition varies with species of fish. Robertson (1993) suggested that non-fatty fish is packed with 30% O_2, 40% CO_2, and 30% NO_2, while packaging for fatty fish the gas composition is usually 40% CO_2 and 60% N_2. High CO_2 level may cause the package to collapse, increase drip, and development of acid flavor due to the CO_2 in some fish species. At this condition, seafood can have a shelf life for about 10-14 d but may reach 20 d with strict temperature control.

Salmonella enteritidis grew rapidly at 10ºC in MAP samples compared to the insignificant growth observed in fresh fish stored at 3ºC (Nychas and Tassou, 1996). Limited growth of *Aeromonas* and *Yersinia enterocolitica* was also observed in cod and trout under MAP (Davies and Slade, 1995). *Y. enterocolitica* growth was inhibited by 80% CO_2 for trout and 60% for cod (Davies, 1995). Silva and White (1994) reported that channel catfish fillets stored in MAP at 2ºC extended the shelf life by 3 weeks with the aerobic plate count of $10^{1.4}$ *Salmonella, Listeria monocytogenes*, and *C. botulinum* were not detected at this storage conditions.

The use of MAP in bulk shipment of meat and fishery products has been accepted. However, proper storage temperature is essential to maintain the safety of food or the benefit of MAP is lost. A major concern that discourages the common practice of MAP for packaging fishery products is the low assurance that the product can be properly stored at temperature below 3ºC at retail level (Robertson, 1993).

To date, no published report is available regarding the effect of MAP on *E. coli* O157:H7 contamination on channel catfish in the U.S.

Possible contamination of channel catfish by *E. coli* 0157:H7

Previous studies suggested that seafood can serve as carriers of human pathogens. Only one study investigated the quality of fresh channel catfish fillets from processing plants for possible contamination with *E. coli* O157:H7 (Fernandez et al. 1997b). However, *E. coli* O157:H7 was not found in the fish fillets. *E. coli* O157:H7 outbreaks have been associated with a variety of foods, excluding fish, prompting us to believe that such an outbreak may occur in freshwater fish. Channel catfish are cultured in areas where agricultural run off is likely to be found. In addition, importation of fresh water fish cultured outside the U. S. can also complicate the scenario. Many of the fish culture facilities outside the U. S. have inadequate water management, and mixing of wastewater with other uses is likely.

Confocal scanning laser microscopy (CSLM)

The principle of confocal microscope relies on the ability to focus the image of a light source on a specimen at a precise depth, and the information is projected to a

detector through a pinhole (Blonk and Aalst, 1993). In essence, a small amount of light probes a tiny region on the specimen and only light from that region is read by the detector. The image is developed due to simultaneous scanning of the image through the focal point and the pinhole of the detector. Computer software aids in the construction of the image (Pawley, 1995).

CSLM has been used in a variety of food application. Food components such as proteins and lipids can be labeled selectively before processing of the product to reveal their microstructure viewed under CSLM. Certain stains such as Nile Blue and sulfonic acid can be used to show the dispersion of fat globules and protein network in cheese (Heerje, et al., 1987). Other stains such as FITC and Rhodamine have also been used as protein stains and Texas Red for lipid (Blonk and van Aalst, 1993). Hassan et al. (2002) observed structure of feta cheese using CSLM. CSLM technique has also been used to observe bacterial attachment to food (Prachaiyo and McLandsborough, 2000; Seo and Frank, 1999; Takeuchi and Frank, 2000). Takeuchi and Frank (2000) employed propidium iodide to distinguished the red to yellow non-viable cells from the viable green fluorescent *E. coli* O157:H7 to study the cell attachment on lettuce. Confocal microscopy is a nondestructive method that allows visualization of microcolony formation on spoiled pork muscle (Delaquis et al., 1992). Kim et al. (1996) observed *Salmonella* attachment using CSLM.

Channel catfish skin mucus

Fish skin is covered with mucus. The mucus is mainly mucopolysaccharides consists of epithelial surface cells (a complex of cell protoplasm), sloughed cells, and any

secreted goblet cell mucus. One of the functions of mucus is the protection against infection (Takashima and Hibiya, 1995) and acts in conjunction with cellular proliferation kinetics to continuously remove microorganisms from the surface (Speare and Mirasalimi, 1992). This type of protection is offered by entraping and continuously sloughing of the mucus which inhibits the colonization of the cuticle (Roberts, 2001). However, the immune response of teleost fish are appreciably less complex than those of higher animals. The efficiency of mucus against microorganism is limited, especially when the microorganism load is greater than what the host defense system can handle (Roberts, 2001).

REFERENCES

Abdul-Raouf, U. M., L. R. Beuchat, M. S. Amar. 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. Appl. Environ. Microbiol. 59:1999-2006.

Ackers, M. L., B. E. Mahon, E. Leahy, B. Goode, T. Damrow, P. S. Hayes, W. F. Bibb, D. H. rice, T. J. Barrett, L. Hutwagner, P. m. Griffin, and L. Slutsker. 1998. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. J. Infect. Dis. 177:1588-1593.

Andrews, W. H., C. R. Wilson, P. L. Poelma, and A. Romero. 1977. Bacteriological survey of the channel catfish (*Ictalurus punctatus*) at the retail level. J. Food Sci. 42:359-363.

Anonymous. 1992. Waterborne *E. coli* O157:H7 implicated in four deaths. Food Chem. News 33:71-72.

Anonymous. 2000. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May –June 2000. Can. Comm. Dis. Report 26:170-173.

Ayulo, A. M. R., R. A.. Machado, V. M. Scussel. 1994. Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. Int. J. Food Microbiol., 24:171-178.

Bean, N. H., J. S. Goulding, C. Lao, and F. J. Angulo. 1996. Surveillance for food-borne disease outbreaks US, 198-1992. MMWR 45.

Belongia, E. A., M. T. Osterlholm, J. T. Soler, D. A. Ammend, J. E. Braun, and K. L. MacDonald. 1993. Transmission of *Escherichia coli* O157:H7 in Minnesota child day-care facilities. JAMA 269:883-888.

Besser, R. E., S. M. Lett, J. T. Webber, M. P. Doyle, T. J. Barrett., J. G. Wells, and P. M. Griffin. 1993. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. JAMA 269:2217-2220.

Beutin, L., G. Geier, H. Steinrück, S. Zimmermann, and F. Scheutz. 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. 31:2483-2488.

Beveridge, M. C. M. 1988. Problems caused by birds at inland waters and freshwater fish farms *in* R. Welcome, Prevention and control of bird predation in aquaculture and fisheries. FAO Fish. Tech. Report Paper 51 (p54-73), Rome.

Black, E. K., and G. R. Finch. 1993. Detection and occurrence of waterborne bacterial and viral pathogens. Water Environ. Res. 65:295-300.

Blake, P. 1998. *Escherichia coli* O157:H7 outbreak among visitors to a water park. Abstract. 537. 36th Ann. Meet. Infec. Dis. Soc. Am., Denver, CO.

Blonk, J. C., and H. V. Aalst. 1993. Confocal scanning light microscopy in food research. Food Res. Int. 26:297-311.

Brewster, D. H., M. I. Brown, D. Robertson, G. L. Houghton, and J. C. M. Sharp. 1994. An outbreak of *Escherichia coli* O157 associated with a children's paddling pool. Epidemiol. Infect. 112, 441-447.

Brody, A. L. 1989. Controlled/modified atmosphere/vacuum packaging of foods. Food Nutrition Press, Inc. Princeton, NJ.

Brown, L. D. and C. R. Dorn. 1977. Fish, shellfish, and human health. J. Food Prot. 40:712-717.

Bryan, F. 1980. Epidemiology of foodborne disease transmitted by fish, shell fish and marine crustaceans in the United States, 1970-1978. J. Food Prot. 43:859-876.

Brewster, D. H., M. I. Brown, D. Robertson, G. L. Houghton, J. Bimson, J. C. Sharp. 1994. An outbreak of *Escherichia coli* O157 associated with a children's paddling pool. Epidemiol. Infect. 112:441-447.

Bui, T. Y., and B. T. Yen. 1992. Combined treatments and irradiation to reduce microbial contamination in dried cuttle fish. Proceedings of the final coordination meeting Bangkok, Thailand 31 Oct-4 Nov. 1988, p. 21-36.

Buras, N. 1993. Microbial safety of produce from wastewater-fed aquaculture, p. 285-295. *In* R. V. C. Pullin, H. Rosenthal, J. L. MacLean (eds.), Environment and aquaculture in developing countries. Proceedings of the 31st ICLARM Conference, Manila, Phillipines.

Cann, D. C. 1988. Modified atmosphere packaging of fishery products. INFOFISH Int. 1, 37-39.

Carter A. O., A. A. Borczyk, J. A. K. Carlson, B. Harvey, J. C. Hockin, M. A. Karmali, C. Krishnan, D. A. Korn, and H. Lior. 1987. A severe outbreak of *Escherichia coli* O157:H7-associated hemorrhagic colitis in a nursing home. New Eng. J. Med. 317:1496-1500.

Catfish Institute. 2002. Quick catfish facts: aquaculture. www.catfishinstitute.com.

Center for Disease Control and Prevention. 1995a. Outbreak of acute gastroenteritis attributable to *Escherichia coli* O104:H21-Helena, Montana, 1994. MMWR 44:501-503.

Center for Disease Control and Prevention. 1995b. Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM-south Australia, 1995. MMWR 44:550-558.

Center for Disease Control and Prevention. 1995c. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami-Washington and California, 1994. MMWR 44:157-159.

Center for Disease Control and Prevention. 1997. Outbreak of *Escherichia coli* O157:H7 infection associated with eating alfalfa sprouts-Michigan and Virginia, June-July 1997. MMWR 46:741-744.

Center for Disease Control and Prevention. 2001. Diagnosis and Management of Foodborne Illnesses. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5002a1.htm. Accessed 10 October 2002.

Chalmers, R. M., H. Aird, F. J. Bolton. 2000. Waterborne *Escherichia coli* O157:H7. J. Appl. Microbiol. 88S:124S-132S.

Chapman, P. A., C. A. Siddons, A. T. Gerdan Malo, and M. A. Harkin. 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs, and poultry. Epidemiol. Infect. 119, 245-250.

Center for Science in the Public Interest. 2001. Outbreak Alert ! Available at http://www.cspinet.org/new/outbreak_alert2001.html. Accessed 3 December 2002.

Dalsgaard, D. 1998. The occurrence of human pathogenic *Vibrio* spp. and *Salmonella* in aquaculture. Int. J. Food Sci. Tech. 33:127-138.

D'Aoust, J. Y., R. Gelinas, and C. Maishment. 1980. Presence of indicator organisms and recovery of *Salmonella* in fish and shellfish. J. Food Prot. 43:679-682.

Davies, A. R. 1995. Fate of foodborne pathogens on modified atmosphere packaged meat and fish. Int. Biodet. Biodeg. 36 (3-4): 407-410.

Davies, A. R. and A. Slade. 1995. Fate of *Aeromonas* and *Yersinia* on modified atmosphere packaged (MAP) cod and trout. Lett. App. Microbiol. 21(6):354-358.

Delaquis, P. J., C. Gariepy, and D. Montpetit. 1992. Confocal scanning laser microscopy of porcine muscle colonized with meat spoilage bacteria. Food Microbiol. 9:147-153.

Dell'Omo, G., S. Morabito, R. Quondam, U. Agrimi, F. Ciuchini, A. Macri, and A. Caprioli. Feral pigeons as a source of verocytotoxin-producing *Escherichia coli*. Vet. Rec. 142, 309-310.

Dev, V. J., M. Main, and I. Gould. 1991. Waterborne outbreak of *Escherichia coli* O157:H7. Lancet 337, 1412.

Doyle, M. P. 1991. *Escherichia coli* O157:H7 and its significance in foods. Int. J. Food Microbiol. 12:289-302.

Doyle, M. P. and J. L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48:855-856

Effler P, M. Isaacson, L. Arntzen, R. Heenan, P. Canter, T. Barrett, L. Lee, C. Mambo, W. Levine, A. Zaidi, P. M. Griffin. 2001. Factors contributing to the emergence of *Escherichia coli* O157 in Africa. Emerg. Infect. Dis. 7:812-819.

FAO, 1999. The state of world fishereies and aquaculture 1998. FAO, Rome, Italy.

Fapohunda, A. O., K. W. McMillin, D. L. Marshall, and W. M. Waites. 1994. Growth of selected cross-contaminating bacterial pathogens on beef and fish at 15 and 35°C. J. Food Prot. 57:337-340.

Farmer, J. I., and B. R. Davis. 1985. H7 antiserum-sorbitol fermentation medium: a single tube screeening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. 22:620:625.

Fattal, B., A. Dotan, Y. Tchorsch. 1992. Rates of experimental microbiological contamination of fish exposed to polluted water. Water Res. 26:1621-1627.

Feng, P. 1995. *Escherichia coli* serotype O157:H7: Novel vehicles of infection and emergence of phenotypic variants. Emerge. Infect. Dis. 1:47-52.

Fernandez, C. F., G. J. Flick Jr., J. Silva, and T. A. McCaskey. 1997a. Influence of processing schemes on indicative bacteria and quality of fresh aquacultures catfish fillets. J. Food Prot. 60:54-58.

Fernandez, C. F., G. J. Flick Jr., J. Silva, and T. A. McCaskey. 1997b. Comparison of quality in aquacultured fresh catfish fillets II. Pathogens *E. coli* O157:H7, *Campylobacter*, *Vibrio*, *Plesiomonas*, and *Klebsiella*. J. Food Prot. 60:1182-1188.

Fletcher, T. C. and A. White. 1973. Lysozyme activity in the plaice (*Pleuronectes platessa*). Experentia 29:1283-1285.

Friedman M.S., T. Roels, J. E. Koehler, L. Feldman, W. F. Bibb, P. Blake. 1999. *Escherichia coli* O157:H7 outbreak associated with an improperly chlorinated swimming pool. Clin. Infect. Dis. 29:298-303

Garrett, E. S., M. L. Jahncke, J. M. Tennyson. 1997. Microbiological hazards and emerging food safety issues associated with seafoods. J. Food Prot. 60:1409-1415.

Genigeorgis, C. A. 1985. Microbial and safety implications of the use of modified atmospheres to extend the storage life of fresh meat and fish. Int. J. Food Microbiol. 1:237-251.

Ghittino, P. 1972. Aquaculture and associated diseases of fish of public health importance. J. Am.Vet. Med. Assoc. 161:1476-1485.

Glass, K. A., J. M. Loeffelholz, J. P. Ford, and M. P. Doyle. 1992. Fate of *Escherichia coli* O157:H7 as affected by pH or sodium chloride and in fermented, dry sausage. Appl. Environ. Microbiol. 58:2513-2516.

Griffin, P. M. and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* 0157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60-98.

Hancock, D. D., T. E. Besser, M. L. Kinsel, P. I. Tarr, D. H. Rice, and M. G. Paros. 1994. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol. Infect 113:199-207.

Hancock, D. D., T. E. Besser, D. H. Rice, E. D. Ebel, D. E. Herriot, and L. V. Carpenter. 1998. Multiple sources of *Escherichia coli* O157:H7 in feedlots and dairy farms in the Northwestern USA. Prevent. Vet. Med. 35:11-19.

Hassan, A. N., J. F. Frank, and M. Corredig. 2002. Microstructure of feta cheese made using different cultures as determined by confocal scanning laser microscopy. J. Food Sci. 67:2750-2753.

Hatha, A. A. M. and P. Lakshmanaperumalsamy. 1997. Prevalence of Salmonella in fish and crustaceans from markets in Coimbatore, South India. Food Microbiol. 14:111-116.

Heerje, I., P. van der Vlist, J. C. G. Blonk, H. A. C. M. Hendrickx and G. J. Brakenhoff. 1987. Confocal scanning laser microscopy in food research. Food Microstruc. 6: 115-120.

Heil, S., and T. W. Peck. 1998. Encyclopedia of American industries vol. 1, 2nd ed. Gale Research, Detroit, MI.

Hilborn E. D., J. H. Mermin, P. A. Mshar, J. L. Hadler, A. Voetsch, C. Wojtkunski, M. Swartz, R. Mshar, M. A. Lambert-Fair, J. A. Farrar, M. K. Glynn, L. Slutsker.

1999. A multistate outbreak of *Escherichia coli* O157:H7 infections associated with consumption of mesclun lettuce. Arch. Internal Med. 159 (15): 1758-1764.

Himelbloom, B. H., E. K. Brown, J. S. Lee. 1991. Microorganisms on commercially processed Alaskan finfish. J. Food Sci. 56:1279-1281.

Holland, G. C. 1980. Modified atmosphere for fresh meat distribution. Proc. Meat Industry Research Conference, pp. 21-39.

Howgate, P. 1998. Review of the public health safety of products from aquaculture. Internat. J. Food Sci. Tech. 33:99-125.

Huss, H. H. 1997. Control of indigenous pathogenic bacteria in seafood. Food Control 8: 91-98.

Huss, H. H., A. Reilly, and P. K. B. Embarek. 2000. Prevention and control of hazards in seafoods. Food Control 11:149-156.

Iyer, T. S. G., K. P. Shrivastava. 1989. On the pattern of *Salmonella* serotypes in fishery products, frog legs and processing environments. Fish. Tech. 26:131-136.

Jay, J. M. 1996. Modern food microbiology, 5th edition, Chapman and Hall, New York, NY.

Karch, H., J. Heesemann, R. lauffs, A. D. O'Brien, C. O. Tacket, and M. M. Levine. 1987. A plasmid of enterohemorrhagic *Escherichia coli* O157:H7 is required for expression of a new fimbrial antigen and for adhesion to epithelial cells. Infect. Immun. 55:455-461.

Keene, W. E., J. M. McAnulty, F. C. Hoesly, L. P. Williams, K. Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. W. Fleming. 1994. A swimming associated

outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. N. Engl. J. Med. 331-579-584.

Keene, W. E., E. Sazie, J. Kok, D. H. Rice, D. D. Hancock, V. K. Balan, T. Zhao, and M. P. Doyle. 1997. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. JAMA 277:1229-1231.

Koenraad, P. M. F. J., R. Ayling, W. C. Hazeleger, F. M. Rombouts, and D. G. Newell. 1995. The speciation and subtyping of *Campylobacter* isolates from sewage plants and waste water from connected poultry abattoir using molecular techniques. Epidemiol. Infect. 115:485-494.

Kim, K. Y., J. F. Frank, and S. E. Craven. 1996. Three dimensional visualization of Salmonella attachment to poultry skin using confocal scanning laser microscopy. Lett. Appl. Microbiol. 22: 280-282.

Kudva, I. T., P. G. Hatfield, and C. J. Hovde. 1996. *Escherichia coli* O157:H7 in microbial flora of sheep. J. Clinical Microbiol. 34:431-433.

Lee, P. K. 1972. Role of Crucian carp in transmission of enteric pathogens. J. Cath. Med. Coll. 23:379.

Leung, C. K., Y. W. Huang, and O. C. Pancorbo. 1992. Bacterial pathogens and indicators in catfish pond environments. J. Food Prot. 55:424-427.

Lobrerra, T. A., L. M. Bulalacao, and A. Tan. 1990. Effect of farming phase in the in-plant processing on the microbiological quality of prawns (*Panaeus monodon*). FAO Fisheries report Supplement 401, Rome.

Lynn, T. V., D. D. Hancock, T. E. Besser, J. H. Harrsion, D. H. Rice, N. T. Stewart, and L. L. Rowan. 1998. The occurrence and replication of *Escherichia coli* in cattle feeds. J. Dairy Sci. 81:1102-1108.

MacDonald, K. L., M. J. O'Leary, M. L. Cohen, P. Norris, J. G. Wells, E. Noll, J. M. Kobayashi, and P. A. Blake. 1988. *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen. JAMA 259:3567-3570.

Maule, A. 2000. Survival of verocytotoxigenic *Escherichia coli* O157:H7 in soil, water and on surface. J. Appl. Microbiol. Symp. Supple. 88:71S-78S.

McCaskey, T., T. C. Hannah, T. Lovell, J. L. Silva, C. E. Fernandes, and G. Flick. 1999. Study shows catfish to be low risk for foodborne illness. Feedstuffs, March 29.

McGowan, K. L., E. Wickersham, and N. A. Strocbine. 1989. *Escherichia coli* 0157:H7 in water. Lancet I:967-968.

Merson, M. H., G. K.Morris, D. A. Sack, J. G. Wells, J. C. Freeley, R. B. Sack, W. B. Creech, A. Z. Kapikian, and E. J. Gangarosa. 1976. Travelers' diarrhea in Mexico. N. Engl. J. Med 294:1299-1305.

Meyer, F. P., and G. L. Bullock. 1973. *Edwardsiella tarda*, a new pathogen of channel catfish. Appl. Microbiol. 25:155.

Montenegro, M. A., M. Bülte, T. Trumpf, S. Aleksic, G. Reuter, E. Bulling, and R. Helmuth. 1990. Detection and characterization of fecal verotoxin producing *Escherichia coli* O157:H7 from healthy cattle. J. Clin. Microbiol.28:1417-1421.

Moore, A. C., B. L. Herwalt, G. F. Craun, R. L. Calderon, A. K. Highsmith, and D. D. Juranek. 1993. Surveillance for waterborne disease outbreaks. MMWR CDC Surveillance Summary 42:1-22.

Morgan, D., C. P. Nawman, D. N. Hutchinson, A. M. Walkes, B. Rowe, and F. Maijd. 1993. Verotoxin producing *Escherichia coli* O157:H7 infections associated with the consumption of yoghurt. Epidemiol. Infect. 111:181-187.

Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli* O157:H7. Clin. Microbiol. Rev. 11:142-201.

National Fisheries Institute. 2002. About our industry. Available at http//www.nfi.org. Accessed 13 January 2002.

National Advisory Committee on Microbiological Criteria for Foods. 1992. Vacuum or Modified Atmosphere Packaging for Refrigerated Raw Fishery Products. Executive secretariat, Food Safety and Inspection Service, USDA, Washington, D.C.

Nilsson, L., H. H. Huss, L. Gram. 1997. Inhibition of *Listeria monocytogenes* on cold smoked salmon by nisin and carbon dioxide. Int. J. Food Microbiol. 38:217-227.

Nychas, G. J. E. and C. C. Tassou. 1996. Growth/survival of *Salmonella enteritidis* on fresh poultry and fish stored under vacuum or modified atmosphere. Lett. Appl. Microbiol. 23:115-119.

O'Brien, A. D. and R. K. Holmes. 1987. Shiga and Shiga-like toxins. Microbiol. Rev 51:206-220.

Ostroff, S. M., J. M. Kobayashi, and J. H. lewis. 1989. Infections with *Escherichia coli* O157:H7 in Washington State. JAMA 262:355-359.

Paludan, M. C., P. Dalgaard, H. H. Huss, L. Gram. 1998. Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum and modified atmosphere packed cold salmon stored at 5C. Int. J. Food Microbiol. 39:155-156.

Park, S., R. W. Worobo, and R. A. Durst. 1999. *Escherichia coli* O157:H7 as an emerging foodborne pathogen: a literature review. Crit. Rev. Food Sci. Nutr. 39:481-502.

Pawley, J. B. 1995. Handbook of biological confocal microscopy. Plenum Press. New York, NY.

Porter, J., K. Mobbs, C. A. Hart, J. R. Saunders, R. W. Pickup, and C. Edwards. 1997. Detection, distribution and probable fate of *Escherichia coli* O157 from asymptomatic cattle on a dairy farm. J. Appl. Microbiol. 83:297-306.

Prachaiyo, P., and L. A. McLandsborough. 2000. A microscopic method to visualize *Escherichia coli* interaction with beef muscle. J. Food Prot. 63:427-433.

Quines, O. D. 1988. Microorganisms: indicators of pollution in integrated livestock-fish farming systems. Environ. Internat. 14:531-534.

Rahn, K., S. A. Renwick, R. P. Johnson, J. B. Wilson, R. C. Clarke, D. Alves, S. McEwen, H. Loir, J. Spika. 1997. Persistence of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment. Epidemiol. Infect. 119:251-259.

Ratnam, S., S. B. March, R. Ahmed, G. S. Bezanson, and S. Kasatiya. 1988. Characterization of *Escherichia coli* serotype O157:H7. J. Clin. Microbiol. 26:2006-2012.

Reddy, N. R., D. J. Armstrong, E. J. Rhodehamel, and D. A. Kautter. 1992. Shelf-life extension and safety concerns about fishery products packaged under modified atmospheres: a review. J. Food Safety 12:87-118.

Reilly, A. and F. Käferstein. 1997. Food safety hazards and the application of the principles of hazard analysis and critical control point (HACCP) system for their control in aquaculture production. Aqua. Res. 28:735-752.

Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGeee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake and M. L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. New Eng. J. Med. 308:681-685.

Rice, E. W., C. H. Johnson, D. K. Wild, and D. J. Reasoner. 1992. Survival of *Escherichia coli* O157:H7in drinking water associated with a waterborne disease outbreak of hemorrhagic colitis. Lett. App. Microbiol. 15:38-40.

Roberts, R. J. 2001. Fish pathology. W. B. Saunders. London.

Robertson, G. L. 1993. Food packaging:principles and practice. Marcel and Dekker, Inc. New York, NY.

Saheki, K., S. Kobayahshi, and T. Kawinishi. 1989. *Salmonella* contamination of eel culture ponds. Nipp. Suis. Gak. 55:675-679.

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. App. Env. Microbiol. 60:1038-1040.

Seo, K. H., and J. F. Frank. 1999. Attachment of Escherichia coli to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. J. Food Prot. 62:3-9.

Silva, J. L., and T. D. White. 1994. Bacteriological and color changes in modified atmosphere-packaged refrigerated channel catfish. J. Food Prot. 57:715-719.

Silvakami, R., G. Premkishore, M. R. Chandran. 1996. Occurrence and distribution of potentially pathogenic *Enterobacteriaceae* in carps and pond water in Tamil Nadu, India. Aqua. Res. 27:375-378.

Smith, J., B. Simpson, and A. Lambert. 1988. Use of modified atmospheres for shelf-life extension of food. Food Sci. Tech. Today 2:250-255.

Solomon E. B., C. J. Potenski, and K. R. Matthews KR. 2002a. Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on lettuce. J. Food Prot. 65: 673-676.

Solomon E. B., S. Yaron, and K. R. Matthews. 2002b. Transmission of Escherichia coli O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. App. Env. Microbiol. 68:397-400.

Speare, D. J. and S. M. Mirasalimi. 1992. Pathology of the mucous coat of trout skin during an erosive bacterial dermatitis. J. Comp. Path. 106:210-211.

Stammen, K., D. Gerdes, F. Carpaso. (1990). Modified atmosphere packaging of seafood. Food Sci. Nut. 29:301-331.

Suhalim, R., Y. W. Huang, and G. Burtle. 2000. Survival of *Escherichia coli* O157:H7 in channel catfish pond water. Abstract at the International Association For Food Protection Annual Meeting, August 6-9, 2000, Atlanta, GA.

Swerdlow, D. L., B. A. Woodruff, R. C. Brady. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann. Intern. Med. 117:812-819.

Takashima, F., and T. Hibiya. 1995. An atlas of fish histology, 2nd edition. Kodansha Ltd., Tokyo, Japan.

Takeuchi, K., and J. F. Frank. 2000. Penetration of Escherichia coli O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. J. Food Prot. 63:434-440.

Troast, J. L. 1975. Antibodies against enteric bacteria in brown bullhead catfish inhabiting contaminated waters. App. Microbiol. 30: 189.

USDA. 1999. Food safety facts. Available at www.foodsafety.gov/~fsg/fs-cook.html. Accessed 13 January 2001.

Wallace, J. S., T. Cheasty, and K. Jones. 1997. Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds. J. Appl. Microbiol. 82:399-404.

Wang, G., and M. P. Doyle. 1998. Survival of Enterohemorrhagic *Escherichia coli* O157:H7 in water. J. Food Prot. 61:662-667.

Wang, G., T. Zhao, and M. P. Doyle. 1996. Fate of enterohemorrhagic *Escherichia coli* O157:H7 in bovine feces. Appl. Environ. Microbiol. 62:2567-2570.

Warburton, D. W., J. W. Austin, B. H. Harrison, and G. Sanders. 1998. Survival and recovery of *Escherichia coli* O157:H7 in inoculated bottled water. J. Food Prot. 61:948-952.

Wentz, B. A, A. P. Duran, A. Swartzentruber, A. H. Schwab, R. B. Read. 1984. Microbiological quality of frozen breaded onion rings and tuna pot pies. J. Food Prot 47:58-60.

Wyatt, L., R. Nichelson, and C. Vanderzant. 1979. Occurrence and control of *Salmonella* in freshwater catfish. J. Food Sci. 44:1067-1069, 1073.

Yambrach, F. 1987. Modified atmosphere storage of fresh seafoods. J. Pack. Tech. 1:154-156.

Youseff, H., A. K. El-Timawy, and S. Ahmed. 1992. Role of aerobic intestinal pathogens of fresh water fish in transmission of human diseases. J. Food Prot. 55: 739-740.

Zhao, T., M. P. Doyle, J. Shere, and L. Garber. 1995. Prevalence of enterohemorrhagic *Escherichia coli* O157:H7 in a survey of dairy herds. Appl. Env. Microbiol.61:1290-1293.

SURVIVAL OF *ESCHERICHIA COLI* O157:H7 ON CHANNEL CATFISH AND IN POND AND HOLDING TANK WATER

Suhalim, R., Y. W. Huang, and G. Burtle. To be submitted to Journal of Food Protection

ABSTRACT

Survival of *Escherichia coli* O157:H7 in channel catfish (*Ictalurus punctatus*), pond and holding tank water was investigated. Water from three channel catfish ponds was inoculated with ampicillin/nalidixic acid-resistant *E. coli* O157:H7 transformed with a plasmid encoding for green fluorescent protein (GFP) at 10^5, 10^6, and 10^7 CFU/ml. Samples were taken from surface, internal organs, and skin scrape of fish and pond water for *E. coli* O157:H7 enumeration on BHI agar containing ampicillin and nalidixic acid. To determine the survival of *E. coli* O157:H7 in catfish holding tank water from two farmers markets the water was inoculated with 10^7 *E. coli* O157:H7 CFU/ml.

E. coli O157:H7 were detected by direct plating for 33 d and 69 d in pond and holding tank water, respectively. A rapid decrease of the pathogen was observed in the first two weeks to reach 2 log CFU/ml. When *E. coli* O157:H7 was not recovered by direct plating, the pathogen was isolated by enrichment in TSB for approximately another 30 d from pond and holding tank water.

The populations of *E. coli* O157:H7 found in the internal organs and skin scrape were 5.5 log and 2.5 log CFU/ml, respectively. *E. coli* O157:H7 from internal organs and water were recovered for at least 12 d. Results suggest that *E. coli* O157:H7 can survive in channel catfish pond and holding tank water and channel catfish may become a potential carrier of the pathogen.

INTRODUCTION

Several human pathogenic bacteria have been associated with water outbreaks: *Aeromonas* spp., *Campylobacter* spp., *Clostridium perfringens*, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Yersinia enterocolitica*, enterococci, *Helicobacter pylori*, *Legionella* spp., *Plesiomonas shigelloides*, *Pseudomonas aeruginosa,* and *Escherichia coli* O157:H7 (*15*). *Escherichia coli* O157:H7 has been recognized as an important foodborne pathogen causing three principal manifestations of illness, hemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (*16*). The pathogen has been detected from cattle, dogs, sheep, birds, deer, and humans (*1*), although most of the outbreaks have been primarily linked to the consumption of undercooked ground beef and raw milk (*5*).

Investigations have also led to the isolation of *E. coli* O157:H7 from both drinking and recreational water (*2*). Several outbreaks associated with drinking reservoir, well water and swimming in recreational lakes have been documented (*9, 20, 21*). McGowan et al. (*14*) first isolated *E. coli* O157:H7 from a countryside reservoir in 1986. The largest waterborne outbreak in South Africa suggested that *E. coli* O157:H7 of bovine origin may be responsible for the contamination of drinking, recreational, and irrigation water. Survival of *E. coli* O157:H7 in lake water was observed up to 4 weeks (*21*).

The culture system of many freshwater fishes, especially channel catfish (*Ictalurus punctatus*), in the southern U.S. depends on water sources including lakes, reservoirs and water from run-offs. *E. coli* O157:H7 contamination may be introduced

into catfish pond water through several routes. Catfish ponds are commonly located within the proximity of farm/pasture land in which cattle carriers of *E. coli* O157:H7 may be present. Occasional flooding following a substantial precipitation, run-off from irrigation water and application of manure in the farm land could reach into the pond area where fish for human consumption are commercially raised. Cattle may also directly introduce *E. coli* O157:H7 through feces into the pond water by grazing around the pond and/or wading in the water. Additionally, wildlife and birds may introduce the pathogens around the farm environment as a result of their feeding habits. Wild birds have been known as a source of *E. coli* O157 and other pathogenic organisms (*20*). Fish from such contaminated culture system may be harvested for human food where it is sold live at farmers markets. The objective of this study was to investigate the survival of *E. coli* O157:H7 in channel catfish pond water, holding tank water, and channel catfish.

MATERIALS AND METHODS

Inocula

A mixture of five strains of *E. coli* O157:H7 E0143 (meat isolate), K262 (human isolate), E0139 (beef jerky isolate), C0083 (cattle isolate), and E318 (ground beef isolate) was used in this study. All strains were transformed with a plasmid encoding for green fluorescent protein (GFP) and ampicillin resistant gene (courtesy of Dr. Jinru Chen and Dr. Mike Doyle). Resistance to nalidixic acid was done through point mutation technique. Each strain was grown separately in brain heart infusion (BHI) (Difco, Detroit, MI) broth containing 100 μg/ml ampicillin (Sigma Chemical Co., St. Louis, MO) and 100

µg/ml nalidixic acid (Aldrich Chemical Co. Milwaukee, WI) at 37ºC for 18 h. Culture broth was centrifuged and washed twice in 0.1% peptone water (Difco). Inocula were diluted in 0.1% peptone water before enumeration by surface plating in duplicate on BHI agar containing the same concentrations of ampicillin and nalidixic acid as the BHI broth. Plates were incubated at 37ºC for 18 h. Fluorescent colonies were observed under long-wave ultraviolet lamp (320-380 nm) and confirmed with *E. coli* O157 agglutination test (Oxoid Limited, Hampshire, England).

Catfish pond water

Surface water was obtained from three channel catfish pond at the University of Georgia Research Station, Tifton, GA. Water (200 ml) was placed in 500 ml autoclaved Erlenmeyer flasks and was inoculated with 10^5, 10^6, and 10^7 CFU/ml of *E. coli* O157:H7 and held at 28ºC. Aeration was applied using home aquarium aerator pump and airstones. Water was sampled every three days, diluted with 0.1% peptone water and spirally plated on BHI agar supplemented with 100 µg naladixic acid and 100 µg ampicillin, and incubated for 48 h at 37ºC. When the pathogen was not detectable by direct plating technique, samples were enriched using Tryptic Soy Broth (TSB, Difco) supplemented with ampicillin and nalidixic acid followed by plating on BHI agar with naladixic acid and ampicillin, and incubated for 18-24 h at 37ºC. Aerobic plate counts (APCs) were enumerated on plate count agar (PCA; Difco) incubated for 48 h at 37°C. Fecal coliform counts were done on mFC agar (Difco) incubated for 24 h at 44°C. Water samples were analyzed for physical and chemical characteristics by the Chemical Analysis Laboratory of the University of Georgia (Table 1.1).

Channel catfish

Channel catfish fingerlings (average 7 cm) were obtained from the University of Georgia Research Station, Tifton, GA. Fish were placed in a 10-gallon aquarium equipped with aeration, and fed at a level of 2% body weight. Water was inoculated with E318 strain *E. coli* O157:H7 at 10^6 CFU/ml. Upon sampling, a net was used to remove the fish from the tank and the net was shaken to remove excess water. Fish were placed in stomacher bags and sacrificed by a sharp blow to the cranial region. Fish were weighed and rinsed in peptone water to give a 1:2 dilution. Skin scrape was obtained using a sterile surgical blade. Internal organs (kidney, liver, digestive tract, and gills) were aseptically removed, pooled and homogenized. Serial dilutions were made in 0.1% peptone water. Water, peptone wash, skin scrape, and internal organ samples were taken daily and plated on BHI containing naladixic acid and ampicillin. Plates were incubated as previously mentioned.

Holding tank water

Channel catfish holding tank water from two farmers markets in Atlanta, GA area was inoculated with 10^7 cfu/ml of a mixture of five strains *E. coli* O157:H7 mentioned earlier. The water profile is shown in Table 1.1 (courtesy of Dekalb County Public Works-Water and Sewer). Water was aerated and held at 15±2ºC (market 1) or 20±2ºC (market 2) according to the holding tank water temperature from the two markets where the live channel catfish were held. Holding tank water was analyzed for *E. coli* O157:H7 in the same manner as the pond water sample mentioned in the previous section.

Statistical analysis

The *E. coli* O157:H7 values were averaged over the three ponds and regression analysis was used to regress the average response value as a function of time. Quadratic regression equations were fit separately for each concentration and the regressions were compared using tests of homogeneity of parameter estimates. The analyses were conducted using SAS (SAS Institute, Inc., Cary, NC).

Holding tank water data were analyzed using the SAS General Linear Models Analysis of variance procedure. Duncan's multiple range test was used to determine differences among means when significant effects were observed ($P<0.05$). All microbiological tests were replicated three times.

RESULTS

Pond water sample

The physical and chemical properties of the three pond waters are shown in Table 1.1. There was no appreciable difference in water quality between the 3 pond waters that would significantly influence the survival of *E. coli* O157:H7. Similar levels of fecal coliform and total aerobic plate counts were observed in all three pond waters (Table 1.2). Consequently, the *E. coli* O157:H7 data from the three ponds were averaged at each inoculation levels.

The shortest *E. coli* O157:H7 survival was noted in pond water inoculated with 10^5 CFU/ml where the pathogen was recovered for up to 24 d by direct plating (Figure

1.1). *E. coli* O157:H7 survived the longest in pond water inoculated at 10^7 and 10^6 CFU/ml in which the pathogen decreased to an undetectable level by direct plating after 33 d. The rate of *E. coli* O157:H7 population decline was not significantly different between the 3 inoculation levels over the study period. An initial significant decline was observed in pond water inoculated with 10^7 CFU/ml compared to the other levels of inoculation.

In general, a rapid (≈ 3-4 log) reduction of *E. coli* O157:H7 was observed for the first 12 d. Following the 12 days the pathogen population stabilized at ≤ 2 log CFU/ml for approximately 2 more weeks and decreased to an undetectable level by direct plating. Enrichment of water samples when no colony was detected by direct plating revealed positive results for several more weeks (Figure 1.1). In contrast to the survival of *E. coli* O157:H7, the total aerobic bacteria decreased by an average of only 1-2 log within 21 days (Figure 1.2), while *E. coli* O157:H7 population was reduced to an undetectable level within the same time period.

Fish sample

E. coli O157:H7 level recovered from peptone rinses and internal organs decreased in similar manner to those in pond water (Figure 1.3). *E. coli* O157:H7 count decreased to undetectable level by direct plating after 12 d for peptone water rinse and pond water. A maximum population of 2.5 log CFU/ml of fluorescent *E. coli* O157:H7 was observed from skin scrape samples. *E. coli* O157:H7 populations isolated from internal organs (kidney, liver, and digestive tract) samples reached 5.5 log CFU/ml) after 2 d and were recovered for at least 12 d (Figure 1.3).

Holding tank water

Water quality results are shown in Table 1.1. The two farmers markets are located in the same county and received water from the same source (*4*). Table 1.2 shows the bacteriological characteristics of holding tank water from the two fish markets in Atlanta, GA. Water obtained from farmers market #2 had higher fecal coliform and total aerobic plate count than farmers market 1. *E. coli* O157:H7 count decreased rapidly for the first 2 weeks of inoculation (Figure 1.4), as also observed in pond water. The pathogen was detected in water for 70 d from market 1 while it was not detected by direct plating after 30 d in market 2. There was a significant difference ($P<0.05$) in the survival of *E. coli* O157:H7 between the water of the two markets (Figure 1.4). On average, *E. coli* O157:H7 in market 1 (held at 15°C) was detected at approximately 1.2 log CFU/ml higher than those detected in market 2 held at 20°C throughout the sampling period.

DISCUSSION

E. coli O157:H7 was detected in channel catfish pond water up to 2 months. A rapid die-off of the pathogen was seen for the first 12 d followed by a constant level (approximately 2 log CFU/ml) in the *E. coli* O157:H7 count for a maximum of 30 d. This low (approximately 2 log CFU/ml) level of *E. coli* O157:H7 observed may represent a natural level of *E. coli* O157:H7 contamination in pond water. *E. coli* O157:H7 was observed to survive at low levels in pond water detected by direct plating for a period of time. This study also showed that the rate of *E. coli* O157:H7 decline was not significantly affected by the levels of inoculation.

E. coli O157:H7 continued to be detected by enrichment method for several more weeks given the right condition for its survival. Outdoor fish ponds are potentially continually exposed to contamination and pathogens, such as *E. coli* O157:H7, which may be introduced daily into the water, thus keeping the pathogen level constant. Daily fluctuation in temperature and other environmental factors may also influence the pathogen survival. In a fish pond, *E. coli* O157:H7 may also enter into the sediment level at the bottom of the pond and survive. *E. coli* O157 was isolated from water-trough sediments in dairy farms in the Northwestern U.S. (*8*).

The total aerobic plate counts were constantly at a high population (5-6 log CFU/ml) throughout the period of observation as compared to the *E. coli* O157:H7 population that was reduced to 1-2 log CFU/ml after the third week (Figure 2). The rapid reduction of *E. coli* O157:H7 population may be an indication of the inability of the pathogen to compete with the resident microflora of the pond water. A previous study using one strain of *E. coli* O157:H7 revealed that the pathogen survived for approximately 2 weeks in pond water (*17*). This short survival rate may suggest that the interaction between strains affords the extended survival of the pathogens observed in the present study.

The pathogen in fish samples (peptone wash water, internal organ, and skin scrape) was reduced to low levels at approximately the same time as the pathogen reduction in the water. The high population recovered from the peptone wash may have been due to the loosely attached *E. coli* O157:H7 cells on the surface of the fish skin. However, *E. coli* O157:H7 that were still present on the thin layer of water surrounding the fish upon sampling could not be discounted. The two factors may have contributed to

the plate counts. The *E. coli* O157:H7 found on the skin scrape sample may have been contributed by the firmly attached cells. Continual shedding of mucus could have explained the low level of *E. coli* O157:H7 present. Fish continually slough off mucus as a natural defense mechanism to prevent colonization by microorganisms. The effect of higher level of *E. coli* O157:H7 inoculation on live fish skin was not investigated. Data in Figure 3 showed that the pathogen was internalized by fish shortly after inoculation into the water. High numbers of *E. coli* O157:H7 were detected from the internal organs. A similar pattern was observed earlier on tilapia exposed to polluted water containing *E. coli* (*6*). The high numbers of pathogen in the internal organs can cause a potential hazard. The pathogen can be spread onto the fish and into the food processing environment either at the plant or retail level.

There were some differences between the water-only observation and the study involving fish. Fish introduced in the fish-water study released organic matter as waste into the water which may have contributed to the increase of the competing aquatic microflora. Organic matter could also come from the feed. Unlike a more dynamic natural pond setting, this study was done in a closed system which could have led to a faster deterioration of water quality. These conditions could have had an adverse effect to the survival ability of the pathogen.

Data in Figure 4 shows the survival of *E. coli* O157 for up to 30 d in water from market 2 while *E. coli* O157:H7 inoculated in water from farmers market 1 survived for >50 d. The two markets received water from the same source and although some water quality data were lacking, the available data were comparable to the pond water. The ability of *E. coli* O157 to maintain longer and higher level of survival in water from

market 1 may have been partly due to the lower holding temperature. Holding tank water temperature from market 1 was about 5º lower than the water temperature in market 2. The pathogen isolated from market 1 water was approximately on average 1.2 log higher than that in market 2 water. A rapid decrease in *E. coli* O157:H7 population was observed in lake water incubated at 25°C, while longer survival was maintained at 15°C (*21*). In the farmers market, the constant water temperature may provide condition for prolonged survival of the pathogen as compared to the fluctuation in outdoor pond water. The shorter *E. coli* O157:H7 survival in water from market 2 may have also been due to the competition with background microflora which was at higher level than in market 1. At low temperature Wang and Doyle (*21*) observed that *E. coli* O157:H7 can persist for a longer period of time in water. It was also indicated that the pathogen survives better in pure water than in lake or reservoir water with higher microbial counts. The pathogen can survive in bottled water for up to >300 days (*22*). Several factors can influence the survival rate of *E. coli* O157:H7 in the water. Indigenous microorganisms which act as competitors in pond water may become a limiting factor that contributes to the rapid decrease of *E. coli* O157:H7 in the water. In addition, temperature, high salinity, compounds found in the agricultural surface water, and presence of chemicals including fertilizer, pesticides, and herbicides reduced survival of *E. coli* O157:H7 (*10, 19*).

ACKNOWLEDGEMENTS

We are grateful to Mr. Travis Ingram, Department of Animal and Dairy Science, The University of Georgia, for his assistance in providing the catfish and pond water, and

Dr. Glenn Ware, School of Forest Resources, The University of Georgia for statistical analysis.

REFERENCES

1. Armstrong, G. L., J. Hollingsworth, and J. G. Morris. 1996. Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. Epidemol. Rev. 18:29-51.
2. Binder, H. J. 1995. A newly recognized source of *Escherichia coli* O157:H7 infections. Gastroenterol. 108:1597-1602.
3. Colwell, R. R., P. R. Brayton, D. J. Grimes, D. R. Rozak. S. A. Huq, and L. M. Palmer. 1985. Viable but non-culturable *V. cholerae* and related pathogens in the environment: implication for release of genetically engineered microorganism. Bio/Tech. 3:817-820.
4. Dekalb County Public Works-Water and Sewer. 2002. Water Quality Standards of the Safe Drinking Water Act. Available at http://projects.ch2m.com/dekalb. Accessed 13 August 2002.
5. Doyle, M. P. T. Zhao, J. Meng, and S. Zhao. 1997. *Escherichia coli* O157:H7 *in* Food microbiology : fundamentals and frontiers, M. P. Doyle, L. R. Beuchat, T. J. Montville, eds. ASM Press, Washington, DC.
6. Fattal, B., A. Dotan, and Y. Tchorsh. 1992. Rates of experimental microbiological contamination of fish exposed to polluted water. Wat. Res. 26:1621-1627.

7. 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. 60:54-58.

8. Hancock, D. D, T. E. Besser, D. H. Rice, E. D. Ebel, D. E. Herriott and L. V. Carpenter. 1998. Multiple sources of *Escherichia coli* O157 in feedlots and dairy farms in the Northwestern USA. Prevent. Vet. Med. 35:11-19.

9. Isaacson, M. P., H. Canter, P. Effler, L. Aentzen, P. Bomans, and R. Heenon. 1993. Haemorrhagic colitis epidemic in Africa. Lancet 341:961.

10. Kaysner, C. A., K. C. Jinneman, P. A. Trost, C. Abeyta, Jr., W. E. Hill, and M. M. Wekell. 1994. Survival of *Escherichia coli* O157:H7 in aquatic and estuarine conditions, p. 383. Abst. 94th Gen. Meet. Am. Soc. Microbiol. 1994. Am. Soc. Microbiol., Washington, D.C.

11. Keene, W. E., J. M. McAnulty, F. C. Hoesly, L. P. Williams, K. Hedberg, G. L. Oxman, T. J. Barrett, M. A. Pfaller, and D. W. Fleming. 1994. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. N. Engl. J. Med. 331:579-584.

12. Lee, K. H., and E. G. Ruby. 1995. Symbiotic role of the viable but nonculturable state of *Vibrio fischeri* in Hawaiian coastal seawater. Appl. Bacteriol. 71:379-382.

13. Margarinos, B., J. L. Romalde, J. L. Barja, and A. E. Toranzo. 1994. Evidence of a dormant but infective state of the fish pathogen *Pasteurella piscida* in seawater and sediment. Appl. Environ. Microbiol. 60:180-186.

14. McGowan, K. L., E. Wickersham, and N. A. Strockbine. 1989. *Escherichia coli* O157:H7 from water. Lancet i:967-968.

15. Palumbo, S. A., K. T. Rajkowski, and A. J. Miller. 1997. Current approaches for reconditioning process water and its use in food manufacturing operations. Trends Food Sci. Tech. 8:69-74.

16. Park, S., R. W. Worobo, and R. A. Durst. 1999. *Escherichia coli* O157:H7 as an emerging foodborne pathogen: a literature review. Crit. Rev. Food Sci. Nutriti. 39:481-502.

17. Suhalim, R., Y. W. Huang, and G. Burtle. 2000. Survival of *Escherichia coli* O157:H7 in channel catfish pond water. Abstract at the International Association For Food Protection Annual Meeting, August 6-9, 2000, Atlanta, GA.

18. Swerdlow, D., B. A. Woodruff, R. C. Brady, P. M. Griffin, S. Tippen, H. D. Donnell, Jr., E. Geldreich, B. J. Payne, and A. Meyher, Jr. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann. Intern. Med. 117:812-819.

19. Terzieva, S. L., and G. A. McFeters. 1991. Survival and injury of *Escherichia coli*, *Campylobacter*, and *Yersinia enterocolitica* in stream water. Can. J. Microbiol. 37:785-790.

20. Wallace, J. S., T. Cheasty, and K. Jones. 1997. Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds. J. Appl. Microbiol. 82:399-404.

21. Wang, G., M. and Doyle. 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. J. Food Prot. 61:662-667.

22. Warburton, D. W., J. W. Austin, B. H. Harrison, and G. Sanders. 1998. Survival and recovery of *Escherichia coli* O157:H7 in inoculated bottled water. J. Food Prot. 6:948-952.

Table 1.1. Physical and chemical characteristics of three channel catfish pond water in Tifton and municipal water of Dekalb County, GA.

	Water source			
Assay	Municipal[a]	Pond 1	Pond 2	Pond 3
pH	9.1	8.5	8.1	8.3
Calcium[b]	24	25.315	31.008	34.335
Sodium[b]	3.4	8.2043	8.5755	5.2632
Cadmium[b]	<0.001	0.0203	0.0060	0.0269
Aluminum[b]	<0.045	3.7435	1.7197	1.2525
Chromium[b]	<0.025	0.0093	0.0097	<0.000
Iron[b]	<0.010	0.7584	0.3875	0.4730
Potassium[b]	NA[c]	11.553	8.2024	8.2024
Magnesium[b]	1.5	7.1240	6.9278	6.1263
Silicon[b]	NA	4.8478	2.9355	6.2335
Strontium[b]	NA	0.1311	0.1294	0.1406
Total nitrogen[b]	NA	3.2615	3.7700	2.6676
Total organic carbon[b]	NA	17.303	18.307	14.085
Total inorganic carbon[b]	NA	24.811	27.046	25.154
Ammonia[b]	NA	0.5700	0.5100	0.4500
Nitrite[b]	<0.2	0.0700	0.0100	0.0100
Total phosphate[b]	NA	1.5	1.4	0.8

[a]Municipal water (from reference *4*) (holding tank water from the two farmers markets)
[b]Parts per million
[c]NA, not available

Table 1.2. Bacteriological characteristics of holding tank water from two farmers markets in Atlanta and water of three channel catfish ponds in Tifton, GA.

Assay	Farmers market 1	Farmers market 2	Pond 1	Pond 2	Pond 3
Total aerobic count[a]	$7.9x10^3$	$6.2x10^4$	$2.6x10^5$	$2.0x10^5$	$2.2x10^5$
Total fecal coliform[a]	$2.0x10^1$	$3.2x10^1$	$1.7x10^2$	$2.0x10^2$	$5.0x10^2$

[a]CFU/ml, three replications

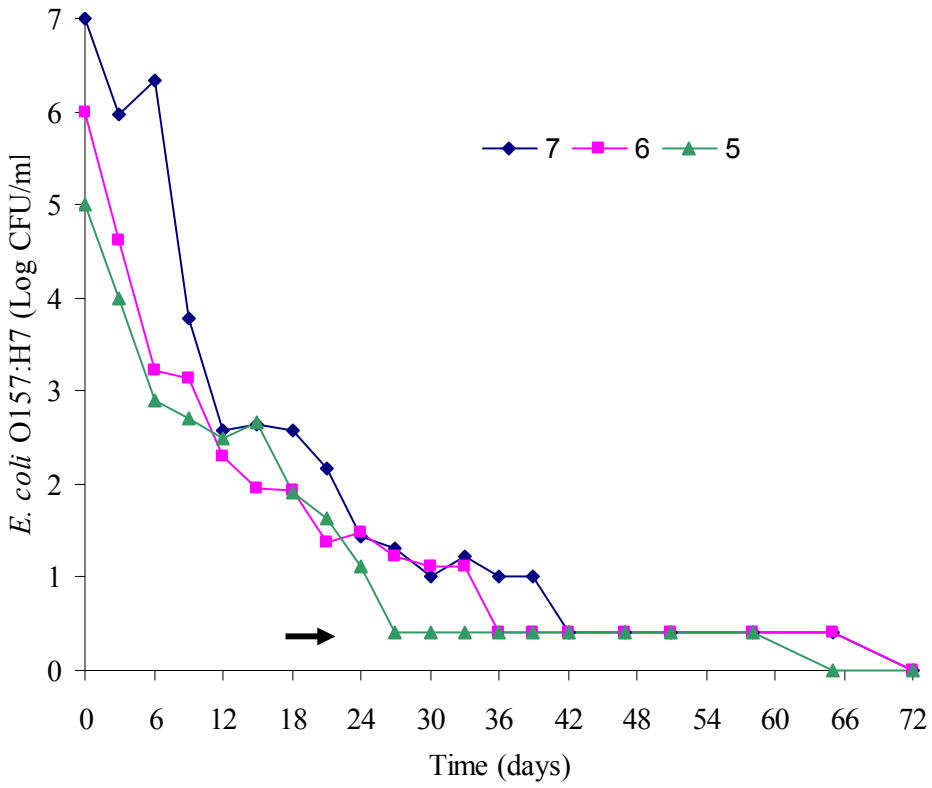


Figure 1.1. Survival of *E.coli* O157:H7 in catfish pond water inoculated at 10^5, 10^6, and 10^7 CFU/ml and held at 28ºC. Arrow: symbols represented on figure corresponding to arrow location indicate detection by enrichment method (cells were not enumerated).

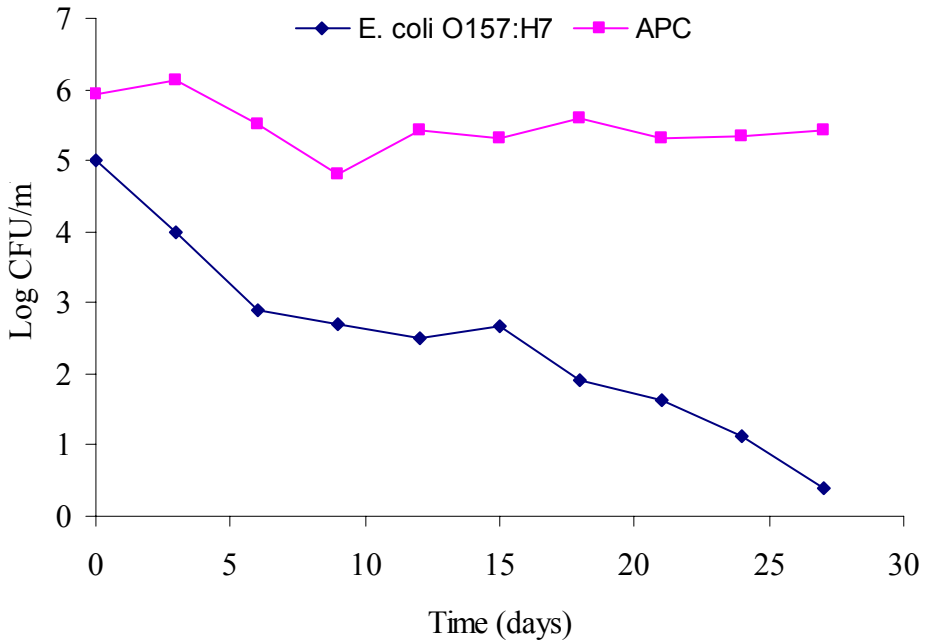


Figure 1.2. Fate of *E. coli* O157:H7 and total aerobic bacteria (APC) count in channel catfish pond water held at 28ºC. Water was inoculated with 10^5 CFU/ml *E. coli* O157:H7.

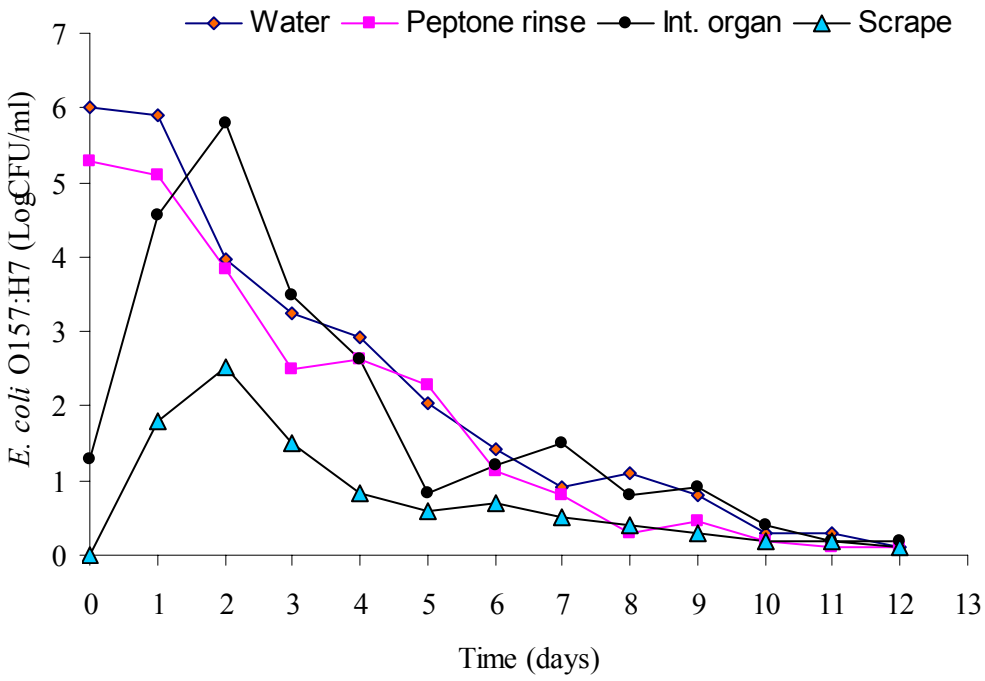


Figure 1.3. *E. coli* O157:H7 isolated from pond water, peptone rinse, internal organs, and skin scrape of channel catfish fingerling. Water was inoculated with 10^6 CFU/ml *E. coli* O157:H7 and held at 25ºC.

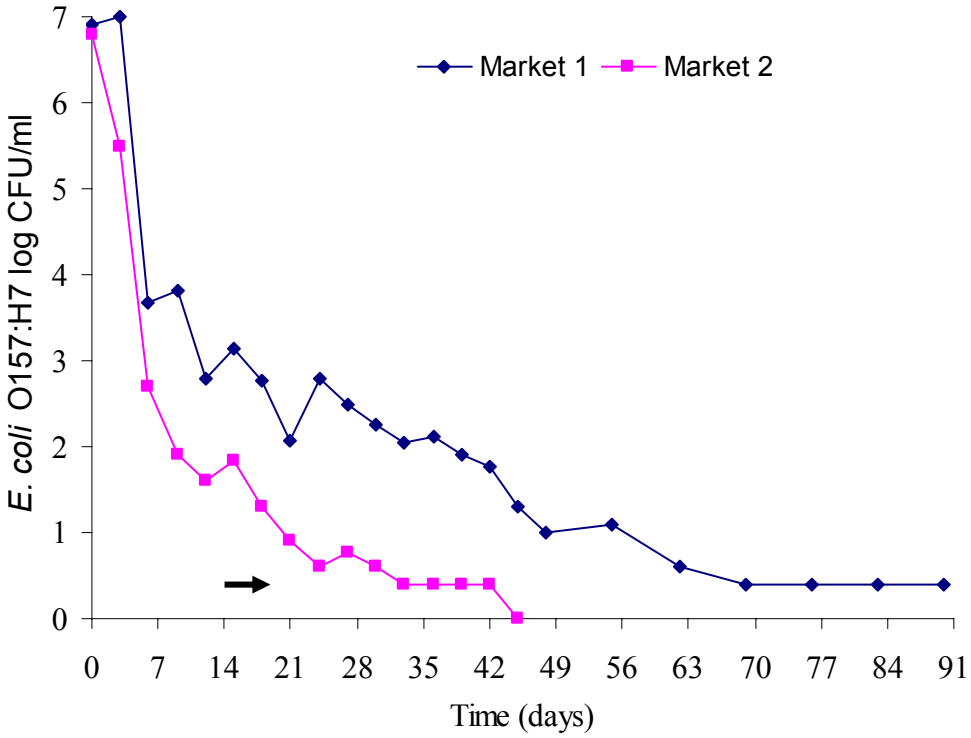


Figure 1.4. Survival of *E. coli* O157:H7 in channel catfish holding tank water from two farmers markets inoculated at 10^7 CFU/ml. Water from markets 1 and 2 were held 15° and 20°C, respectively. Arrow: symbols represented on figure corresponding to arrow location indicates detection by enrichment method (cells were not enumerated).

FATE OF *ESCHERICHIA COLI* O157:H7 INOCULATED ON CHANNEL CATFISH FILLETS AS AFFECTED BY MODIFIED ATMOSPHERE PACKAGING

Suhalim, R., Y. W. Huang. To be submitted to Journal of Food Science

ABSTRACT

The effects of modified atmosphere (MA, 30% CO_2) and overwrapped packaging on the survival of *Escherichia coli* O157:H7 on channel catfish (*Ictalurus punctatus*) fillets were investigated. Fish and non-fish products from adjacent seafood display from 2 farmers markets were also assayed for with *E. coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus,* and *Listeria* spp. Channel catfish fillets, with and without skin, were inoculated with 10^6 CFU/ml *E. coli* O157:H7, packaged in MA or overwrapped, and were stored at 0ºC and 10ºC for 20 days. Aerobic and psychrotrophic plates were incubated at 37º and 4ºC, respectively.

E. coli O157:H7 survived in overwrapped and MA packaging for 20 d. There was a significant difference ($P<0.05$) on the survival of the organism between MA and overwrapped packaging at 0ºC and between MA packaged fillets stored at 0º and 10ºC. A 0.5 and 1 log reduction of *E. coli* O157:H7 in MA was observed after 5 d storage on fillets stored at 10º and 0ºC, respectively. There was no significant difference between the survival of *E. coli* O157:H7 on fillets with and without skin either in MA or overwrapped packages. Aerobic bacterial populations at the end of the 20 d storage increased by 1 and 3 log CFU/g while psychrotropic populations increased by 2 and 3 log CFU/g for the 0 and 10ºC storage, respectively.

Low (1-2 log CFU/g) levels of *S. aureus* were isolated from the farmers markets samples. Presumptive colonies of *Salmonella* spp. and *Listeria* spp. were isolated from the raw and processed samples ranging from 0-20%. No *E. coli* O157:H7 was detected in any sample. Although *E. coli* O157:H7 was not detected from fish samples, other

pathogenic bacteria in raw and processed muscle foods from adjacent display cases were present.

INTRODUCTION

The microbial profile of aquatic food is reflected by the environmental conditions and microbiological quality of the water. Enteric bacteria, such as *E. coli* O157:H7, may be introduced into aquaculture such as channel catfish ponds by human waste and animal manure, directly from animal droppings, and/or through application of improperly composited manure fertilizers in agriculture land. *E. coli* O157:H7 has been found on raw vegetables grown in agricultural systems irrigated with contaminated water (Abdul-Raouf et al., 1993). Although *E. coli* O157:H7 has not been implicated in catfish, seafoods as carriers of human pathogens is of concern (Bal'a et al., 1999). Channel catfish as a possible vehicle of foodborne pathogens has been reported (Andrews et al., 1977; Cotton and Marshall, 1998; Leung et al. 1982; Meyer and Bullock, 1973). Previous study indicated that *E. coli* O157:H7 can survive in the channel catfish pond water for two weeks under controlled conditions (Suhalim et al., 2000). Live fish may become a vehicle for *E. coli* O157:H7 from pond water during production and carry the pathogen to processing facility and/or retail food outlet. Enterotoxigenic (ETEC) *E. coli* has been isolated from seafood (Ayulo et al., 1994). *Listeria* spp. and *Salmonella* spp. have also been isolated in a variety of fish and shellfish at processing plants and retail level (Andrews et al., 1977; Hatha and Lakshmanaperumalsamy, 1997; McCaskey et al. 1999; Davies et al., 2000).

In addition to pathogenic *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes,* and *Staphylococcus aureus* have been isolated from various foods including poultry, meat and fishery products (Andrews et al. 1977; Doyle and Schonei,

1987; Farber and Peterkin, 1991; Fernandes et al. 1997a; Fernandes et al. 1997b; Feldhusen, 2000; Leung et al., 1992) and water (Swerdlow et al., 1992; Wang and Doyle, 1998; Warburton et al., 1998). Food can come into contact with these pathogens during slaughtering, processing, handling, packaging, or cross-contamination from other food. Raw and processed fish products may be cross-contaminated by other muscle food products during handling and processing at retail level and/or at home (Fapohunda et al. 1994).

Numerous studies have been done regarding the use of modified atmosphere packaging (MAP) for seafood products (Cai et al., 1997; Silva and White, 1994; Statham, 1984; Stiles, 1991) and low temperature storage to effectively extend the shelf-life of food (Ogrydziak and Brown, 1992). Comparison of MAP studies on seafood is hampered by the variety of gas concentrations and storage conditions used by the researchers. Proper refrigeration conditions and MAP have prolonged shelf-life of food due to the elevated level of carbon dioxide that retards microbial growth (Stiles, 1991). Under proper storage temperatures, MAP has doubled the shelf-life of seafood products (Reddy et al., 1997; Statham, 1984).

Currently, only a few studies have investigated the effect of MAP on the fate of pathogens on seafood products, with the exception of *Clostridium botulinum*. *C. botulinum* type E has been the major concern of seafood product under MAP due to the growth of the pathogen and development of toxin under anaerobic environment without any significant sensory or visual sign of spoilage (Conner et al., 1989). No studies have been attempted to investigate the fate of *E. coli* O157:H7 on channel catfish fillet packaged under MAP. Previous studies on fish biology indicated the antimicrobial

activity in the skin of channel catfish (Ourth, 1980; Robinette et al., 1998). Thus, the current research also attempted to observe the role of skin mucus, if any, against *E. coli* O157:H7 on packaged catfish fillet.

The objectives of this study were 1) to investigate the survival of *E. coli* O157:H7 on channel catfish fillets under MAP and overwrapped packaging conditions and 2) to isolate *E. coli* O157:H7 and other foodborne pathogens in catfish and adjacent muscle food products sold at farmers markets. The latter objective was to observe the possibility of cross-contamination with *E. coli* O157:H7, *Salmonella* spp., *Listeria* spp., and *Staphylococcus aureus* between fishery and non-fishery products at retail level.

MATERIALS AND METHODS

Modified atmosphere packaging and storage of catfish fillet

Inoculum

A ground beef isolate (E318) of *E. coli* O157:H7 transformed with a plasmid encoding for green fluorescent protein (GFP) and ampicillin resistant gene (courtesy of Dr. Jinru Chen) was used in this study. Resistance to nalidixic acid was done through point mutation technique. Bacteria were grown in brain heart infusion (BHI) (Difco, Detroit, MI) broth containing 100 µg/ml ampicillin (Sigma Chemical Co., St. Louis, MO) and 100 µg/ml nalidixic acid (Aldrich Chemical Co. Milwaukee, WI) at 37ºC for 18 h. Culture broth was centrifuged and washed twice in 0.1% peptone water (Difco). Inoculum was diluted in 0.1% peptone water before enumeration by surface plating in

duplicate on BHI agar containing the same concentrations of ampicillin and nalidixic acid as the BHI broth. Plates were incubated at 37ºC overnight. Fluorescent colonies were observed under long-wave ultraviolet lamp (320-380 nm) and selected colonies confirmed with *E. coli* O157 agglutination test (Oxoid Limited, Hampshire, England).

Fish packaging

Live channel catfish (1-2 lb) were purchased from a farmers market in Atlanta, GA. Fish were transported on ice and filleted at the University of Georgia processing plant and stored at 4ºC until further use within 24 h. Fish fillets were packaged in a gas mixture of 30% CO_2 balanced with N_2 using a Dyno Sealing machine 462 (Schmid Maschinenbau GmbH, Germany). Expanded polystyrene trays (0.1 cc/tray/24h OTR) and Saran coated polyester film (12cc/m^2/24h) were used (Cryovac, Duncan, SC). Packaged fillets, with and without skin, were inoculated with 10^6 CFU/ml *E. coli* O157:H7 with a syringe and 16G needle on 5 x 5 cm inoculation area. Inoculation was performed by puncturing the barrier film and was quickly sealed with vinyl tape. Packaged fillets were placed in a household cooler filled with ice and stored in a 4ºC walk-in incubator to achieve a 0ºC storage temperature. Abused temperature packaged fillets were stored at 10ºC. All fillets were stored up to 15 d and representative samples were taken every 5 d. A Pac Check 650 Oxygen and CarbonDioxide Analyzer (Mocon) was used to measure CO_2 and O_2 concentration. Gas mixture samples were taken by using a 10 ml filtered-

syringe before injecting into the analyzer. Surface pH of fillets was also taken with an ISFET surface pH meter probe (Beckman Instrument, Inc. Fullerton, CA).

Overwrap packaging and storage of catfish fillets

Fillets were placed on styrofoam trays (Genpack, SC) and wrapped with Clear Plastic Wrap (BI-LO, Inc., Mauldin, SC). Packaged fillets were stored at 0ºC in the same manner as the MAP samples.

Microbiological evaluation

Samples were taken at 0, 5, 10, 15, and 20 d following inoculation. The 5 X 5 cm area was excised and placed in stomacher bag and homogenized in 0.1% peptone water for 60 s at normal speed (Tekmar Stomacher, Cincinnati, OH). Serial dilutions of bacteria were prepared in 0.1% peptone water. The samples were spirally plated and enumerated on brain hearth infusion (BHI) agar supplemented with 100 μg/ml ampicillin and nalidixic acid. BHI plates were incubated at 37ºC for 48 h. Fluorescent *E. coli* O157:H7 colonies were visualized with a portable ultraviolet light source and selected colonies were confirmed by O157 agglutination test (Oxoid, UK). Aerobic and psychrotrophic populations were enumerated on plate count agar (PCA, Difco) and plates were incubated at 37°C for 24 h and 4ºC for 10 d, respectively.

Farmers market samples

Catfish fillets, iced whole catfish, processed fish products (fish balls and fried fish patties), ground beef, precut chicken, whole uncooked chicken, and rotisserie chicken were obtained from two farmers markets in Atlanta, GA. All samples were examined using the enrichment procedures for *Salmonella* spp., *Listeria* spp., and *E. coli* O157:H7. Isolation of *S. aureus* was done by direct plating of diluted samples (Donnelly et al.1992;

Flowers, et al., 1992; Lancette and Tatini, 1992). Sampling of farmers market food items were done to investigate the hygienic practice of the food handlers in the market. Improper handling practice between raw and processed food in the same vicinity could lead to cross-contamination of microorganisms to foods.

Isolation of Salmonella *spp.,* Listeria *spp.,* Staphylococcus aureus*, and,* E. coli O157:H7*, and Aerobic Plate Counts*

Procedures for *Salmonella* spp. isolation were according to Flowers et al. (1992). Briefly, samples were pre-enriched in lactose broth (Difco) for 24 h at 37ºC followed by incubation in tetrathionate (TT, Difco) broth and selenite cystine (SC, Difco) broth. Samples were plated on bismuth sulfite (BS, Difco) and xylose lysine desoxychocolate citrate (XLD, Difco) agar. Suspected *Salmonella* spp. colonies were confirmed by inoculating them in triple sugar iron (TSI, Difco) and lysine iron (LI, Difco) agars. Incubation was done at 37ºC for 24-48 h.

Samples for the detection of *Listeria spp.* were initially enriched in University of Vermont (UVM, Oxoid) enrichment broth incubated for 24 h at 30ºC. Subcultures were made into Fraser secondary enrichment broth (Difco) and incubated for another 24 h at 37ºC. The incubated samples were then plated on modified Oxford agar (MOX, Oxoid). Plates were incubated at 37ºC for 24-48 h. Suspected *Listeria* colonies were gram stained and tested for motility (Donnelly et al., 1992). MR-VP (Difco) media were also used to test for *L. monocytogenes* (Hitchins, 1992).

Staphylococcus aureus was isolated and enumerated by direct plating. A 25 g sample was placed into sterile stomacher bags with 225 ml of 0.1% peptone water and

was pummeled in a stomacher for 60 s on normal speed. Homogenate was diluted in 0.1% peptone water and plated onto Baird-Parker (BP, Difco) agar. BP agar plates were incubated at 37°C for 24 h. Colonies typical of *S. aureus* were isolated and grown in tubes of tryptic soy broth (TSB, Difco) for 24 h incubation at 37ºC. Randomly selected colonies were confirmed with coagulase plasma test (Lancette and Tatini, 1992).

For the isolation of *E. coli* O157:H7, 25 g samples were enriched in TSB and incubated for 24 h at 37ºC. The following day, sample mixtures were streaked on MacConkey sorbitol agar (Oxoid). Sorbitol negative colonies typical of *E. coli* O157:H7 were confirmed with *E. coli* O157 (Oxoid) and H7 (Difco) agglutination tests.

Serial dilutions of the samples were prepared using 0.1% peptone water. Total aerobic and non-pathogenic *E. coli* populations were determined by plating on aerobic plate count and *E. coli* count plate containing Violet Red Bile (VRB, 3M Petrifilm™, St. Paul, MN), respectively, and incubated at 37°C for 24 h. Blue colonies with gas on petrifilm agar were counted as *E. coli*. All samples for microbial analysis were done in duplicates.

Statistical analysis

Data were analyzed using the SAS General Linear Models procedure. Duncan's multiple range test was used to determine differences among means when significant effects were observed ($P<0.05$).

RESULTS

Modified atmosphere packaging of channel catfish fillet

E. coli O157:H7 was detected throughout the 20 d storage from MAP fillets (Figure 2.1). Approximately 1 and 0.5 log reductions of *E. coli* O157:H7 populations were observed over the first 5 d of storage at 0 and 10ºC, respectively. *E. coli* O157:H7 populations continued to decrease but remained at 3.5 and 4.5 log CFU/g at the end of the 20 d storage at 0 and 10ºC, respectively. There was a significant ($P<0.05$) difference in the survival of the pathogen between 0º and 10ºC. The survival of *E. coli* O157:H7 on fillets with skin showed no significant difference from those without skin at either storage temperature. However, in general *E. coli* O157:H7 populations on fillets with skins were slightly lower (<1 log) than those without skin. Off odor was noticeable from fillets stored at 10ºC after 5 d.

Aerobic bacteria counts at the end of the 20 d storage increased by 1 and 3 log CFU/g while psychrotrophic populations grew by 2 and 3 log CFU/g for the 0º and 10ºC storage, respectively (Figures 2.2, 2.3). There was a significant ($P<0.05$) difference for the aerobic and psychrotrophic bacterial populations observed between 0º and 10ºC storage. However, no significant ($P>0.05$) difference was noted between the skin and no skin at 0º or 10ºC storage for their respective aerobic and psychrotrophic populations. The headspace gas (CO_2) concentration ranged between 23 and 25% for the 0ºC and 25 and 30 for the 10ºC storage (Figure 2.4). The surface pH fluctuated slightly over the 20 d period with the initial pH of 6.5 and a final value ranging from 6.3-6.4 for MAP fillets, while surface pH of overwrapped fillets increased from 6.8 to 7.3.

Overwrapped fillets

Approximately 3 log CFU/g reduction of *E. coli* O157:H7 population was observed after 20 d of storage (Figure 2.5). There was a significant ($P<0.05$) difference in the survival of *E. coli* O157:H7 populations between MAP and overwrapped fillets stored at 0ºC. No significant difference was noted in the populations between skin and no-skin overwrapped fillets. Psychrotrophic and aerobic plate counts on overwrapped fillets increased by approximately 4 log CFU/g (Figures 2.6 and 2.7). Onset of spoilage of overwrapped fillets was observed after 5 d of storage. The surface pH increased from 6.8 to 7.3.

Isolation of Pathogens from Meat and Fish Samples

The microbiological profile of the fish and non-fish sample from the two farmers markets are shown in Table 2.1. Among the fish items, catfish fillets had the highest (6.3 log CFU/g) aerobic bacteria count and chicken rotisserie had the lowest count (1 log CFU/g). Generic *E. coli* ranged from 1 log CFU/g (fish cake and chicken rotisserie) to 6 log CFU/g (raw chicken). In general, low (1-2 log CFU/g) levels of *S. aureus* were found in all sample. Presumptive colonies of *Salmonella* spp., and, *Listeria* spp. were recovered from up to 20% of the raw and processed samples. Raw and processed fish products were positive for *Salmonella* spp. 10-15% and 0-5% of the time, while raw and processed non fish products were S*almonella* spp. positive in 10-20% of the cases, respectively. *Listeria* spp. were observed in raw (0-15%) and processed (5%) fish product and in raw (0-10%) and processed (10%) non-fish products. *E. coli* O157:H7 was not detected in any sample.

Although colonies typical of *L. monocytogenes* grew on the MOX plates, further biochemical MR-VP test revealed negative results.

DISCUSSION

The combination of CO_2 and low temperature in MAP results in the reduction in microbial growth by extending the lag phase (Ogrydziak and Brown, 1992; Silva et al., 1993). In this study, the survival of *E. coli* O157:H7 was significantly higher on fillets in MAP at 10ºC than at 0ºC. The 30% CO_2 gas dissolved in the fish tissue at 0ºC in MAP may have an effect on the reduction of the pathogen populations. A slight reduction of CO_2 was observed in 0ºC MAP fillets, while CO_2 continued to rise in MAP at 10ºC. *E. coli* O157:H7 is relatively tolerant to CO_2, however inhibition of growth is more effective at high CO_2 concentration and pH<6.0 (Sutherland et al. 1997). Inhibition of the pathogen was more pronounced in ground beef packed in 60% CO_2/40% N_2 (Nissen et al., 2000).

The survival of the pathogen on the fillets with or without skin was not significantly different. However, it was noted in this study that *E. coli* O157:H7 populations on fillets with skins were slightly lower (<1 log) than those without skin, and it was more obvious on fillets stored at 0ºC than at 10ºC. This may have been an indication that mucus offered limited inhibition against the pathogen at lower temperatures. Previous studies indicated that purified skin mucus extracted from channel catfish possessed an antimicrobial activity (Ourth, 1980; Robinette et al., 1998). Mucus

of fish provides protection to the host through the presence of immunoglobulin, lysozymes, and a number of other nonspecific antimicrobial factors (Roberts, 2001). No information is currently available regarding the role of intact mucus against foodborne pathogenic bacteria.

There is an inverse relationship between the aerobic-psychrotrophic population and *E. coli* O157:H7. Aerobic and psychrotrophic populations from both packaging types continued to increase over the 20 d period, while *E. coli* O157:H7 population declined. Vold et al. (2000) observed the inhibition of *E. coli* O157:H7 in inoculated ground beef spiked with its native background bacteria. Bacterial competition could have contributed to the greater inhibition of *E. coli* O157:H7 in overwrapped package stored at 0ºC with higher number of aerobic and psychrotrophic bacteria than on MAP fillets stored at 0ºC.

The pH of MAP fillets decreased slightly (approximately 0.2 log) by the end of the storage. Lower pH in MAP has been attributed to the conversion of CO_2 to carbonic acid (Brody, 1989) and formation of lactic acid from lactobacilli (Gray et al., 1983). In contrast, pH of overwrapped fillets increased from 6.8 to 7.3. The increase of pH may be attributed to the production of volatile basic compounds such as ammonia by the spoilage bacteria (Stammen at al., 1990). The surface pH data observed in the present study were in agreement with previously published data (Reddy, et al., 1997; Silva and White, 1994,). The combination of factors such as temperature, CO_2 concentration, and background microflora may have contributed to the reduction of *E. coli* O157:H7 in MAP observed in this study.

In this study non-fish and fish products displayed at farmers markets were analyzed for the presence of pathogenic bacteria, with the focus on contamination to fish

items. The close proximity of the preparation area may increase the likelihood of cross-contamination between the muscle foods. Among the food analyzed from the two markets, none contained *E. coli* O157:H7. To date, no *E. coli* O157:H7 fish-related outbreak has been reported where fish is the primary vehicle. One small *E. coli* O157:H7 outbreak in Washington state involving fish was documented. However, further investigation revealed that fish was cross-contaminated with *E. coli* O157:H7-containing ground beef product in a food service institution (CSPI, 2001). Other non-O157:H7 *E. coli* have been found to be associated with seafood. Enterotoxigenic (ETEC) *E. coli*, enterophatoghenic (EPEC) and shiga-like toxin-producing *E. coli* (SLTEC) have previously been isolated from a variety of seafood (Ayulo et al., 1994; Sack et al., 1977; Samadpour et al., 1994; Youssef et al., 1992). Samadpour et al. (1994) also found SLTEC from fresh meat and poultry samples and suggested that food could have been contaminated during processing at retail level.

S. aureus were detected at low number from fish and non-fish foods. This organism is not a natural microflora component of fish. At low levels *S. aureus* would not compete well with the high background microflora present on the food. The aerobic population of the food observed in this study would have caused spoilage on the product before *S. aureus* can multiply. *S. aureus* ranging from 0.1 to 4 log CFU/g have been isolated from small to large catfish processing plants (Fernandes et al., 1997a). Raw (10-15%) and processed (0-5%) fish products contained *Salmonella* spp. from the two markets. *Salmonella* from the raw non-fish items displayed adjacent to the fish products from the same market ranged from 10 to 20%. These raw and processed food items from both markets were displayed either in the same or different display cases but located next

to each other. The latter was observed for the ready-to-eat rotisserie chicken displayed next to raw seafood and raw poultry in one of the farmers markets. In some situations, food workers handled both raw fish and non-fish items when serving customers. Observation of food handling practices at these markets was done when purchasing samples for this study. Cross-contamination could occur through direct handling of the product or through shared utensils.

In the U.S., raw domestic and imported seafood were found to be *Salmonella* positive approximately 10% of the time (Heinitz, et al. 2000) and approximately 6% of the catfish from processing plants were *Salmonella* positive (Andrews et al. 1977). It was suggested that *Salmonella* contamination in fish may occur during culture in polluted water. However, the more likely route of contamination is through improper post harvest handling and processing (Dalsgaard, 1998; Feldhusen, 2000). In other muscle food, approximately 28% poultry, 10% pork, and 0.5% cooked meat were positive for *Salmonella* spp. from retail markets (Duffy et al. 1999; Harrison et al., 2001). *Listeria* spp. was found in 0-15% raw and 5% processed fish items from both farmers markets. *L. monocytogenes* and *L. innocua* are among the 2 most common species associated with food, with the latter species being more common in seafood (Farber, 1991; Jineman et al., 1999). *L. innocua* has been isolated in 46% of seafood, 42% of beef and poultry (Skovgaard and Morgen, 1988; Weagant et al., 1988). In the U.S., *L. monocytogenes* was isolated from approximately 6% of the catfish tested from processing plants (McCaskey et al., 1999).

Catfish can be contaminated by the pathogen during production, through farm/wild animals and water, and processing. Increases in contamination levels with the

progress of processing steps either at the processing plant or retail level have been documented (Boonmar et al., 1998; Iida, 1998; Lawrence and Gilmour, 1995). Microflora from gut and skin can spread to edible muscle and food contact surface during processing (Bal'a et al., 1999). The close proximity of or a common processing and display area could contribute to the cross contamination of the bacteria from seafood to muscle foods and vice versa. In the event when certain muscle food is contaminated with *E. coli* O157:H7, this pathogen can be a potential hazard to nearby food items. *A. hydrophila* cross-contamination to beef could be a concern when jointly handled with fish (Ingram and Simonsen, 1980). Like wise the presence of human enteric organisms on fishery products implies contamination from a terrestrial source (Fapohunda et al., 1994). In the present study, cross contamination, abusive storage temperature, and/or excessive handling may explain the higher aerobic plate counts on fillets or iced fish than live fish from both markets. It is believed that food is most likely to be cross-contaminated at retail level during preparation (Duffy et al., 1999; Plummer et al., 1995). Cross contamination with pathogenic microorganisms was demonstrated to occur from hands, cutting board, and knives to food products during preparation in the kitchen (Zhao et al., 1998).

Proper sanitation and/or food preparation procedures should be maintained to avoid cross-contamination between the raw foods at retail level. This is particularly important since some species of fish are lightly cooked or consumed raw. In addition, fish contaminated with pathogens may not necessarily show the typical signs of spoilage on the product, thus giving a false sense of safety. Prevention of cross contamination by

proper storage temperature, separation of raw and processed food, sanitation, and discriminate use of utensils is critical to ensure safe food.

AKNOWLDGEMENTS

We would like to thank Mr. James Belcher, Cryovac, Sealed Air Co., SC, for providing the MAP supplies, Mr. David Peck and Dr. Romeo Toledo, Food Process Research and Development Laboratory, The University of Georgia, for the packaging technical assistance.

REFERENCES

Abdul-Raouf, U. M., L. R. Beuchat, and M. S. Ammar. 1993. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. App. Environ. Microb. 59:1999-2006.

Andrews, W. H., C. R. Wilson, P. L. Poelma. 1977. Bacteriological survey of the channel catfish *(Ictalurus punctatus*) at the retail level. J. Food. Sci. 42:359-363.

Ayulo, A. M. R., R. A.. Machado, V. M. Scussel. 1994. Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. Int. J. Food Microbiol. 24:171-178.

Bal'a, M. F. A., R. Podolak, and D. L. Marshall. 1999. Steam treatment reduces the skin microflora population on deheaded and eviscerated whole catfish. Food Microbiol. 16:495-501.

Boonmar, S., A. Bangtrakkulnonth, S. Pornrunangwong, N. Marnrim, K. Kaneko, and M. Ogawa. 1998. *Salmonella* in broiler chickens in Thailand with special reference to contamination of retail meat with *Salmonella enteritidis*. J. Vet. Med. Sci. 60:1233-1236.

Brody, A. L. 1989. Controlled/modified atmosphere/vacuum packaging of foods. Food Nutrition Press, Inc. Princeton, NJ.

Cai, P. M. A. Harrison, Y. W. Huang, and J. L. Silva. 1997. Toxin production by *Clostridium botulinum* type E in packaged chan nel catfish. J. Food. Prot. 60:1358-1363.

Conner, D. E., V. N. Scott, D. T. Bernard, and D. A. Kautter. 1989. Potential *Clostridium botulinum* hazards associated with extended shelf-life refrigerated foods: a review. J. Food Safety 10:131.

Cotton, L. N., and D. L. Marshall. 1998. Predominant microflora on catfish processing equipment. Dairy Food Environ. Sanit. 18:650-654.

Center for Science in the Public Interest. 2001. Outbreak Alert ! Available at http://www.cspinet.org/new/outbreak_alert2001.html. Accessed 3 December 2002.

Dalsgaard, D. 1998. The occurrence of human pathogenic *Vibrio* spp. and *Salmonella* in aquaculture. Int. J. Food Sci. Tech. 33:127-138.

Davies, A. R. 1996. Fate of food-borne pathogens on modifies–atmosphere packaged meat and fish. Intl. Biodet. and Biodeg. 1995:407-410.

Davies, A. R., C. Capell, D. Jehanno, G. J. E. Nychas, and R. M. Kirby. 2000. Incidence of foodborne pathogens on European fish. Food Control 12:67-71.

Donnelly, C. W., R. E. Brackett, S. Doores, W. H. Lee, and J. Lovett. 1992. *Listeria*, p. 637-663. *In* C. Vanderzant, and D. Splittstoesser (eds.), Compendium of methods for the microbiological examination of foods, 3rd ed. APHA, Washington, DC.

Doyle, M. P. and J. L. Schonei. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh market and poultry. Appl. Environ. Microbiol. 53:2394-2396.

Duffy, G., O. M. Cloak., M. G. O'Sullivan, A. Guilet, J. J. Sheridan, I. S. Blaor, and D. A. McDowell. 1999. The incidence and antibiotic resistance profiles of *Salmonella* spp. on Irish retail meat products. Food Microbiol. 16:623-631.

Fapohunda, A. O., K. W. McMillin, D. L. Marshall, and W. M. Waites. 1994. Growth of selected cross-contaminating bacterial pathogens on beef and fish at 15 and 35°C. J. Food. Prot. 57:337-340.

Farber, J. M. 1991. *Listeria monocytogenes* in fish products. J. Food Prot 54:922-924.

Farber, J. M. and P. I. Peterkin. 1991. *Listeria monocytogenes*, a foodborne pathogen. Microbiol. Review 55:476-511.

Feldhusen, F. 2000. The role of seafood in bacterial foodborne diseases. Microb. Infect. 2:1651-1660.

Fernandes, C. F., G. J. Flick, J. L. Silva, and T. A. McCaskey. 1997a. Influence of processing schemes on indicative bacteria and quality of fresh aquacultured catfish fillets. J. Food Prot. 60:54-58.

Fernandes, C. F., G. J. Flick, J. L. Silva, and T. A. McCaskey. 1997b. Comparison of quality in aquacultured fresh catfish fillets II. Pathogens *E. coli* O157:H7, *Campylobacter*, *Vibrio*, *Plesiomonas*, and *Klebsiella*. J. Food Prot. 60:1182-1188.

Flowers, R. S., J. Y. D'Aoust, W. H. Andrews, and J. S. Bailey. 1992. *Salmonella*, p. 371-422. *In* C. Vanderzant, and D. Splittstoesser (eds.), Compendium of methods for the microbiological examination of foods, 3rd ed. APHA, Washington, DC.

Gray, R. J. H., D. G. Hoover, and A. Muir. 1983. Attenutation of microbial growth on modified atmosphere packaged fish. J. Food. Prot 46:610-613.

Harrison, W. A., C. J. Griffith, D. Tenant, and A. C. Peters. 2001. Incidence of *Campylobacter* and *Salmonella* isolated from retail chicken and associated packaging in South Wales. Lett. Appl. Microbiol. 33:450-454.

Hatha, A. A. M. and P. Lakshmanaperumalsamy. 1997. Prevalence of *Salmonella* in fish and crustaceans from markets in Coimbatore, South India. Food Microbiol. 14:111-116.

Heinitz, M. L., R. D. Ruble, D. E. Wagner, and S. Tatini. 2000. Incidence of *Salmonella* in fish and seafood. J. Food Prot. 63:579-592.

Hjelmeland, K., M. Christie, and J. Raa. 1983. Skin mucus protease from rainbow trout, and its biological significance. J. Fish Biol. 23:13-22.

Hitchins, A. D. 1992. *Listeria monocytogenes*. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.

Iida, T., M. Kanzaki, A. Nakama, Y. Kokubo, T. Maruyama, and C. Kaneuchi. 1998. Detection of *Listeria monocytogenes* in humans, animals and foods. J. Vet. Med. Sci. 60:1341-1343.

Ingram, M. and B. Simonsen. 1980. Meat and meat products. Pp. 33-409. In ICMSF (ed.). Microbial ecology of foods. Vol II. Academic Press, New York, NY.

Jinneman, K. C. M. M. Wekell, and M. W. Eklund. 1999. Incidence and behaviour of *Listeria monocytogenes* in fish and seafood products, p. 631-655. *In* E. T. Ryser, and E. H. Marth (eds.), *Listeria*, listeriosis and food safety. Marcel Dekker, New York, NY.

Lancette, G. A. and S. R. Tatini. 1992. *Staphylococcus aureus*, p. 533-550. *In* C. Vanderzant, and D. Splittstoesser (eds.), Compendium of methods for the microbiological examination of foods. APHA, Washington, DC.

Lawrence, L. M and A. Gilmour. 1995. A characterization of *Listeria monocytogenes* isolated from poultry products and from the poultry-processing environment by random amplification of polymorphic DNA and multilocus enzyme electrophoresis. Appl. Environ. Microbiol. 61:2139-2144.

Leung, C. K. Y. W. Huang, and M. A. Harrison. 1992. Fate of *Listeria monocytogenes* and *Aeromonas hydrophila* on packaged channel catfish fillets stored at 4ºC. J. Food Prot. 55:728-730.

McCaskey, T., T. C. Hannah, T. Lovell, J. L. Silva, C. E. Fernandes, and G. Flick. 1999. Study shows catfish to be low risk for foodborne illness. Feedstuffs, March 29.

Meyer, F. P. and G. L. Bullock. 1973. *Edwarsiella tarda*, a new pathogen of channel catfish. Appl. Microbiol 25:155.

Nissen, H, O. Alvseike, S. Bredholt, A. Holck, and T. Nesbakken. 2000. Comparison between the growth of *Yersinia enterocolitica*, *Listeria monocytogenes*,

Escherichia coli O157:H7 and *Salmonella* spp. in ground beef packed by three commercially used packaging techniques. Int. J. Food Microbiol. 59:211-220.

Ogrydziak, D. M. and W. D. Brown. 1982. Temperature effects in modified atmosphere storage of seafood. Food Tech. 36(5):86-96.

Ourth, D. D. 1980. Secretory IgM, lysozyme, and lymphocytes in the skin mucus of the channel catfish. Dev. Comp. Immunol. 4:65-74.

Plummer, R. S., S. J. Bissett, and C. E. R. Dodd. 1995. *Salmonella* contamination of retail chicken products sold in the U.K. J. Food Prot 58:843-846.

Reddy, N. R., M. G. Roman, M. Villanueva, H. M. Solomon, D. A. Kautter, and E. J. Rhodehamel. 1997. Shelf-life and *Clostridium botulinum* toxin development during storage of modified atmposphere-packaged fresh catfish fillets. J. Food Sci. 4:877-884.

Roberts, R. J. 2001. Fish pathology, 3rd ed. W. B. Saunders, London, UK.

Robertson, G. L. 1993. Food Packaging: principle and practice. Marcel Dekker, New York, NY.

Robinette, D., S. Wada, T. Arroll, M. G. Levy, W. L. Miller, and E. J. Noga. 1998. Antimicrobial activity in the skin of the channel catfish: characterization of broad spectrum histone-like antimicrobial proteins. CMLS 54:467-475.

Sack, R. B., D. A. Sack, I. J. Mehlman, F. Orskov, and I. Orskov. 1977. Enterotoxigenic *Escherichia coli* isolated from food. J. Infect. Dis. 135, 313-317.

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. App. Environ. Microbiol. 60:1038-1040.

SAS. 1987. SAS User's guide statistics. SAS Institute, Cary, NC.

Silva, J. L., E. Harkness, and T. D. White. 1993. residual effect of CO_2 on bacterial counts and surface pH of channel catfish. J. Food. Prot 56:1051-1053.

Silva, J. L. and T. D. White. 1994. Bacteriological and color changes in modified atmosphere-packaged refrigerated channel catfish. J Food Prot. 57:715-719.

Skoovgaard, N. and C. A. Morgen. 1988. Detection of *Listeria* spp. in faeces from animals, in feeds, and in raw foods of animal origin. Int. J. Food Microbiol. 6:229-242.

Smith, A. C. and F. Ramos. 1976. Occult haemoglobin in fish skin mucus as an indicator of early stress. J. Fish. Biol. 9:537-541.

Stammen, K., D. Gerdes, and F. Caporaso. 1990. Modified atmosphere packaging of seafood. CRC Crit. Rec. Food Sci. Food Sci. Nutr. 29:301-331.

Statham, J. A. 1984. Modified atmosphere storage of fiheries products: the state of the art. Food Technol. Aust. 36:233.

Stiles, M. E. 1991. Scientific principles of controlled/modified atmosphere packaging, p.18-25. *In* B. Ooraikul and M. E. Stiles (eds.), Modified Atmosphere Packaging of Food. Ellis Horwood, London.

Suhalim, R., Y. W. Huang, and G. Burtle. 2000. Survival of *Escherichia coli* O157:H7 in channel catfish pond water. Abstract at the International Association For Food Protection Annual Meeting, August 6-9, 2000, Atlanta, GA.

Sutherland, J. P., A. J. Bayliss, D. S. Braxton, and A. L. Beumont. 1997. Predictive modeling of *Escherichia coli* O157:H7: Inclusion of carbon dioxide as a fourth factor in a pre-existing model. Int. J. Microbiol. 37:113-120.

Swerdlow, D., B. A. Woodruff, R. C. Brady, P. M. Griffin, S. Tippen, H. D. Donnell, Jr., E. Geldreich, B. J. Payne, and A. Meyher, Jr. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann. Intern. Med. 117:812-819.

Vold, L., A. Holck, Y. Wasteson, and H. Nissen. 2000. High levels of background flora inhibits growth of *Escherichia coli* O157:H7 in ground beef. Int. J. of Food Microbiol. 56:219-225.

Warburton, D. W., J. W. Austin, B. H. Harrison, and G. Sanders. 1998. Survival and recovery of *Escherichia coli* O157:H7 in inoculated bottled water. J. Food Prot. 61:948-952.

Wang, G. and M. Doyle. 1998. Survival of enterohemorrhagic *Escherichia coli* O157:H7 in water. J. Food Prot. 61:662-667.

Weagant, S. D., P. N. Sado, K. G. Colburn, J. D. Torkelson, F. A. Stanley, M. H. Krane, S. C. Shields, and C. F. Thayer. 1988. The incidence of *Listeria* species in frozen seafood products. J. Food Prot. 51:655-657.

Youssef, H., A. K. El-Timawy, and S. Ahmed. 1992. Role of aerobic intestinal pathogens of fresh water fish in transmission of human diseases. J. Food Prot. 55:739-740.

Zhao, P., T. Zhao, M. P. Doyle, J. R. Runino, and J. Meng. 1998. Development of a model for evaluation of microbial cross-contamination in the kitchen. J. Food Prot. 61:960-963.

Table 2.1. Microbial profile of raw and processed poultry, catfish, and meat samples from two farmer markets. Aerobic plate count (APC), *E. coli*, and *S. aureus* (mean (range) (log CFU/g). *E. coli* O157:H7, *Salmonella* spp., and *Listeria* spp. (positive/total).

Sample	APC	*E. coli*	*S. aureus*	*E. coli* O157:H7[1]	*Salmonella* spp.[1]	*Listeria* spp.[1]
Market 1:						
Catfish fillet	6.3 (4.6-7.0)	3.7 (2.6-4.2)	2.9 (1.8-3.5)	0/20	2/20	3/20
Live catfish	3.5 (3.1-3.6)	2.7 (1.9-3.0)	2.6 (1.4-2.6)	0/20	2/20	0/20
Chicken rotisserie	3.7 (1.0-4.3)	3.1 (1.0-3.4)	2.7 (2.0-3.0)	0/10	1/10	1/10
Chicken (raw)	4.9 (3.6-5.3)	3.6 (2.3-4.1)	1.7 (0.0-2.1)	0/10	2/10	1/10
Market 2:						
Processed fish						
Fish balls	4.2 (3.3-4.4)	3.6 (3.4-3.7)	2.7 (2.2-3.0)	0/20	1/20	1/20
Fish cake	3.2 (2.5-4.0)	2.8 (1.0-3.6)	1.5 (1.4-2.0)	0/20	0/20	1/20
Live catfish	4.7 (3.3-5.2)	2.8 (2.0-3.0)	4.3 (2.0-4.7)	0/20	3/20	2/20
Iced whole catfish	5.6 (3.4-6.1)	4.6 (3.3-5.0)	3.2 (2.2-3.4)	0/20	3/20	1/20
Ground beef	5.2 (4.0-5.6)	2.6 (2.3-2.9)	2.5 (0.7-2.9)	0/20	2/20	4/20
Chicken						
Whole (raw)	4.4 (4.3-6.6)	5.6 (3.0-6.0)	2.6 (1.6-2.9)	0/10	2/10	1/10
Precut (raw)	5.0 (3.7-5.4)	4.8 (2.7-5.1)	2.7 (2.5-2.8)	0/10	2/10	0/10

[1]Positive samples/total

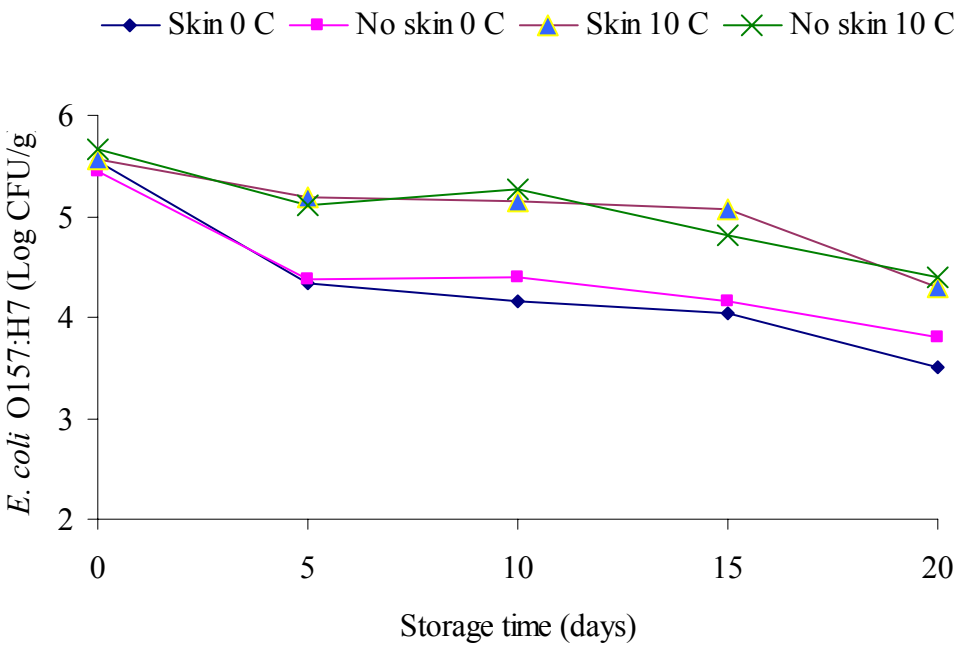


Figure 2.1. Survival of *E. coli* O157:H7 inoculated on fillets of channel catfish with and without skin stored under modified atmosphere packaging at 0º and 10ºC.

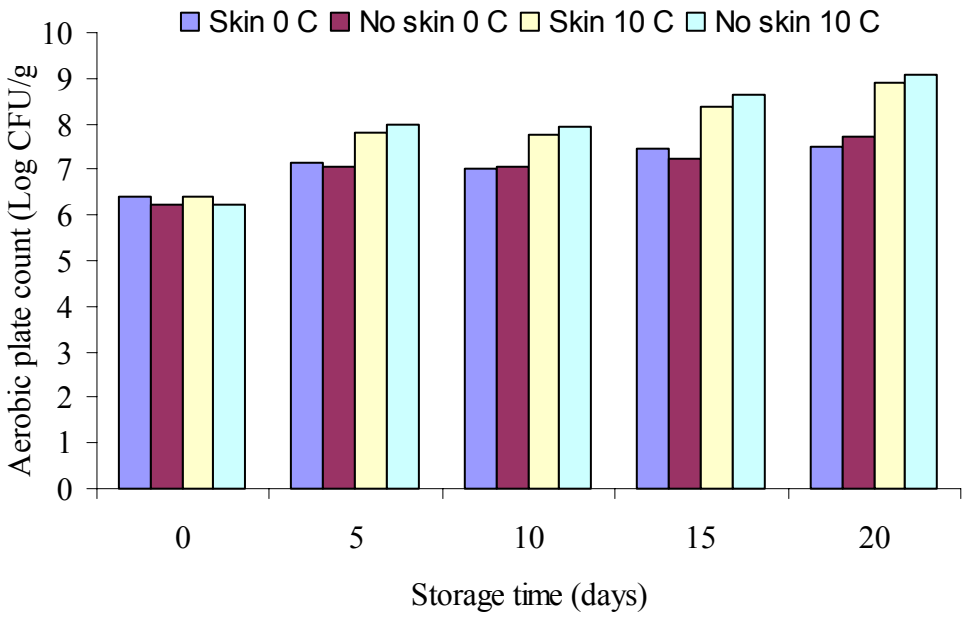


Figure 2.2. Aerobic plate counts of channel catfish fillet with and without skin inoculated with *E. coli* O157:H7 and stored under modified atmosphere packaging at 0º and 10ºC.

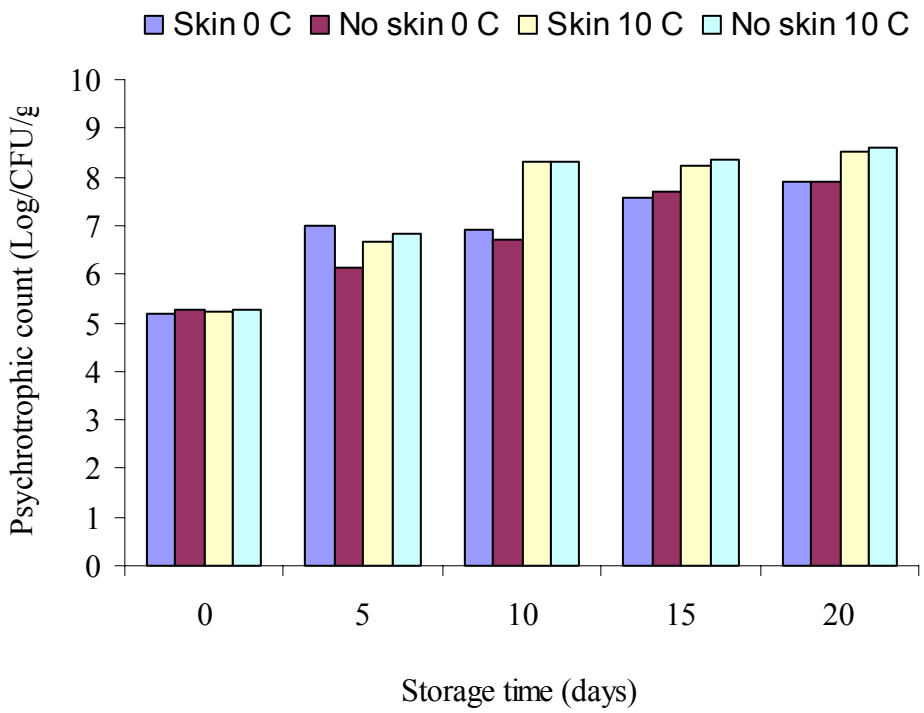


Figure 2.3. Psychrotrophic counts of channel catfish fillet with and without skin inoculated with *E. coli* O157:H7 and stored under modified atmosphere packaging at 0º and 10ºC.

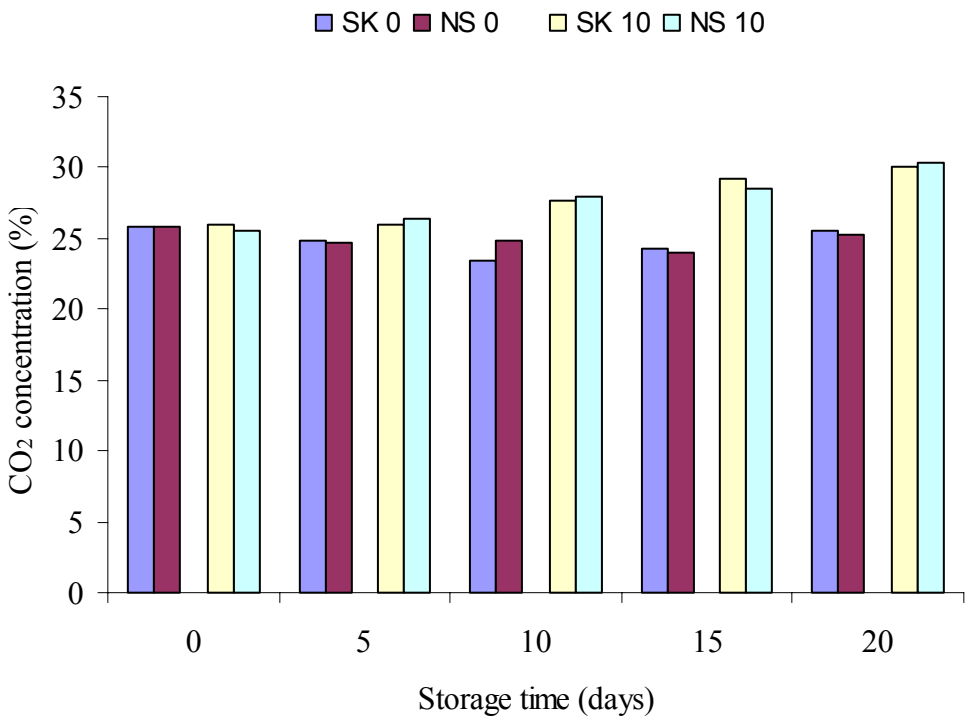


Figure 2.4. Carbon dioxide concentrations of modified atmosphere packaged fillets of channel catfish with (SK) and without (NS) skin inoculated with *E. coli* O157:H7 stored at 0º and 10ºC.

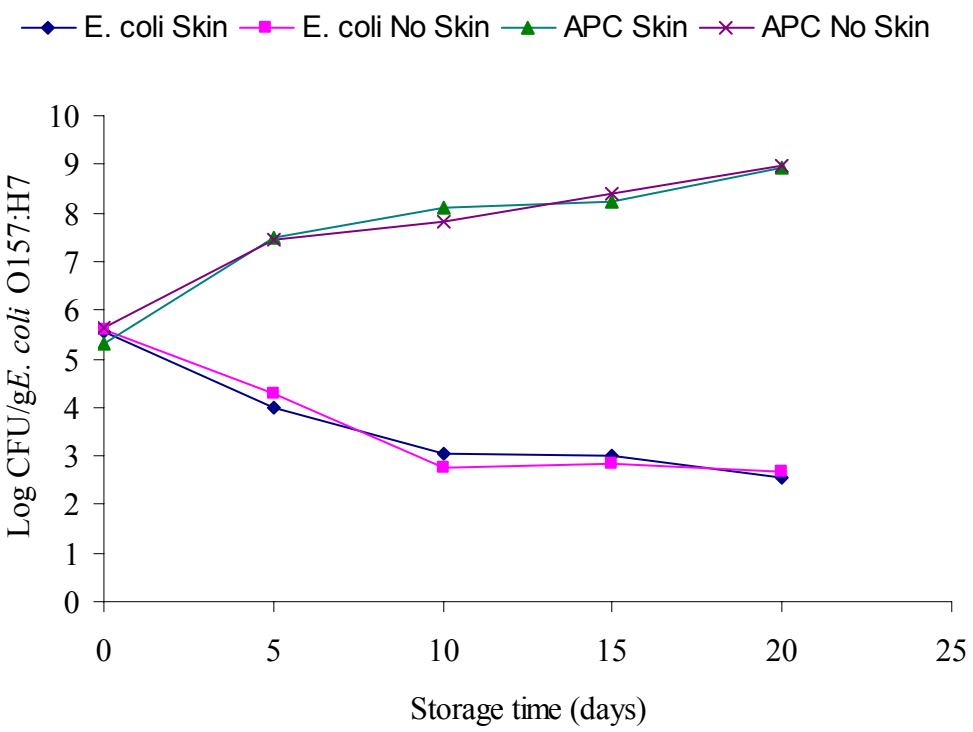


Figure 2.5. Survival of *E. coli* O157:H7 inoculated on fillets of channel catfish with and without skin stored under overwrapped packaging at 0ºC. Aerobic plate counts were shown for comparison.

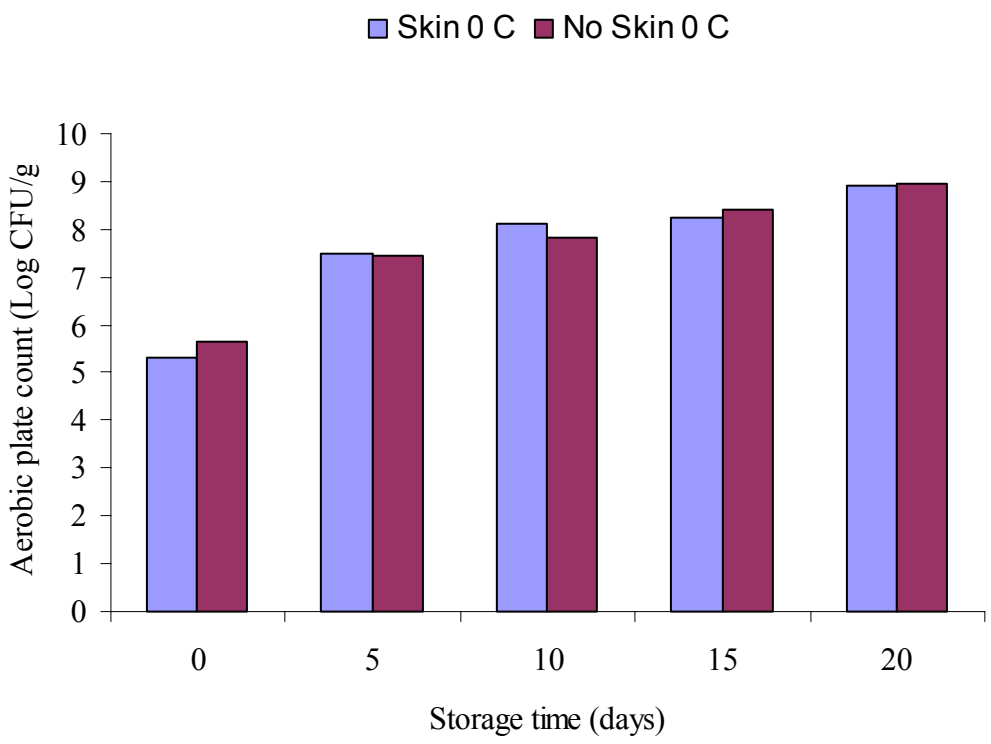


Figure 2.6 Aerobic counts of channel catfish fillet with and without skin inoculated with *E. coli* O157:H7 stored under overwrapped packaging at 0^{o}C.

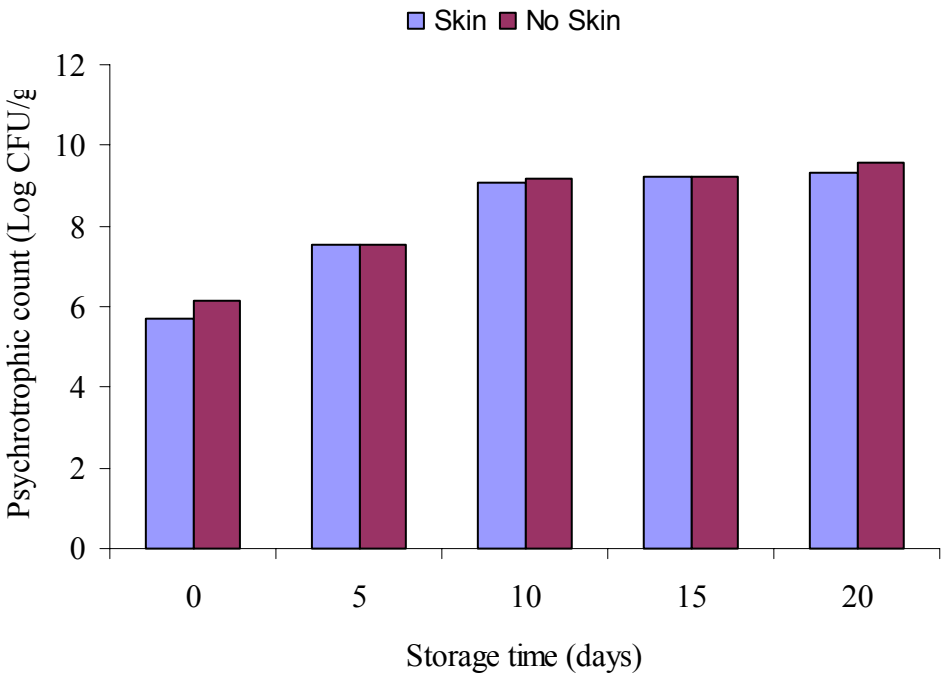


Figure 2.7. Psychrotrophic counts of channel catfish fillet with and without skin inoculated with *E. coli* O157:H7 stored under overwrapped packaging at 0ºC.

PENETRATION OF *ESCHERICHIA COLI* O157:H7 INTO CHANNEL CATFISH SKIN MUCUS DETECTED BY CONFOCAL SCANNING LASER MICROSCOPY AND PLATE COUNT METHODS

Suhalim, R., Y. W. Huang. To be submitted to Journal of Food Safety

ABSTRACT

Confocal scanning laser microscopy (CSLM) was used to observe the distribution of *Escherichia coli* O157:H7 containing green fluorescent protein (GFP) into channel catfish skin mucus. Bacteria were observed to attach to catfish mucus as confirmed by CSLM images. *E. coli* O157:H7 were seen at 15 μm into the mucus layer. Propidium iodide stain distinguished yellow dead cells from the green (GFP) viable cells on catfish skin. Plate count revealed a half a log decrease in *E. coli* O157:H7 population/cm^2 on skin without mucus than on skin with mucus, while a 1-log decrease in population/cm^2 was observed on skin without mucus than on skin with mucus by microscopic count. Approximately 18 and 16% of all the cells observed under CSLM appeared to be dead cells on skin with or without mucus, respectively. Limited *E. coli* O157:H7 inhibition by fish mucus was observed as clearing zone with a diameter of approximately 0.7 cm for the first 12 h.

This study suggests that *E. coli* O157:H7 attached and was penetrated into skin mucus of channel catfish. The pathogen was embedded into the skin mucus and may become a source of contamination during processing.

INTRODUCTION

Escherichia coli O157:H7 outbreaks have been associated with a variety of foods and water (Doyle and Schonei, 1987; Swerdlow et al., 1992). Although *E. coli* O157:H7 has not caused public health concerns on fishery products, seafood can serve as carriers of human pathogens. Channel catfish (*Ictalurus punctatus*) as a possible vehicle of foodborne pathogens has been reported (Andrews et al., 1977; Cotton and Marshall, 1998; Leung et al. 1982; Meyer and Bullock, 1973). Channel catfish may carry pathogenic bacteria such as *E. coli* O157:H7 from the growing pond water as a result of runoff from adjacent agricultural land or droppings from domestic and wild farm animals. Contamination of catfish by pathogenic microorganisms may also occur during processing. Steps during catfish processing can spread microorganisms from skin and gut to the processing environment (Bal'a et al., 1999). Catfish skin is believed to be an important source of microbial contamination during processing (Kim and Marshall, 2000). Channel catfish is commercially available both in the form of skinless fillets or whole dressed fish with skin intact.

Fish mucus has the ability to entrap microorganisms and inhibit their colonization by continuous sloughing (Roberts, 2001). The mucus contains specific immunoglobins and lysozyme. As part of the defense system of fish, it is believed that these entities have anti-pathogen activity to remove microorganism from the surface (Speare and Mirasalimi, 1992). Previous studies indicated that channel catfish mucus contains substances with antimicrobial activity such as histone-like proteins and lysozyme which were effective against *Aeromonas hydrophila* (Robinette et al., 1998) and *Salmonella*

parathyphi (Ourth, 1980). To date, reports on the penetration and possibly inhibition of *E. coli* O157:H7 on fish mucus is lacking.

Confocal scanning laser microscopy (CSLM) techniques have been used to observe food structure (Hassan et al., 2002) and bacterial attachment to food (Prachaiyo and McLandsborough, 2000; Seo and Frank, 1999; Takeuchi and Frank, 2000). This technique allows observation of specimen in a fully hydrated state (Blonk and van Aalst, 1993). The major advantage of CSLM is its ability to focus within a specific depth by optically section specimen and construct a three-dimensional image (Amos and Fordham, 1997, Blonk and van Aalst, 1993). CSLM provides info about the location and distribution of the cells in addition to the ability to quantify the bacteria cells without extensive preparation and sample fixation. The ability to distinguish cells under CSLM by using stains is also an advantage.

Skin of live catfish may offer protection against certain microorganisms. However, not much is known regarding the role of fish skin mucus post-harvest. Fish skin may become a source of microbial contamination during handling and thus posses a food safety concern during processing. The objective of this study were to 1) observe the distribution and degree of penetration of *E. coli* O157:H7 on channel catfish mucus skin using CSLM technique, and 2) observe the ability of intact mucus to inhibit *E. coli* O157:H7.

MATERIALS AND METHODS

Inoculum

E. coli O157:H7 isolated from ground beef (E318) containing plasmid encoding for green fluorescent protein (GFP) was used in this study (courtesy of Dr. Jinru Chen, The University of Georgia). This GFP transformed bacteria has an excitation wavelength at 488 nm. Bacteria were grown for 18 h at 37ºC in brain heart infusion broth (BHI, Difco Laboratories, Detroit, MI) supplemented with 100 μg/ml of ampicillin (Sigma, St. Loius, MO) and nalidixic acid (Aldrich Chem Co., Milwaukee, WI). Bacteria were centrifuged at 5,000 X g for 20 min, and pellet was washed in 0.1% peptone water (Difco). Cultures were suspended in 0.1% peptone. Serial dilutions of bacteria in 0.1% peptone water were prepared to reach the desired concentration. GFP colonies were viewed under a long-wave UV light. Enumeration was done on BHI agar supplemented with 100 μg/ml of ampicillin and nalidixic acid. BHI agar plates were incubated for 18 h at 37ºC.

Channel catfish

Fish were purchased from the farmers market in Atlanta, GA and were transported live to laboratory. Live fish were maintained in 40 L aquaria until use. On the day of the experiment, fish were sacrificed and skin was removed with sterile surgical blade. Mucus was removed by scraping the skin surface with sterile surgical blade to obtain skin without mucus.

Sample preparation for *E. coli* O157:H7 enumeration by direct plate count

A 0.1% peptone water suspension containing approximately 10^9 CFU *E. coli* O157:H7/ml was used to inoculate the catfish skin samples by complete immersion for 20 min at room temperature (RT, 22ºC). Following inoculation, skin was rinsed twice by immersion in sterile distilled water (SDW) at RT for 3 min each. Pieces of skin were placed in sterile stomacher bags containing 20 ml of 0.1% peptone water. Samples were pummeled for 2 min at high speed (Tekmar, Cincinnati, OH) and serially diluted in 0.1% peptone prior to surface plating on BHI agar containing 100 µg ampicillin and nalidixic acid. Duplicate plates were incubated for 24 h at 37ºC and colonies were viewed under long-wave UV light and counted. Confirmation of selected *E. coli* O157:H7 was done using a latex agglutination test (Oxoid Limited, Hampshire, England). Three replicate samples were done. Enumeration was reported as CFU/cm^2 of the area of the skin.

Antibacterial activity assay

Approximately 0.1 ml of 10^5 to 10^6 CFU *E. coli* O157:H7/ml was surface plated with a sterile glass rod on Tryptic Soy Agar (TSA, Difco) plates. After bacteria were allowed to absorb for 30 min into the agar, catfish skin with and without mucus of approximately 0.5 cm in diameter was placed mucus-side down on the bacteria lawn on TSA. Plates were incubated for 24 h at 37ºC. The presence of clearing zone around the skin sample on *E. coli* O157:H7-inoculated TSA agar indicated antimicrobial activity. Sterile 0.5 cm discs impregnated with 5% acetic acid were used as a control.

Sample preparation for *E. coli* O157:H7 enumeration by CSLM

Catfish skin samples with and without mucus were inoculated by immersion in a suspension of approximately 10^9 CFU/ml of *E. coli* O157:H7 at RT for 20 min. Sample were rinsed twice by immersion in SDW for 3 min at RT. Following rinsing, samples were treated by flooding with propidium iodide (PI, Molecular Probes, Inc., Eugene, OR) for 5 min at RT. The 7.5 μM PI enabled the distinction of dead (red stained) and live (GFP) cells. Two immersion rinses in SDW were done for 3 min at RT.

Skin samples were placed on cover slips for maximum contact surface and the latter inverted onto a hanging drop slide. The skin thickness was important to prevent unnecessary pressure from the cover slip that could damage the mucus layer. Specimen was viewed with CSLM Leica DM RBE (Leica Microsystems Inc., Exton, PA) with 100x objective lens. GFP cells were imaged with argon laser at 488 nm ex/543 nm em and PI was scanned with green neon at 543 nm. Reflectance mode was used to visualize mucus and skin and to aid in depth determination. Leica confocal software Version 2.0 Build 0770 (Exton, PA) was used for data acquisition and analysis.

E. coli O157:H7 cells were enumerated at various depths along the z axis until no GFP cells were observed. Optical slices were taken at approximately every 1 μm to avoid repeated counting of the same cell. *E. coli* O157:H7 counts obtained from CSLM observation was multiplied by two in order to give a similar counting effect as the two-sided skin surface done on plate count.

Statistical analysis

Data were analyzed using the generalized linear model (GLM) of the statistical analysis software (SAS Institute, Cary, NC). Duncan's multiple range test was used to determine significant difference between means.

RESULTS

E. coli O157:H7 cells attached primarily to the area where mucus was present (Figure 3.1A), while skin without mucus had less number of bacteria (Figure 3.1B). Viable cells stained green while non-viable cells stained red to yellow under CSLM (Figures 3.1A and 3.1B). Bacteria were observed as deep as 15 μm into the mucus layer and were decreasing in number at a deeper layer, with majority found embedded within the first 5 μm. Free moving bacteria were observed in greater number on skin without mucus than on skin with mucus. Approximately 18 and 16% of all the cells observed under CSLM were dead cells on skin with or without mucus, respectively. Skin without mucus had less *E. coli* O157:H7 than skin with mucus both on the plate and direct microscope counts (Table 3.1) Lower counts were also obtained from CSLM observation.

Approximately 0.7 cm in diameter of clearing zone around the skin samples was observed on TSA plates inoculated with *E. coli* O157:H7. However, clearing zone was only observed for the first 12 h of incubation. By 18 h, the clearing zone was less obvious due to the overgrown bacteria surrounding the skin sample. The clearing zone due to the acetic acid control was observed throughout incubation.

DISCUSSION

CSLM has been used to study the attachment, penetration, and location of *E. coli* O157:H7 on apples and lettuce leaf (Burnett et al., 2000; Seo and Frank, 1998; Takeuchi and Frank, 2000). In the present study we observed the attachment of *E. coli* O157:H7 on the skin mucus of catfish by CSLM. Bacterial attachment did not occur randomly but rather in the area where mucus was abundant and *E. coli* O157:H7 cells were able to penetrate into the mucus layer of catfish. In contrast, fish skin without mucus had fewer bacteria.

Fish skin is covered with mucus that is composed of mucopolysaccharides (Takashima and Hibiya, 1995) and its continual production facilitates in trapping microorganisms (Ourth, 1980). Extracellular polymers, such as polysaccharide, were believed to aid in bacterial adherence (Fletcher and Floodgate, 1973; Marshall et al., 1971). Bacterial attachment to mucus may be supported by the hydrophilic nature of fish skin (Haas et al., 2002) and the non-hydrophobic surface of *E. coli* O157:H7 may also assist in the attachment (Dewanti and Wong, 1995). Several studies showed attachment and colonization of bacterial pathogens on mucus surface of mammals (Freter et al., 1981; Laux et al., 1984) and Sajjan and Forstner (1990) found that *E. coli* O157:H7 adhered to rat intestine in vitro. In regards to fish, Balebona et al., (1995) suggested that gilt-head sea bream skin mucus provided an optimal substrate for accumulation of aquatic bacteria, while Krovacek et al. (1987) observed increase accumulation of *Vibrio anguillarum* and *A. hydrophyla* on fish mucus. However, in live fish, the continual

sloughing of the mucus helps in the removal of bacteria and may also inhibit colonization of bacteria on fish skin.

Plate count results suggested that skin without mucus contained a high number of bacteria. This high count could have been attributed to the bacteria that adhered to the film layer of water on the skin after rinsing. A large number of free-moving bacteria on skin without mucus visualized under CSLM may have contributed to the high plate counts. Although the mucus had been significantly removed, a small amount of mucus was still present which allowed the bacteria to attach. In contrast, the majority of the bacteria on the skin with mucus attached to the mucus and penetrated at deeper layer, as visualized through the scanned images, rather than free-moving on the surface. In both skin types, plate counts were higher than direct counts. The high values may have been due to the stomaching of the sample. Stomaching may have dislodged additional bacteria that attached to the edges of the skin and to the muscle side of the skin which tended to be irregular in thickness and surface area. In addition, the disruption of cell aggregates on skin surface during stomaching may have also contributed to the higher number of microorganisms enumerated by plate counting. Cell enumeration by direct observation was restrained to counting the cells that were not in clusters.

Higher plate counts may also have been due to the ability of injured cells to grow on agar media, while the same cell may have appeared dead under CSLM due to penetration of PI stain that was able to gain access to the leaky cell membrane. The percentage of dead cells found on skin with and without mucus observed under CSLM did not suggest antibacterial activity of mucus against *E. coli* O157:H7.

The 20 min inoculation period may not be sufficient to observe the mucus antibacterial activity. Longer time effect on mucus against *E. coli* O157:H7 was not examined.

Although previous studies demonstrated the active compounds in catfish mucus against bacteria (Ourth, 1980; Robinette et al., 1998), the nature of bacteriolysis in the skin mucus of catfish is not known (Ourth, 1980). In this study, some inhibition of *E. coli* O157:H7 by catfish mucus was observed for the first 12 h. Bacterial colonies started to grow around the clearing zone after 18 h incubation. This may have been contributed by the less susceptible nature of *E. coli* O157:H7 to mucus or by the insufficient level of active compound in mucus against the pathogen. The inhibitory level of fish mucus against the pathogen has not been investigated. Other aquatic bacteria also have varying susceptibility level against fish mucus as described later in this chapter. The methods described in previous studies to demonstrate the antibacterial activity in mucus require extensive extraction and purification procedures which may have limited application in food safety. Intact skin mucus was used in the present study to observe antibacterial activity on the skin as fish is being exposed to bacterial contaminants during processing. The potential of mucus as an agent against bacteria may assist in the reduction of contamination from pathogenic organisms on fish as a food product. However, mucus may lose its activity after the fish is slaughtered. Certain inhibitors, such as blood serum, greatly reduced the antimicrobial activity of fish mucus (Hjelmeland et al., 1983; Smith and Ramos, 1976). Fish could easily be contaminated with blood during processing. Boiling the sample is also needed to stabilize the activity of the mucus. It is believed that proteases present in mucus not receiving heat treatment can inactivate the antibacterial compound of the mucus (Robinette et al., 1998). Other factors such as temperature abuse,

bacterial load, and health condition of the fish may affect the mucus effectiveness against bacteria. No information is available regarding the antibacterial role of intact mucus on fish against foodborne pathogenic bacteria as fish undergoes food processing scheme.

Fish mucus also shows a varying degree of inactivation against aquatic bacteria. Mucus extracts from rainbow trout were demonstrated to show antibacterial activity against *Planococcus citreus*, a gram positive bacteria, but not *Psychrobacter immobilis*, a gram negative bacteria, both of which were isolated from fish (Smith et al., 1999). In catfish, mucus has been demonstrated to display antibacterial activity against *S. paratyphi* (Ourth, 1980) and certain *A. hydrophyla* isolates (Robinette et al., 1998), but the latter study did not observe inhibition against *S. aureus*, *Edwardsiella ictaluri* and one other aeromonad isolate tested. With respect to food safety, catfish skin is thought to be an important source of bacterial contamination (Kim and Marshall, 2000). Firstenberg-Eden (1980) believed that attachment of bacteria to surfaces is the first step in product contamination. *E. coli* O157:H7 contamination on live catfish can be a potential problem since it can be easily transferred to other food contact surface and muscle during skinning; increasing food safety hazard to consumers. In addition, since the infectious dose of *E. coli* O157:H7 is low (Nataro and Kaper, 1998), the presence of a few viable cells on fish skin can be a potential hazard. The effectiveness of catfish decontamination during processing was also affected by the presence of mucus. Studies suggested that mucus of catfish slightly reduced the effectiveness of lactic acid decontamination treatment against *Salmonella* Typhimurium (Kim and Marshall. 2000), *Edwardsiella tarda*, and *L. monocytogenes* (Kim and Marshall, 2001). In these studies they also found

that the gram negative bacteria more readily attached to catfish skin mucus than did gram positive bacteria.

ACKNOWLEDMENTS

We are grateful to Drs. Ashraf Hassan, Department of Food Science and Technology, and John Shields, the Center for Ultrastructural Research, The University of Georgia, for their valuable technical assistance with the CSLM application.

REFERENCES

Amos, W. B. and M. Fordham. 1997. An evaluation of confocal versus conventional imaging of biological structures by fluorescence light microscopy. J. Cell Biol. 105:41-48.

Andrews, W. H., C. R. Wilson, P. L. Poelma. 1977. Bacteriological survey of the channel catfish *(Ictalurus punctatus*) at the retail level. J. Food. Sci. 42:359-363.

Aranishi, F., and N. Mano. Antibacterial cathepsin in different types of ambicoloured Japanese flounder skin. Fish Shellfish Immunol. 10:87-89.

Bal'a, M. F. A., R. Podolak, and D. L. Marshall. 1999. Steam treatment reduces the skin microflora population on deheaded and eviscerated whole catfish. Food Microbiol. 16:495-501.

Balebona, M. C., M. A. Morinigo, A. Faris, K. Krovacek, I. Manson, M. A. Bordas, and J. J. Borrego. 1995. Influence of salinity and pH on the adhesion of pathogenic *Vibrio* strains to *Sparus aurata* skin mucus. Aquaculture 132:112-120.

Blonk, J. C.G., and H. van Aalst. 1993. Confocal scanning light microscopy in food research. Food Res. Int. 26:297-311.

Burnett, S.L., J. Chen, and L. R. Beuchat. 2000. Attachement of *Escherichia coli* O157:H7 to the surfaces and internal structures of apples as detected by confocal scanning laser microscopy. App. Environ. Microbiol. 66:4679-4687.

Cotton, L. N., and D. L. Marshall. 1998. Predominant microflora on catfish processing equipment. Dairy Food Environ. Sanit. 18:650-654.

Delaquis, P. J., C. Gariepy, and D. Montpetit. 1992. Confocal scanning laser microscopy of porcine muscle colonized by meat spoilage bacteria. Food Microbiol. 9:147-153.

Dewanti, R., and A. C. L. Wong. 1995. Influence of culture condition s on biofilm formation by *Escherichia coli* O157:H7. Int. Food. Microbiol. 26:147-164.

DiConza, J. J. 1970. Some characteristics of natural haemagglutinins found in serum and mucus of the catfish. Aust. J. Exp. Biol. Med. Sci. 48:515-523.

Doyle, M. P. and J. L. Schonei. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh market and poultry. App. Environ. Microbiol. 53:2394-2396.

Firstenberg-Eden, R. 1980. Attachment of bacteria to meat surface: A review. J. Food Prot. 44:602-607.

Fletcher, M., and M. D. Floodgate. 1973. An electron-microscopic demonstration of an acidic polysaccharide involved in the adhesion of a marine bacterium to solid surfaces. J. Gen. Microbiol. 74:325-334.

Fletcher, T. C. and A. White. 1973. Lysozyme activity in the plaice. Experientia 29:1283-1285.

Freter, R., B. Allweis, P. C. M. O'Brien, S. A. Halstead, and M. S. Macsai. 1981. Role of chemotaxis in the association of motile bacteria with intestinal mucosa: in vitro studies. Infect. Immun. 34:241-249.

Hass, W., P. Stiegeler, A. Keating, B. Kullman, H. Rabenau, E. Schonamsgruber, and B. Haberl. 2002. *Diplostomum spathaceum* cercariae respond to a unique profile of cues during recognition of their fish host. Int. J. Parasitol. 32:1145-1154.

Hassan, A. N., J. F. Frank, and M. Corredig. 2002. Microstructure of feta cheese made using different cultures as determined by confocal scanning laser microscopy. J. Food Sci. 67:2750-2753.

Hellio, C., A. M. Pons, C. Beaupoil, N. Bourgougnon, and Y. L. Gal. 2002. Antibacterial, antifungal and cytotoxic activities of extracts from fish epidermis and epidermal mucus. Int. J. Antimic. Agents. 20:214-219.

Hjelmeland, K., M. Christie, and J. Raa. 1983. Skin mucus protease from Rainbow trout, and its biological significance. J. Fish Biol. 23:13-22.

Kenney, S. J., S. L. Burnett, and L. R. Beuchat. 2001. Location of *Escherichia coli* O157:H7 on and in apples as affected by bruising, washing, and rubbing. J. Food Prot. 64:1328-1333.

Kim, K. Y., J. F. Frank, and S. E. Craven. 1996. Three dimensional visualization of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. Letters Appl. Microbiol. 22:280-282.

Kim, J., and D. L. Marshall. 2000. Lactic acid inactivation of *Salmonella Typhymurium* attached to catfish skin. J. Food Safety 20:53-64.

Kim, J., and D. L. Marshall. 2001. Effect of lactic acid on *Listeria monocytogenes* and *Edwardsiella tarda* attached to catfish skin. Food Microbiol. 18:589-596.

Kim, J., and D. L. Marshall. 2002. Influence of catfish skin mucus on trisodium phosphate inactivation of attached *Salmonella Typhymurium*, *Edwardsiella tarda* and *Listeria monocytogenes*. J. Food Prot. 65:1146-1151.

Krovacek, K., A. Faris, W. Ahne, and I. Masson. 1987. Adhesion of *Aeromonas hydrophyla* and *Vibrio anguilarum* to mucus-coated glass slides. FEMS Microbiol. Lett. 42:85-89.

Laux, D. C., E. F. Sweegan, and P. S. Cohen. 1984. A new test based on 'salting out' to measure relative surface hydrophobicity of bacterial cells. Biochim. Biophys. Acta 677:471-476.

Leung, C. K. Y. W. Huang, and O. C. Pancorbo. 1992. Bacterial pathogens and indicators in catfish and pond environments. J. Food Prot. 55:424-427.

Margarinos, B. F. Pazos, Y. Santos, J. L. Romalde, A. E. Toranzo. 1995. Responses of *Pasteurella piscida* and *Flexibacter maritimus* to skin mucus of marine fish. Dis. of Aquatic Org. 21:103-108.

Marshall, K. C., R. Stout, and R. Mitchell. 1971. Mechanism of the initial events in the sorption of marine bacteria to surfaces. J. Gen. Microbiol. 68:337-348.

Meyer, F. P., and G. L. Bullock. 1973. *Edwarsiella tarda*, a new pathogen of channel catfish . Appl. Microbiol 25:155.

Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev. 11:142-201.

Ourth, D. D. 1980. Secretory IgM, lysozyme and lymphocytes in the skin mucus of the channel catfish, *Ictalurus punctatus*. Dev. Comp. Immunol. 4:65-74.

Prachaiyo, P., and L. A. McLandsborough. 2000. A microscopic method to visualize *Escherichia coli* interaction with beef muscle. J. Food Prot. 63:427-433.

Roberts, R. J. 2001. Fish pathology. W. B. Saunders, London.

Robinette D., S. Wada, T. Arroll, M. G. Levy, W. L. Miller, and E. J. Noga. 1998. Antimicrobial activity in the skin of the channel catfish: characterization of broad spectrum histone-like antimicrobial proteins. CMLS 54:468-475.

Romalde, J. L., B. Magariños, S. Nuñez, J. L. Barja, A. E. Toranzo. 1996. Host range susceptibility of *Enterococcus* sp. strains isolated from diseased turbot: possible routes of infection. Appl. Environ. Microbiol. 62:607-611.

Sajjan, S. U., and J. F. Forstner. 1990. Characteristics of binding of *Escherichia coli* O157:H7 strain CL-49 to purified intestinal mucin. Infect. Immun. 58:860-867.

Seo, K. H., J. F. frank. 1999. Attachment of *Escherichia coli* to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using confocal scanning laser microscopy. J. Food Prot. 62:3-9.

Silva, J. L., and T. D. White. 1994. Bacteriological and color changes in modified atmosphere-packaged refrigerated channel catfish. J. Food Prot. 57:715-719.

Smith, V. J., J. M. O. Fernandes, S. H. Jones, G. D. Kemp, and M. F. Tatner. 2000. Antibacterial proteins in rainbow trout. Fish Shellfish Immunol. 10:243-260.

Smith, A. C., and F. Ramos. 1976. Occult haemoglobin in fish skin mucus as an indicator of early stress. J. Fish Biol. 9:537-541.

Speare, D. J., and S. M. Mirasalimi. 1992. Pathology of the mucous coat of trout skin during an erosive bacterial dermatitis. J. Comp. Pathol. 106:210-211.

Swerdlow, D., B. A. Woodruff, R. C. Brady, P. M. Griffin, S. Tippen, H. D. Donnell, Jr., E. Geldreich, B. J. Payne, and A. Meyher, Jr. 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann. Intern. Med. 117:812-819.

Takashima, F., and T. Hibiya. 1995. An atlas of fish histology, 2nd edition. Kodansha Ltd., Tokyo, Japan.

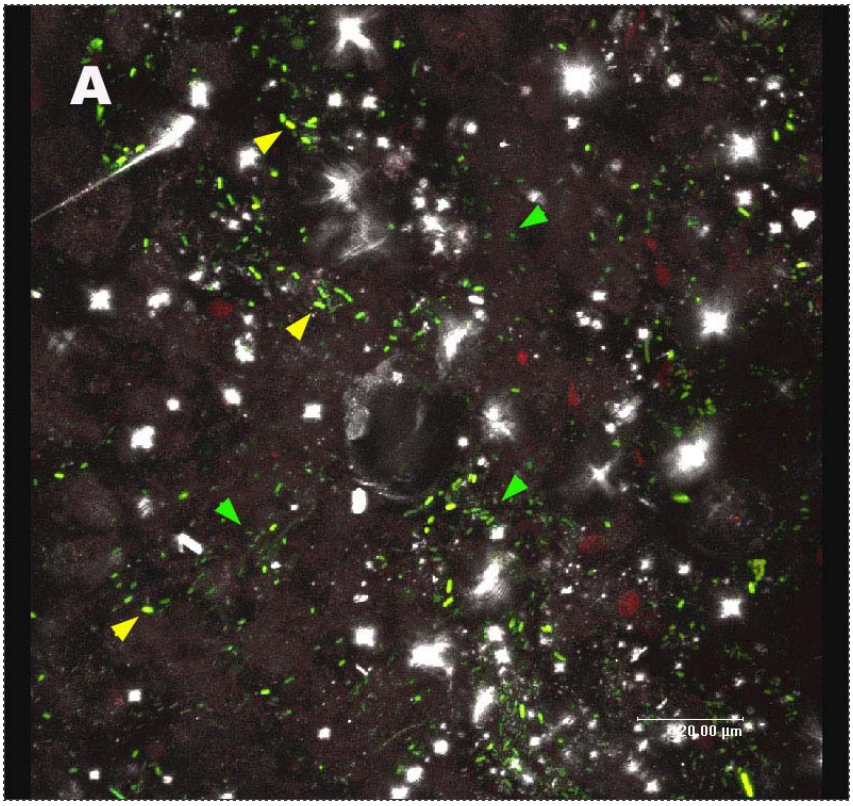
Takeuchi, K., and J. F. Frank. 2000. Penetration of *Escherichia coli* O157:H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. J. Food Prot. 63:434-440.

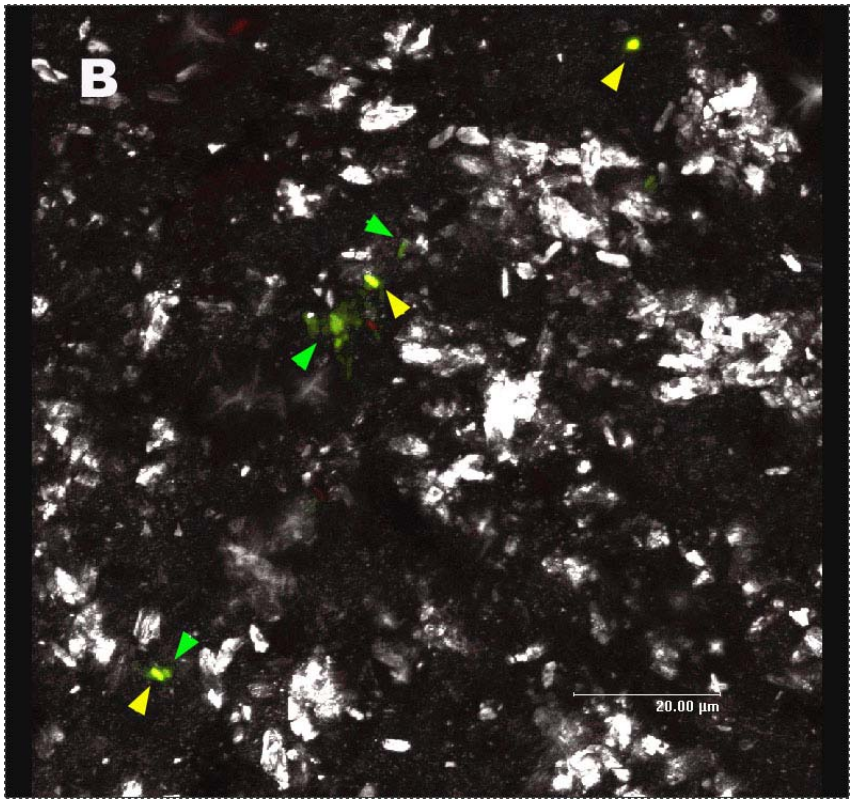
Table 3.1. Enumeration (mean±sd) by plate and direct microscopic count of *E. coli* O157:H7 inoculated on catfish skin with and without mucus.

	Treatment	
	Skin with mucus	Skin without mucus
Plate count (CFU/cm^2)	7.8±0.2a[1]	7.3±0.1b
CSLM (Cell/cm^2)	5.5±0.2a	4.5±0.2b

[1]Means on the same row followed by the same letter are not significantly different.

Figure 3.1. Attachment of GFP *E. coli* O157:H7 on channel catfish skin with mucus (A) and without mucus (B) stained with PI (red dead cell stain) visualized under CSLM. Images were taken at 10 µm (A) and 5 µm (B). Green and yellow arrow heads indicate live and dead *E. coli* O157:H7 cells, respectively.


A
20.00 µm


B
20.00 µm

SUMMARY AND CONCLUSION

Surface water runoff carrying *E. coli* O157:H7, wildlife and livestock may introduce contamination to the fish culture system and pose risks to the aquatic food chain. This study indicated that *E. coli* O157:H7 can survive in channel catfish pond water for > 30 d at 25ºC. Longer survival rates may be possible at lower temperatures as indicated in the holding water study. Fish, as carriers of pathogens, may become a contamination source throughout the food processing scheme as indicated in the *E. coli* O157:H7 penetration into channel catfish skin mucus observed in this study. This condition may cause a health hazard for consumers due to cross-contamination through handling and preparation of foods. Good agricultural and manufacturing practices should be in place to prevent/reduce contamination of pathogen into foods in farms and/or in food processing environment.

Although fishery products are generally safe, as with all foods, there are some risks. Many of the foodborne pathogens associated with other food muscle can also be isolated from seafood. This study showed that *E. coli* O157:H7 survived on catfish fillets packaged under modified atmosphere packaging (MAP) and overwraped up to 20 d stored at proper refrigeration temperature. Proper storage (0ºC) of fillets inhibits growth of *E. coli* O157:H7 population but the risk of cross contamination is still prevalent. The effectiveness of MAP will depend on initial microbial load, fish species, CO_2 concentration, ratio of gas and product volume, and temperature. Higher (>30%) CO_2 concentration may be able to retard/eliminate *E. coli* O157:H7 more effectively.

Although the sale of MAP fish is not permitted in the U. S. at this time due to the lack of assurance in storage temperature during distribution, this study illustrates the risk and survival ability of pathogenic *E. coli* on catfish fillet in MAP condition.

Although, *E. coli* O157:H7 was not recovered from fish or non-fish foods purchased from the farmers markets, the presence of other pathogenic bacteria could be a potential health hazard. Cross contamination of pathogens that are not native to fish products can occur in fish due to inappropriate methods of handling during food processing and preparation. Catfish can be potentially contaminated by *E. coli* O157:H7 during production and post harvest processing. Good manufacturing practice (GMP) and proper sanitation standard operating procedure (SSOP) in an effective HACCP program would result in the reduction of microbial load of food products during processing and handling and thus producing safer food.

Mucus is a non-specific protective barrier for fish but it is also a suitable media for microbial attachment once the fish is slaughtered. Cross contamination with pathogens, such as *E. coli* O157:H7, from skin to muscle is possible when the pathogen has previously been introduced in the water during production or post-harvest processing. Proper skinning of catfish is recommended to reduce the cross contamination and/or growth of potential foodborne pathogens on mucus.