Aquaponics and Food Safety

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You never really understand something unless you can explain it to your grandmother. - **Albert Einstein**

So I left him, and as I went away, I said to myself, 'Well, although I do not suppose that either of us knows anything, I am better off than he is. For he knows nothing, and thinks that he knows. I neither know nor think that I know. In this latter particular, I seem slightly to have the advantage of him'. **- Socrates**

On the antiquity of microbes: 'Adam had 'em'. - **Anon**

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1. Introduction

Aquaculture provides approximately 20 million of the 140 million metric tons of fish and shellfish consumed in the world annually. The remaining 120 million metric tons are harvested from naturally existing populations, principally from marine fisheries, many of which are at their maximum sustainable yields, are in decline, or have completely collapsed. China dominates the world in the aquacultural production of fish and shellfish, of which more than half by weight are raised in China. However, several countries in Europe and North America are among the top 10 producers. Total global production by aquaculture is expected to grow from 20 to 55 million metric tons by 2025, with no increase, and possibly even declines, in harvests from the capture fisheries (Georgiadis *et al*, 2000).

In North America, the three principal species of fish reared by aquaculture are salmon (*Oncorhynchus* spp.), rainbow trout (*O. mykiss*) and channel catfish (*Ictalurus punctatus*). In 1999, the total production for both Pacific and Atlantic salmon in Canada and the USA was about 72,000 metric tons, for a value of $450 millions; for rainbow trout, production was 24,000 metric tons valued at $85 millions; for channel catfish, production was 271,000 metric tons valued at $424 millions.

In the USA in 2002, the consumption of seafood had increased 7.1%, with Americans eating 4.5 billion pounds of domestic and imported seafood (Anon, 2003d)(**Table 1**).

Table 1. Top Ten Seafoods in the USA, 2000-2002– Consumption per Person

2000	2001	2002
Canned tuna/3.50 lb	Shrimp/3.40 lb	Shrimp/3.7 lb
Shrimp/3.200 lb	Canned tuna/2.90 lb	Canned tuna/3.1 lb
Pollock/1.595 lb	Salmon/2.023 lb	Salmon/2.021 lb
Salmon/1.582 lb	Pollock/1.207 lb	Pollock/1.13 lb
Catfish/1.050 lb	Catfish/1.044 lb	Catfish/1.103 lb
Cod/.752 lb	Cod/0.577lb	Cod/.658 lb
Clams/.473 lb	Clams/.465 lb	Crabs/.568 lb
Crabs/.375 lb	Crabs/.437 lb	Clams/.545 lb
Flatfish/.423 lb	Flatfish/.387 lb	Tilapia/.401 lb
Scallops/.269 lb	Tilapia/.348 lb	Flatfish/.317 lb
Tilapia/.264 lb	Scallops/.342 lb	Scallops/.313 lb

2. Aquaponics

Aquaponics is a refined branch of aquaculture. The word 'aquaponics' is derived from a combination of 'aquaculture' (fish farming) and 'hydroponics' (growing plants without soil), and refers to the integration of hydroponic plant/vegetable production with aquaculture*. It is a bio-integrated system linking recirculating aquaculture with hydroponic production of plants such as vegetables, ornamental flowers, and culinary or medicinal herbs, etc.. A brief history of aquaponics and its evolution have been provided by Jones (2002). Helfrich (2000) also examined food production through hydroponics and aquaculture.

A variation of the aquaponic process as proposed by Nuttle (2003a) involves algalculture, aquaculture and aquaponics. In this system, quail provide

* Cover, Aquaculture Journal, 2002

the manure to supplement nutrients needed for algalculture, as well as supplying some eggs and meat. Tilapia (*Oreochromis* spp.) are used to consume surplus micro-algae and to supplement diets. Manure from the fish, effluent and algal water may be used to fertilize and irrigate ('fertigate') nearby aquaponic crops. A tank system is used to produce a manure effluent that is filtered by sand before being used in algalculture or aquaponic systems; manure solids are used to make an organic compost. To date, no disease problem has occurred in this system (Nuttle, 2003b). Algae are harvested weekly, sun-dried and crushed into algal powder, blended with bread flour and/or soup to add proteins, vitamins, minerals, omega-3 oils and multi-nutrient supplements ('nutraceuticals') for end users. Since one third of the 2.3 billion people in the world are known to be impoverished, such a combination of algalculture, aquaculture and aquaponics has the potential to help resolve many health problems caused by poverty.

In Australia, aquaculture is a fast-growing industry that utilizes low-density pond-rearing systems that, to a great extent, are limited by the lack of freshwater resources because of recent severe drought – hence, these systems are becoming increasingly wasteful of a precious resource. Because of these concerns, the aquacultural industry in Australia is evolving toward the use of the more efficient (in terms of water use) indoor re-circulating systems. As well, increasingly stringent environmental regulations make aquaponics a major answer to these critical problems (Lennard, 2004).

Although the word 'aquaponics' tends to imply the use of freshwater systems, there is ongoing work in Israel and Australia on saltwater aquaponics in the production of saltwater algae and seaweed as the plant elements, and sea finfish, sea crustaceans (shrimp), sea urchins and sea mollusks (shellfish such as abalone) as the animal element (Wilson, 2003). In Israel, saltwater aquaponics provide a *'holistic but profitable approach based on algal sunlight-dependent assimilation of excess nutrients and their conversion into microalgal …. biomass. The algae produced can be sold either as a primary commodity (the world wide seaweed market handles annually around nine million metric* *tons) or fed on-site to saltwater algivores such as fish, crustaceans, mollusks or echinoderms (sea urchins) that feed on algae.*' (Wilson, 2003).

The science of aquaponics helps agricultural production through the implementation of certain principles:

- the products from one system serve as food or fuel for a second biological system;
- the integration of fish and plants is a type of polyculture that increases diversity and by this means, enhances stability of the system;
- biological water filtration removes nutrients from the water before it leaves the system;
- the sale of greenhouse products generates income that supports the local economy.

Nutrient wastes from tanks are used to fertilize production beds via the water. The roots of plants and associated rhizosphere bacteria remove nutrients from the water. These nutrients, generated from the feces of fish, algae and decomposing feed, are contaminants that could otherwise increase to toxic levels in the tanks. Instead they act as liquid fertilizer for hydroponically grown plants. In turn, the hydroponic beds function as biofilters, and the water can be recirculated to the tanks. Bacteria in the gravel and associated with the roots of the plants have a critical role to play in the cycling of nutrients; without these organisms, the system would stop functioning (Rakocy, 1999a,b; Diver, 2000).

A number of advantages of aquaponics for greenhouse managers include:

- water carrying feces from fish is a source of organic fertilizer that allows plants in the system to grow well;

- hydroponics is viewed as a method of biofiltration that facilitates intensive recirculating aquaculture;
- aquaponics is seen as a method to introduce organic hydroponically-grown products into the market place, because the only fertility introduction is feed, and all of the nutrients pass through a biological process;
- food-producing greenhouses, yielding two products from one production unit, are naturally appealing for niche marketing and green labeling;
- in arid regions where water is scarce, aquaponics is an appropriate technology that allows food production with re-used water;
- aquaponics is a working model of sustainable food production in which plant and animal systems are integrated, and the recycling of nutrients and water filtration are linked;
- in addition to its commercial applications, aquaponics has become a popular training aid in integrated bio-systems in vocational agriculture and biology classes (Rakocy, 1999a ; Diver, 2000).

An additional advantage of aquaponics includes improved efficiency in the use of water, especially in areas with a limited supply of water (McMurtry et al, 1997). Some methods of aquaponic production have been described at: www.aquaponics.com/infohydromethods.htm ; attra.ncat.org/attra-pub/aquaponic.html.

Graham (2003a) has examined aquaponics in Alberta from a business perspective.

3. Plants

Common plants that do well in aquaponic systems include any leafy lettuce, pak choi, spinach, arugula, basil, mint, watercress, chives, and most common house plants, etc.. Species of plants that have higher nutritional demands and will do well only in heavily stocked, well established aquaponic systems include tomatoes, peppers, cucumbers, beans, peas, and squash, among others (Rakocy, 1999a).

Aquaponic plants are subject to many of the same pests and diseases that affect field crops, although they seem to be less susceptible to attack from soil-borne pests and diseases. Because plants may absorb and concentrate therapeutic agents used to treat parasites and infectious diseases of fish, these products cannot be used in aquaponic systems. As an example related to pond culture, Avault (2001) reported the catastrophic loss of crawfish in an integrated rice-crawfish facility, after the use of the pesticide fipronil (ICON®) for the control of the rice water weevil. Even the common practice of adding salt to treat parasitic diseases of fish or to reduce nitrate toxicity would be deadly to plants. Instead, non-chemical methods are used, ie, biological control (resistant cultivars, predators, antagonistic organisms), barriers, traps, manipulation of he environment, etc.). It also seems that plants in aquaponic systems may be more resistant to diseases that affect those in hydroponic systems. This resistance may be due to the presence of some organic matter in the water, creating a stable, ecologically balanced growing environment with a wide diversity of microorganisms, some of which are antagonistic to pathogens that affect the roots of plants (Rakocy, 1999a).

Adler *et al* (2000) discussed the economics of an aquaponic system in the production of lettuce, sweet basil and rainbow trout, but did not indicate temperature levels for the growth of plants or fish.

In aquaponic environments, one of the concerns in the growth of plants is the effect of insect pests. For reasons mentioned previously, pesticides are not a practical answer in dealing with problems with insects in aquaponic environments. Some control strategies include the use of the bacterium *Bacillus thuringiensis* and insecticidal soaps.

In addition, phage therapy (page 69) has been suggested for the control of some diseases such as bacterial spot on tomatoes and *Erwinia* sp. infections of fruit trees (fire blight) and root crops (soft rot) (Brabban *et al*, 2003).

a) *Bacillus thuringiensis*

As noted, one of the concerns in the aquaponic systems is the control of insect pests of plants. However, the use of man-made chemical pesticides to control these insects is not a viable option in aquaponic systems. A practical method to aid in the control of insect pests on aquaponic plants may be through the use of strains of the *Bacillus thuringiensis* (*Bt*). This bacterial organism occurs naturally in the environment and has been isolated from insects, soil and the surfaces of plants. Its value lies in the fact that it produces substances that are toxic to insects. In 1961, it was registered as a pesticide in the USA and later, in 1998, it was re-registered (Anon, 2000).

The classification of *Bt* is difficult because of the close genetic relationship among *B. thuringiensis, B. cereus, B. anthracis* (the cause of anthrax), and *B. mycoides*. *Bacillus thuringiensis* is a Gram-positive, spore-forming rod that often has insecticidal properties. It belongs to the '*Bacillus cereus* complex' which includes those species mentioned previously. The taxonomic relationships among members of the *B. cereus* group are not clear, and are the cause of some concern, since the differences between *B. cereus* and *Bt* are small and possibly plasmid-based. The main characteristic separating *Bt* from the other *Bacillus* spp. listed is the formation of insecticidal crystalline proteins (Glare and O'Callaghan, 1998).

During sporulation, some strains of *Bt* produce one or more inclusions or parasporal bodies within a sporangium. The parasporal body is often toxic to specific groups of insects, and many different insecticidal crystal proteins (б-endotoxin) can be found in different strains and subspecies of *Bt*.

For example, products of *Bacillus thuringiensis israelensis* (*Bti*) contain the spores and parasporal crystals of *Bti* H-14 serotype that must be ingested by the larval stage of the insect to cause mortality. Following ingestion, the parasporal crystals are solubilized in the alkaline midgut of the larvae, followed by proteolytic activation of the soluble insecticidal crystalline proteins. The toxin binds to a receptor on the cell membrane of the midgut, and results in pore formation in the cell, and death of the larvae. Insecticidal effect is caused by the parasporal crystal, which for *Bti* usually contains four major proteins (27, 65, 128, 135 kDa). The crystalline toxins of *Bti* are designated Cry4A, Cry4B, Cry11Aa and Cyt1Aa (Glare and O'Callaghan, 1998).

The toxicity of *Bt* is insect-specific. There are subspecies of the organism that affect different organisms, eg, subspecies *aizaiwa* and *kurtstaki* affect moths, *israelensis* affects mosquitoes and flies, and *tenebrionis* affects beetles, etc.. These organisms are applied to food and non-food crops, green houses, forests and outdoor home use. As well, researchers have inserted genes from *Bt* in some crops (called *Bt* crops), such as corn, cotton and potatoes (Anon, 2000).

Summarizing their study on *Bt*, and *Bti* in particular, Glare and O'Callaghan (1998), commented as follows:

- Strains and varieties of *Bt* are pathogenic to a number of insect pests, including Lepidoptera and Diptera. In 1978, the discovery of *Bti*, a variety specific to Diptera, especially mosquitoes and black flies, has led to the development of many products based on this species of bacteria.
- There is a well-documented history of environmental safety of strains of *Bt* used in pest control. This fact, coupled with the nature of its toxicity and level of specificity for target hosts, has led to the use of *Bt* in many pest control programs in environmentally sensitive areas.
- The mode of action of *Bti* involves the synergistic interaction of four toxic proteins; toxicity to insects is related to the crystalline proteins formed during sporulation. The organism rarely recycles in natural environments.
- Aspects of the environmental impact that need to be considered include mammalian and non-target safety, effect on the environment, persistence and occurrence in the natural environment, and possible resistance of the

host. For microbial-based pesticides, such as *Bt*, gene transfer has to be considered.

- The close genetic relationship among *Bt, B. cereus* (an occasional human pathogen) and *B. anthracis* has raised concerns about possible implication of *Bt* in human gastrointestinal illnesses and other health problems caused by *B. cereus*. However, after extensive field use, no such ill effect has been detected. A specific identification system for strains of *Bt* would assist monitoring of future applications.
- After application, *Bti* does not persist in the environment. In general, reports of activity after application show a decline in efficacy within days, and little residual activity after several weeks. (On plant surfaces, *Bt* products degrade rapidly; they are moderately persistent in soil but their toxins degrade rapidly; *Bt* is not native to water, and is not likely to multiply in water; *Bt* is practically nontoxic to birds and fish; there is minimal toxicity of most strains to bees [Anon, 2000]).
- Some of the toxic proteins of *Bt* are encoded by genes residing on extra-chromosomal DNA (plasmids) which can be exchanged among strains and species by conjugation and/or transformation. Although genetic transfer between *Bt* and other soil bacteria has been demonstrated in the laboratory, it hasn't been shown in the field. Unexpected pathogens have not resulted from extensive application of *Bt*, which suggests that, although gene transfer may have implications for genetically modified strains, it is a lesser concern for wild-type strains.
- Some insects, especially Lepidopterans, have become resistant after constant application of strains of *Bt*. However, resistance has not occurred after the application of *Bti,* possibly as a result of the complex mode of action involving synergistic interaction among up to four proteins. The use of *Bti* for over 10 years in Africa, USA and Germany has not resulted in the development of resistance.

- Over 40 tons of *Bti* were applied in west Africa alone, without reports of safety or non-target concerns.

Glare and O'Callaghan (1998) listed a wide range of acari, amphibians, fish, crustaceans and insects, etc. that are not susceptible to *Bti.* Numerous references are also provided in this publication.

b) Insecticidal Soaps

Compared with traditional pesticides, insecticidal soaps control many targeted pests with fewer potentially adverse effects to the user, beneficial insects, and the environment – important factors in aquaponic systems. Insecticidal soaps are effective only on direct contact with the pests. The most common soaps are made of the potassium salts of fatty acids, which disrupt the structure, and permeability of cell membranes in insects. The contents of injured cells are able to leak from these cells, and the insect dies quickly. There is no residual insecticidal activity once the soap spray has dried.

Insecticidal soaps function best on soft-bodied insects such as aphids, mealybugs, spider mites, thrips, and whiteflies. It can also used for caterpillars and leafhoppers, though these large bodied insects can be more difficult to control with soaps alone. The addition of horticultural oils can increase the effectiveness of soap for harder to kill insects. Adult lady beetles, bumble bees and syrphid flies are relatively unaffected. Soap can be used with many beneficial insects, however predatory mites, larvae of green lacewing, and small parasitic wasps (such as the *Encarsia, Trichograma* and *Aphidius* spp. wasps) can be harmed with soap. Once the spray has dried, beneficial insects can be reintroduced safely into the treated area.

Soaps have low toxicity for mammals. However, they can be mildly irritating to the skin or eyes. Insecticidal soaps are biodegradable, do not persist in the environment, and they do not contain any organic solvents. It is less likely that resistance to insecticidal soaps will develop as quickly as it will to the more traditional pesticides. Resistance within the insect tends to develop more quickly with materials that have a very specific mode of action. There is a greater chance that resistance will develop to a material that affects the nervous system of an insect, for example, in a shorter period of time. Mixtures with foliar nutrients or pesticides containing metallic ions, such as zinc or iron, may be physically incompatible or phytotoxic.

Once an insecticidal soap spray has dried, there is no residual activity because soaps are effective only on contact. Therefore, if an insect has not been coated with the spray, it will not be affected by contact with or ingesting plant material that has been treated with soap.

Insecticidal soaps should be applied when conditions favor slow drying to provide maximum effectiveness, e.g., in the early morning hours with dew coverage or in the early evening. Treating with soaps on hot sunny afternoons promotes rapid drying of the material. Thorough coverage is vital for the soap to be effective. All soaps are long chain fatty acids, but not all soaps have insecticidal properties. Insecticidal soaps are specifically formulated to have high insect-killing properties, while being safe for most plant species. The soaps have no residual activity toward insects, but repeated applications may have damaging effects on some types of plants.

Hard water reduces the effectiveness of insecticidal soaps. Calcium, magnesium and iron precipitate the fatty acids and render them useless against the insects. Good spray coverage is essential for adequate results.

Insecticidal soaps may cause signs of phytotoxicity, such as yellow or brown spotting on the leaves, burned tips or leaf scorch on certain plants. In general, some crops and certain ornamentals are sensitive to burn caused by soaps. Multiple applications in a short time interval can aggravate phytotoxicity. In addition, water-conditioning agents can increase phytotoxicity. A precipitate may be formed when the metallic ions (e.g., calcium, iron or magnesium) found in hard water bind to the fatty acids in the soap (Anon, 2004b).

Some operators of aquaponic systems simply use a mixture of ordinary soap and water, and find it to be effective in controlling insects. One recipe is:

one teaspoon of liquid soap such as mild Dove®, Pure Ivory Soap®, Sunlight® or pure castille soap, per quart of water.

4. Fish

In Canada as well as other areas of the world today it is common to grow Nile tilapia (*Oreochromis niloticus*), a warmwater species, and in some cases, rainbow trout (*Oncorhynchus mykiss*), a coldwater species, in aquaponic systems. Tilapia appear to be one of the most popular species of fish reared in aquaponic systems.

Selection of a desirable *Tilapia* sp. depends on the rate of growth and their tolerance to cold. Rankings for the growth rate of *Tilapia* sp. in ponds are: *T. nilotica* > *T. aurea* > *T. rendalli* > *T. mossambica* ≥ *T. hornorum.* Tolerance to cold becomes increasingly important, especially for pond-rearing in more northern latitudes. *Tilapia aurea* is generally regarded as the most cold-tolerant of *Tilapia* spp.. The geographic range for culturing tilapia in outdoor ponds depends on temperature. The preferred temperature range for optimal growth of tilapia is 28-30°C (82-86°F). Growth diminishes significantly below 20°C (68°F), and death will occur below 10°C (50°F). Thus, *Tilapia* spp. are ideal for indoor aquaponic systems because the warm temperatures are also needed for the growth of plants (Rakocy and McGinty, 1989).

Other species of fish that are reared in aquaponic systems in other countries include largemouth bass (*Micropterus salmoides*), sturgeon (*Acipenser* spp.), hybrid and koi carp (*Cyprinus* spp), and baramundi (*Lates calcarifer*), etc.. Other common species used in aquaponic systems include sunfish (Family Centrarchidae), bream (*Abramis brama*), crappie, pacu (Family Characidae), red claw lobster or crayfish, and ornamental fish such as angelfish (*Pterophyllum scalare*), guppies (*Poecilia reticulata*), tetras (Family Chiracidae), gouramis (Family Belontiidae), swordfish (Family Xiphiidae), mollies (Family Poeciliidae), etc..

5. Water

From the perspective of food safety, the source of water used in aquaponic systems has the potential to have a significant bearing on the quality of the final products, whether they are fish or plants. In Alberta, deep wells or municipal supplies of water are the most common sources of water for experimental or commercial aquaponic systems, all of which are currently indoor facilities. According to Hutchings (2003), at least two of the deep-well sources of water for privately owned aquaponic systems in the province have a high total-salt content and generally, are not suitable for the growth of plants or freshwater fish.

In terms of water quality, and the concentrations of salts and minerals needed for the production of sweet basil (or general guidelines), Racozy (2003b) noted: '*Our general guideline is to feed fish at a ratio of 57 grams per m*^{2} *of plant growing area per day. This ratio provides good nutrient levels. We supplement with equal amounts calcium hydroxide and potassium hydroxide to maintain pH near 7.0. Every three weeks we add 2 mg/L of iron in the form of a chelated compound.*

In a commercial-scale aquaponic system at UVI (University of the Virgin Islands) that was to produce lettuce continuously for 2.5 years, nutrient concentrations varied within the following ranges (mg/L) that would have produced excellent sweet basil growth':

Calcium - 10.7-82.1	Phosphate P - 0.4-15.3	Copper - 0.01- 0.11
Magnesium - 0.7-12.9	Sulfate S - 0.1-23.0	Zinc - 0.11-0.80
Potassium - 0.3-192.1	Iron - 0.13-4.3	Boron - 0.01-0.23
Nitrate N - 0.4-82.2	Manganese - 0.01-0.19	Molybdenum - 0.00-0.17

Tests for water quality by the producer have been outlined by Mitchell (1998). Other papers relevant to effluent, waste management, and standards of water quality in aquaculture in general include those of Buttner *et al* (1993), Chen (1998), Boyd and Gautier (2000), Negroni (2000), and Lutz (2001).

Water can be a carrier of many microorganisms including pathogenic strains of bacteria, such as *Escherichia coli, Salmonella* spp., *Vibrio cholerae*, *Shigella* spp., and the microscopic parasites *Cryptosporidium parvum*, *Giardia lamblia*, *Cyclospora cayetanensis*, *Toxoplasma gondii*, and the Norwalk and hepatitis A viruses. Even small amounts of contamination with some of these organisms can result in foodborne illness in humans. The quality of water, how and when it is used, and the characteristics of the crop influence the potential for water to contaminate produce. In general, the quality of water in direct contact with the edible portion of produce may need to be of better quality compared to uses where there is minimal contact.

Other factors that influence the potential for contact with waterborne pathogens, and their likelihood of causing food-borne illness, include the condition and type of crop, the amount of time between contact and harvest, and post-harvest handling practices. Produce that has a large surface area (such as leafy vegetables) and those with topographical features (such as rough surfaces) that foster attachment or entrapment of organisms may be at greater risk from pathogens if they are present, especially if contact occurs close to harvest or during post-harvest handling. Some sectors of the produce industry use water containing antimicrobial chemicals to maintain water quality or minimize surface contamination (Anon, 1998c).

The quality of agricultural water will vary -- particularly surface waters that may be subject to intermittent, temporary contamination, such as the discharge of waste water or polluted runoff from livestock operations located upstream. Ground water that is influenced by surface water, such as older wells with cracked casings, may also be vulnerable to contamination. Practices to help ensure adequate water quality may include ensuring that wells are properly constructed and protected, treating water to reduce microbial loads, or using alternative methods of application to reduce or avoid water-to-produce contact. The feasibility of these and other practices will depend on available sources of water, the intended use of the water, and the needs and resources of the particular produce operation (Anon, 1998c).

Water supplies for onshore facilities have a very much higher risk of being contaminated by intestinal bacteria than do those for offshore operations. Feces from birds, animals and humans can enter bodies of water directly or from runoff from the land. For example, Strauss (1985) (cited by Howgate, 1998) reported the results of a global health-related environmental monitoring program of 110 rivers in four regions, namely, North America, South America, Europe, and Asia-Pacific. The median fecal coliform count in these rivers was in the range of 10^3–10^4 organisms/100 mL of water.

There are very few reports concerning the presence of pathogenic intestinal bacteria in farmed fish cultivated in unfertilized systems. Some studies have reported *Salmonella* spp. in ponds holding catfish, and on the skin and in the intestines of these harvested fish; the incidence was higher in samples taken in the summer compared with those collected in the winter. In Japan, this organism was found at low level in ponds holding eels, and in the intestines of fish in one pond. Several organisms including *Listeria monocytogenes, Plesiomonas shigelloides,* and *Shigella dysenteriae* (but not *Salmonella* spp.) were cultured from hybrid striped bass (*Morone saxitalis* x *M. chrysops*) reared in three freshwater systems in Maryland, USA. Two of the sites used well water, and the third used river water (Howgate, 1998).

The few data reported come from countries with temperate climates, and indicate a very low incidence of intestinal pathogens in fish cultured in unfertilized surface waters. However, the data point to an increased hazard during warm seasons. Of greater concern are the widespread practices of using human and animal waste as fertilizers in pond aquaculture, and of raising fish in waste waters. A number of studies cited by Howgate (1998) point up the health hazards of this practice.

An extensive microbiological study of water in, and fish cultured in, ponds filled with a mixture of waste waters found that bacterial loads were very high in these waters (Buras *et al*, 1987). Numbers of fecal organisms were in the order of 10^6 MPN (most probable number)/100mL of water, and those of *Salmonella* spp. were in the order of 10^2 MPN/100mL of water. At the end of the growing season, carp and tilapia in one pond had fecal coliforms in their tissues, including the muscle. *Salmonella* spp. were not detected in any tissue from these fish, but were detected in the digestive tract of tilapia from other ponds on other sampling occasions. The authors compared the bacterial counts in the tissues of fish and those in the water, and concluded that, in the water, there was a limiting count of 10^4/mL (standard plate count), below which bacteria of any kind did not penetrate tissues of the fish.

Other workers showed that in pond water, there is a 'threshhold' of about 10^3 organisms/mL above which the enteric organisms, *E. coli* and *Salmonella typhi*, will be found in the muscle of exposed fish (Pal and Dasgupta, 1991, cited by Howgate, 1998). Although the data were limited, there was evidence indicating that fish can be cultured in wastewater-treated ponds, without a significant risk to public health, as long as some safeguards are in place.

The bacterial load on/in contaminated fish can be reduced by allowing them to 'depurate' in clean water for a number of days, but the rate of reduction of the bacterial counts is very slow, and 'depuration' is likely not practical under commercial conditions. Some authors have proposed maximum counts of some bacterial species as guidelines for the management of aquacultural products from wastewater systems (Howgate, 1998).

In its 'Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables' (Anon, 1998c), the USDA recognizes certain basic principles and practices associated with minimizing hazards of microbial contamination of food from the field through the distribution of fresh fruits and vegetables. *However, it is important to note that these recommendations focus primarily on the common field methods of production, and NOT those of aquaponic/aquacultural methods*. Nevertheless, they provide some useful guidelines.

Some of the general principles recommended by the USDA include:

Principle 1. Prevention of microbial contamination of fresh produce is favored over reliance on corrective actions once contamination has occurred.

Principle 2. To minimize microbial food safety hazards in fresh produce, growers, packers, or shippers should use good agricultural and management practices in those areas over which they have control.

Principle 3. Fresh produce can become microbiologically contaminated at any point along the farm-to-table food chain. The major source of microbial contamination with fresh produce is associated with human or animal feces.

Principle 4. Whenever water comes in contact with produce, its source and quality dictate the potential for contamination. It is important to minimize the potential of microbial contamination from water used with fresh fruits and vegetables.

Principle 5. Practices using manure from animals or municipal biosolid wastes should be managed closely to minimize the potential for microbial contamination of fresh produce.

Principle 6. Hygienic measures and sanitation practices during production, harvesting, sorting, packing, and transportation play a critical role in minimizing the potential for microbial contamination of fresh produce.

It is important to note that infected employees may transmit a wide range of communicable diseases and infections through food or utensils. A partial list of infectious and communicable diseases that may be transmitted through produce include (**Table 4**):

Pathogens Often Transmitted by Food Contaminated by Infected Employees*	
1. Hepatitis A virus	Fever, Jaundice
2. *Salmonella typhi*	Fever
3. *Shigella* spp.	Diarrhea, Fever, Vomiting
4. Norwalk and Norwalk-like viruses	Diarrhea, Fever, Vomiting
5. *Staphylococcus aureus*	Diarrhea, Vomiting
6. *Streptococcus pyogenes*	Feve; Sore throat with fever

*****The symptoms of diarrhea, fever, and vomiting are also those of several other pathogens transmitted occasionally by food contaminated by infected employees.

Plumb (1999) noted that cyprinids (which include various species of carp, as well as minnows indigenous to Alberta) are extremely susceptible to infections by columnaris organisms, the cause of Bacterial Gill Disease in a variety of species of fish. Both mortality and acuteness of disease increase with temperature (Noga, 1996). In general, water from pond or irrigation sources would not likely be good sources for aquaponic systems because of the potential of introducing minnows and other species of fish, along with a variety of disease-causing agents, ie, viral, bacterial, parasitic, fungal, etc..

a) Algae

In the context of suitable sources of water for aquaponic systems, certain species of cyanobacteria (blue-green algae)-like bodies (CLB) have been reported to cause a prolonged syndrome of diarrhea, loss of appetite and fatigue lasting a range of 4 –107 days in humans in Chicago, USA and in the country of Nepal (Kocka *et al*, 1991). In this study, analysis of water from various sources, raw vegetables and cow manure detected CLB on one head of lettuce from which an affected patient had eaten two days before the onset of illness. Analysis of 184 stool samples submitted from affected patients at the end of an outbreak in Nepal, detected CLB in six (3%) patients.

Cyanobacteria are a diverse collection of primitive unicellular to multicellular photosynthetic bacteria usually found in water or very moist environments. In Alberta, species of cyanobacteria (commonly called blue-green algae) are well known for their role in poisoning cattle and other species when they ingest algal blooms that are concentrated by prevailing winds in areas of shoreline frequented by these animals when they come to drink.

When nutrients are in rich supply, some species of cyanobacteria may grow without light. The CLB are so named because they possess some morphological and reproductive characteristics similar to those of the Order Chroococcales of cyanobacteria. However, CLB don't have all the characteristics of any known type of cyanobacteria. These bodies may be seen on light microscopic examination of fresh stools as nonrefractile hyaline cysts measuring 8-9 µm in diameter (Kocka *et al*, 1991).

In 1989, the salmon farming industry in the Sechelt Inlet, BC, a well-protected but poorly flushed fjord, was heavily affected by algae (*Heterostigma* and *Chaetoceros* spp.). It is believed that, as the plume of the Fraser river turns northward, it has several effects: it tends to prevent water from leaving Sechelt Inlet, thereby reducing flushing action and creating the stability favorable to the growth of algae, as well as supplying nutrients that support the growth of these algae. In particular, the damaging effects of *Chaetoceros* sp. are better understood than those of *Heterostigma* sp.. In response to gill damage caused by algal spikes, even small numbers of *Chaetoceros* sp. (5 organisms/mL of seawater) were sufficient to stimulate the production of massive amounts of mucus, which inhibited the uptake of oxygen. This process caused the fish to convert to anaerobic metabolism, and ultimately led to death caused by one of three factors: 1) microbial infections of damaged gills, 2) hemorrhage of capillaries in the gills, or 3) suffocation as the result of the production of excess mucus. As well, damage to the gills allowed for the introduction of bacterial pathogens such as those of bacterial kidney disease and vibriosis that killed the affected fish (Stewart, 1997).

In another study, the blue-green alga, *Lyngbya* sp., was found to be abundant in several ponds in which catfish had an extreme off-flavor (Brown and Boyd, 1982).

However, the most significant public health problems caused by harmful algae are:

- Amnesic Shellfish Poisoning, caused by *Pseudo-nitzschia* sp. (domoic acid),
- Ciguatera Fish Poisoning, caused by a variety of algal species including *Gambierdiscus toxicus, Prorocentrum* spp., *Ostreopsis* spp., etc. (ciguatoxin, maitotoxin),
- Diarrhetic Shellfish Poisoning, caused by *Dinophysis* sp. (okadaic acid),
- Neurotoxic Shellfish Poisoning, caused by *Gymnodinium breve* (brevetoxins),
- Paralytic Shellfish Poisoning, caused by *Alexandrium* spp., *Gymnodinium catenatum* (saxitoxins).

Anamnesic shellfish and paralytic shellfish poisonings can be life-threatening, whereas the others listed cause illnesses from which recovery does occur. Recovery time following ciguatera fish poisoning may take weeks, months and even years. These toxicities occur in various coastal waters of the USA and around the world (Anon, 2003g).

b) Contaminants in Water

i) Hormones

In 2003, a study at St Mary's College of Maryland, USA, showed that minnows located immediately downstream from a large cattle feedlot in Nebraska had significant alterations in their reproductive biology. Male fish had one-third less testosterone and their testes were about half as big as those of unexposed fish; females had 20% less estrogen and 45% more testosterone compared with females from an uncontaminated stream. These findings indicated that effluent from feedlots is hormonally active, whether from natural or synthetic hormones injected into the cattle (Anon, 2003e).

The feeding of methyl testosterone (MT) in tilapia fry to produce a uniformly male population could suggest the presence of residues of this hormone. However, since these fish are fed MT for only a few days early in life, residues of this hormone are unlikely to be of concern in these fish at the time of marketing.

The injection of hormones such as human chorionic gonadotropin (HCG), a glycoprotein, in the spawning of fish has raised questions about residues in the tissues of these fish. However, Kelly and Kohler (1994) showed that in fish injected with HCG to induce ovulation and sperm production, heating (such as in cooking), as well as human digestive enzymes, will destroy residues of this hormone. In addition, these authors found that HCG was not detected in fish injected with this hormone after an average of 19 days (range 14-35 days) post-injection, depending on the species. It is interesting to note that tests for HCG in hybrid tilapia (*O. mossambicus* x *O. niloticus*) used in these experiments were negative at 14 days post-injection.

ii) Drugs

A study from Oslo, Norway found that marine fish near an Arctic city had been receiving a mix of caffeine and painkillers from a local sewer. As well, samples taken from a sewer outlet near a psychiatric hospital had measurable amounts of anti-epileptic drugs and anti-depressants. Also found was ibuprofen, an anti-inflammatory drug often used to treat arthritis (Anon, 2003f). Hirsch *et al* (1999) cited a number of papers dealing with medications, including antibiotics, antiphlogistics (anti-inflammatory drugs), lipid regulators and beta-blockers found in aquatic environments in a number of countries. These studies would appear to represent 'the tip of the iceberg'.

A study to be released by the government of Alberta into levels of drugs and antibiotics in the Bow river reflects current concerns about the safety of sources of water in this province.

iii) Polychlorinated biphenyls

Not only biological agents, but also pollutants are of great concern (Arkoosh *et al*, 1998). As we live in a virtually inescapable worldwide sea of polluted air, water and soil, it seems impossible to guarantee that our food supplies are completely free of contaminants. For example, it has been reported that seven of 10 farmed salmon purchased at grocery stores in Washington DC, San Francisco, California, and Portland, Oregon were contaminated with polychlorinated biphenyls (PCBs) at levels that raise health concerns. The report, released by the Environmental Working Group (EWG), has claimed that farmed salmon are likely the most PCB-contaminated protein source in the US food supply, and contain 16 times the amounts found in wild salmon, four times the level in beef, and 3.4 times the amount found in other seafood. The source of these PCBs is believed to be the fishmeal (most supplies are from Iceland, Peru, Chile and Denmark) fed to these salmon, although the origin of these salmon was not indicated (Anon, 2003b). However, Whelan (2003) discounted these claims and questioned the credibility of the EWG as a shadowy, non-scientific group.

Despite these objections, a report in early 2004 from CBC-TV indicated that there are definite risks to human health from the consumption of more than one meal of farmed salmon every two months, because contaminants such as PCBs are 10 times higher in these fish than in wild salmon (Anon, 2004d). In a subsequent press release, Health Canada reported that levels of PCBs in farmed (and wild) salmon are within the 2 ppm safety guideline, and thus, are safe for human consumption (Anon, 2004).

In a scientific publication however, Krümmel *et al* (2003) showed that wild stocks of sockeye salmon (*Onchorhynchus nerka*) returning from the sea to spawn in pristine lakes in Alaska can act as bulk-transport vectors of PCBs. When these fish die after spawning, PCBs are released into the sediment of these lakes and increase in concentration by more than seven-fold in some instances when the density of returning salmon is high. The source of PCBs in this case is believed to be distant industrial activities that release these pollutants into the atmosphere and oceans.

In an experimental study, Arkoosh *et al* (1994) found that B-cell mediated immunity was suppressed in juvenile Chinook salmon (*O. tshawytsha*) after exposure to either a polycyclic aromatic hydrocarbon or to PCBs. Johnson *et al* (2003) provided a detailed document on the public health implications of human exposure to PCBs: the finding of elevated levels of PCBs in human populations, plus the presence of developmental and neurological problems in children whose mothers ate PCB-contaminated fish, have serious implications in public health.

iv) <u>Organic Pollutants -- Pesticides, Herbicides, etc.</u>

Aquacultural systems can be affected by acute and chronic discharges of organic pollutants. Acute pollution results from single, short-lived discharges such as accidental spillages from chemical plants into water supplies or by the grounding of sea vessels. Most industrial and agricultural chemicals are readily degraded by chemical and biological processes in soil and water, and do not accumulate to any large extent, and are rapidly eliminated from fish. Some studies have measured the uptake and loss of several agricultural chemicals by/from fish, and showed that these chemicals had low accumulation co-efficients and short half-lives of the order of hours. In one study, a single dose of parathion was added to a pond. Within two days, the fish concentrated this pesticide about 100-fold compared with the concentration in water, but 'depurated' it to very low levels by a month after exposure (Howgate, 1998).

More difficult to control is chronic contamination. In aquaculture, the main routes of chronic contamination are the use of polluted water, leaching of agricultural or industrial chemicals from treated or contaminated soil into surface waters, and deposition from the atmosphere. Many chlorinated compounds are discharged into, or are present in, the aquatic environment, but three groups in particular are of concern: 1) chlorinated insecticides such as DDT, dieldrin, lindane and their degradative products, 2) PCBs and 3) polychlorinated dibenzo-*p*-dioxins (PCDDs) and –difurans (PCDFs). Summaries and reviews of environmental impacts and the fate and significance to human health, of chlorinated organic compounds and other contaminants in the aquatic environment have been provided by several authors cited by Howgate (1998).

According to Howgate (1998), a hazard that apparently hasn't been investigated in aquacultural products is the presence of persistent organochlorines. High concentrations of these contaminants have been found in fish from some freshwater environments. For example, there is official advice against consuming fish from some parts of the Great Lakes because of high levels of organochlorines. By analogy, it is possible that fish in freshwater aquaculture could be affected similarly. There are theoretical reasons related to the physical properties of these contaminants and to aquacultural practices, for <u>fish from freshwater aquaculture to pose only a low risk of harm to humans</u> – however, measurements are needed for confirmation of this point. The flux of organic contaminants in aquatic ecosystems, their distribution among different compartments of the system, and their accumulation through trophic chains have been modeled and applied successfully to field situations (several references, cited by Howgate, 1998). It would be useful to apply these models to some representative aquacultural systems in order to predict how persistent chlorinated hydrocarbons, if present, would be distributed in these systems.

Kennish and Ruppel (1996) found contamination by chlordane (1,2,4,5,6,7,8,8-octachloro-3☟-tetra-hydro-4,7-methanoindane) that is used in formulations of pesticides, at levels ranging from 5-2150 ppb wet weight in the tissues of four species of finfish and one of shellfish from estuarine and coastal marine waters of New Jersey, USA. In terms of contamination of water by pesticides and herbicides, several studies have been conducted by Agriculture Canada and/or AAFRD, and have shown levels of these products in a number of samples from Alberta (Hill *et al*, 1996; Hill *et al*, 2000; Ontkean *et al*, 2000; Hill, 2001). As well, Miller *et al* (1992) and Olson *et al*, (2003) reported on the effects of agricultural practices on water quality in Alberta.

A joint study by Agriculture Canada and AAFRD on the effects of agricultural management practices on water quality in southern Alberta detected the presence of selected herbicides at significant concentrations in surface runoff, effluent from subsurface drainage and ground water, under surface and sprinkler irrigation. As well, significant concentrations of nitrate were found in ground water under irrigated soils subjected to high applications of manure from feedlots (Miller *et al,* 1992).

In a more recent study on the Crowfoot Creek watershed near Strathmore, Alberta, Ontkean *et al* (2000) determined that levels of total phosphorus, total coliform bacteria and total dissolved solids often exceeded both Alberta and Canadian guidelines for water quality. In addition, levels of several pesticides often exceeded guidelines. Five pesticides were detected in this study; MCPA exceeded guidelines at least 50% of the time, and Dicamba met the irrigation guideline less than 30% of the time.

v) Manure

A study on the application of manure and its effects on the quality of soil and ground water under irrigation in southern Alberta, found that repeated application of manure, especially at high annual rates (60-120 mega grams/ha/year) significantly affected the quality of soil and ground water, with a buildup of nutrients in the soil, and the movement of nitrate and chloride into ground water. The report also indicated that even at low rates of application, phosphorus will concentrate in soil at the surface, a concern for potential contamination of surface water by phosphorus through surface runoff (Olson *et al*, 2003).

vi) Metals

Many metals and metalloids of concern for human health exist in a number of forms and valency states, and the chemistry of their fate in water is complex. The pH of water plays a large part, and for metals, solubility decreases with increasing pH. Fresh waters tend to be alkaline, and aquacultural systems in ponds are usually maintained at a pH above 8.0. As well, ponds usually have an aerobic, organic-rich sediment, conditions under which metals tend to precipitate in the sediment as insoluble sulfides or hydrated oxides.

The concentration of metals in edible portions of aquacultural products rather than in the water in which fish are reared, is relevant to public health. Although metals can enter fish by absorption through the gills or by absorption from feed, the latter is the more important route of the two. Metals are accumulated in tissues in which their concentrations are greater than in water or feed. In vertebrate species of fish, concentrations of metals are lowest in muscle, and tend to concentrate in kidney and liver. Sewage often contains high levels of heavy metals, but measurements in farmed fish, even those in sewage-fertilized systems, with the possible exception of mercury, are below regulatory or recommended limits (Howgate, 1998).

The significant exception to the regulation of metals in muscle by vertebrate fish is mercury in its organic form of methylmercury. Inorganic mercury can be methylated by biological, predominantly microbiological, processes. This organic form is taken up by aquatic organisms, and as a result, the concentration in tissues can be orders of magnitude greater than that in the water. Because methlymercury accumulates up the trophic chain, the highest concentrations are found in predatory fish. More than 95% of the total mercury in the edible portions of fish and invertebrates is in the form of methylmercury (Howgate, 1998; Gorski *et al* 1999). Ward and Neumann (1999) described seasonal variations in concentrations of mercury in the axial muscle of largemouth bass (*Micropterus salmoides*).

vii) Odors, Off Flavors, etc..

As a point of interest, an examination of the strong odor of freshly chopped cucumbers in the Australian grayling (*Prototroctes maraena*) correlated it with trans-2-*cis*-6-nonadienal, an organic compound known to have the intense fragrance of cucumbers. The authors of this study also noted that the natural odor of cucumbers is shared by a number of identified salmoniform fish. There would not appear to be an issue of food safety in this finding (Berra *et al*, 1982).

Off-flavor in pond-reared channel catfish has been reported to be a frequent problem for farmers and has been viewed as a water quality-related phenomenon. Results of experiments conducted by Brown and Boyd (1982) indicated several possible causes that included a high rate of feeding. Although correlations between chlorophyll-α and chemical oxygen demand (COD) were not significant, ponds with the lowest concentrations of chlorophyll-α and COD contained the best-tasting fish. The blue-green alga, *Lyngbya* sp., was abundant in several ponds in which fish had an extreme off-flavor.

The aforementioned studies represent a sample of many investigations to determine the presence of pollutants of various kinds in water in southern Alberta and other sites; they indicate that, indeed there are pollutants present in water for agricultural use. A more detailed examination of several other published studies on pollutants in water is beyond the scope of this document. Pertinent citations of environmental and experimental studies on pollutants/toxins may be found in several of the references in this section and in the Supplementary References section.

Over all, it would seem that the best sources of water for aquaponic operations are likely to be treated municipal water supplies, or those from drilled wells or springs. All such supplies of water, especially those from wells, should be analyzed prior to use for their levels of chemical constituents and contaminants, to determine their suitability for both plants and fish (Mitchell, 1998).

6. Feed

In human food supplies, hazards that may be related to feed for animals may include salmonellosis, mycotoxicosis (toxins from molds), and the ingestion of unacceptable levels of veterinary drugs and agricultural and industrial chemicals. The link between BSE and variant Jakob-Creutzfeldt disease in humans is another example of contamination from livestock feeds (Orriss, 1997.)

Mycotoxins are secondary metabolites produced by various genera of fungi that grow on agricultural products before or after harvest, or during transportation or storage. Some species such as *Aspergillus* and *Penicillium* can invade grain after harvest and produce toxins, whereas others such as *Fusarium* spp. typically infest grains and produce toxins before harvest. In some cases, *Aspergillus* spp. can grow and produce toxins before the crop is harvested (Orriss, 1997).

Mycotoxins may be carcinogenic (ie, aflatoxins B_1, ochratoxin A, fumonisin B_1), estrogenic (zearalenone, and I and J zearalenols), nephro (kidney) toxic (ochratoxins, citrinin, oosporeine), dermo (skin) necrotic (trichothecenes), or immunosuppressive (aflatoxin B_1, ochratoxin A and T-2 toxin). These toxins are regularly found in ingredients for animal feeds – maize (corn), sorghum grain, rice meal, cottonseed meal, peanuts, legumes, wheat and barley. Most are relatively stable and aren't destroyed by processing, and may even be concentrated in screenings. For humans, the main source of mycotoxins is contaminated grains and cereal, rather than animal products. Hence, the hazard is much greater in developing countries in which maize and other grains form the staple diet (Orriss, 1997). Experimental studies on the effects of aflatoxin in channel catfish have been reported by Jantrarotai *et al* (1990), and by Jantrarotai and Lovell (1990).

According to Tacon (2000), aquaculture consumes about 35% of the world supply of fishmeal, and the expectation is that by 2010, this level will rise to 56% of the entire supply. The use of commercial feeds containing fish meal seems to be a subject of current concern, given the findings of contamination with polychlorinated biphenyls (PCBs) in farmed (Anon, 2003b) and wild salmon (Krümmel *et al*, 2003). Most supplies of fishmeal, which is the suspected source of PCBs for farmed salmon, originate from Iceland, Peru, Chile and Denmark.

Prior to the introduction of pelleted, expanded and extruded feeds for fish, *Salmonella* spp. could be recovered from feeds. At present, given the high cooking temperature used in modern processes, these bacteria are rarely, if ever, detected in feed.

Feed is an ideal vehicle for the delivery of various 'neutraceuticals' in support of the immune system of fish and other species (de Wet, 2002).

Massie (2003) discussed the commercial production of environmentally friendly feeds for aquaponic systems.

7. Food Safety

The safety of food for human consumption is becoming increasingly important/significant on a worldwide level. The recent, devastating industry-wide problems associated with the discovery of a single case of Bovine Spongiform Encephalopathy (BSE, 'Mad cow') that occurred in Alberta, plus the discovery in late 2003 of an Alberta-born BSE-affected Holstein cow in Washington state, USA, are only two examples. Another is the reverberations that continue to this day in connection with human illness and deaths caused by exposure to suspected animal-origin, water-borne *E. coli* serotype O157:H7 in Walkerton, Ontario in 2000. As well, among other examples, this bacterial serotype was the cause of serious illness in Japanese school children (Nickelson, 1998), in addition to illness associated with unpasteurized apple cider produced from fallen apples (ie, apples that had fallen from the trees prior to harvest) contaminated with livestock manure (Mshar *et al*, 1997). Cyclosporiasis, caused by a coccidia-like parasite that appears to be specific to humans, in raspberries imported into the USA (Hofmann *et al*, 1996; Nickelson, 1998; Anon, 1998a,b; Sterling and Ortega, 1999), and cryptosporidiosis (Mshar *et al*, 1997) associated with fallen apples, are further examples of the growing issue of the safety of the food supply in a shrinking world.

In spite of the many positive aspects of aquaculture/aquaponics -- such as the nutritional benefits of farmed fish (Hardy, 1998) -- in terms of food safety, it is important to examine the subject to determine the possible impact of factors that can adversely affect the final product.

Nickelson (1998) noted that the per capita consumption of seafood (a combination of salt-and-freshwater fish) in the USA in 1998 was only 7.7% of all meat (**Table 2).**

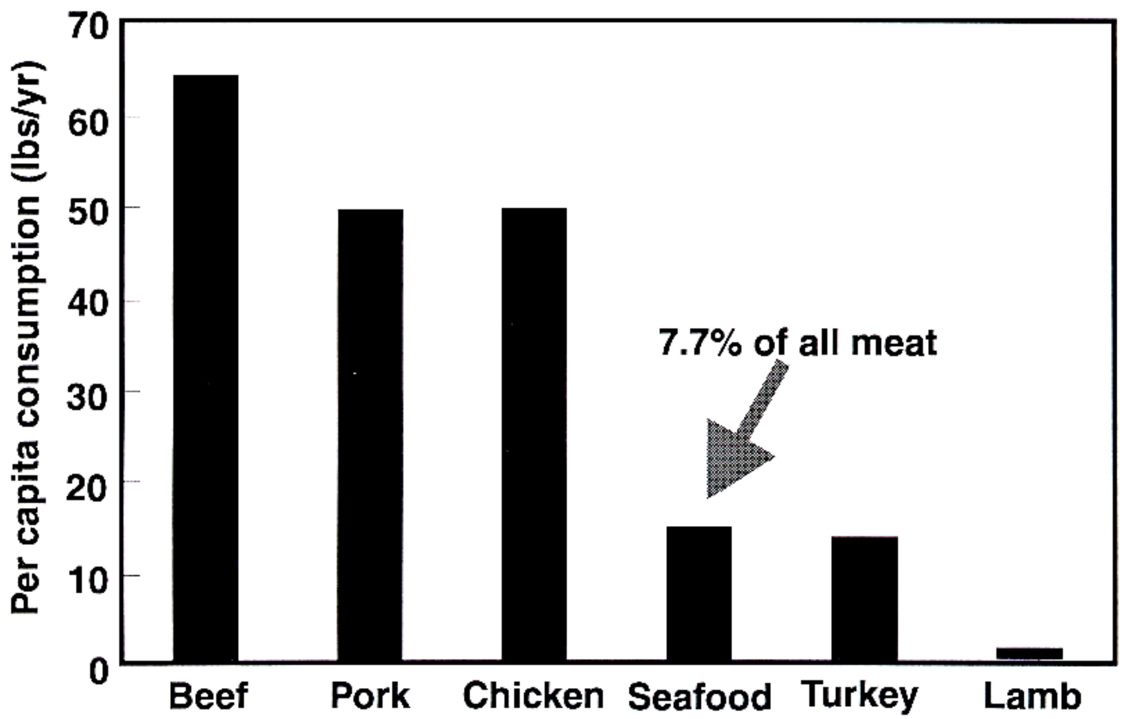

Table 2. Per capita consumption of meat, poultry and seafood in the USA (From: Nickelson, 1998).

However, the percentage of confirmed outbreaks of food-borne disease was highest in seafood at almost 17%, compared with approximately 6% for beef, 5% for chicken, 2% for turkey, and just over 1% for pork, [even though the per capita consumption of seafood was much lower than it was for beef, pork and chicken (**Table 3**)].

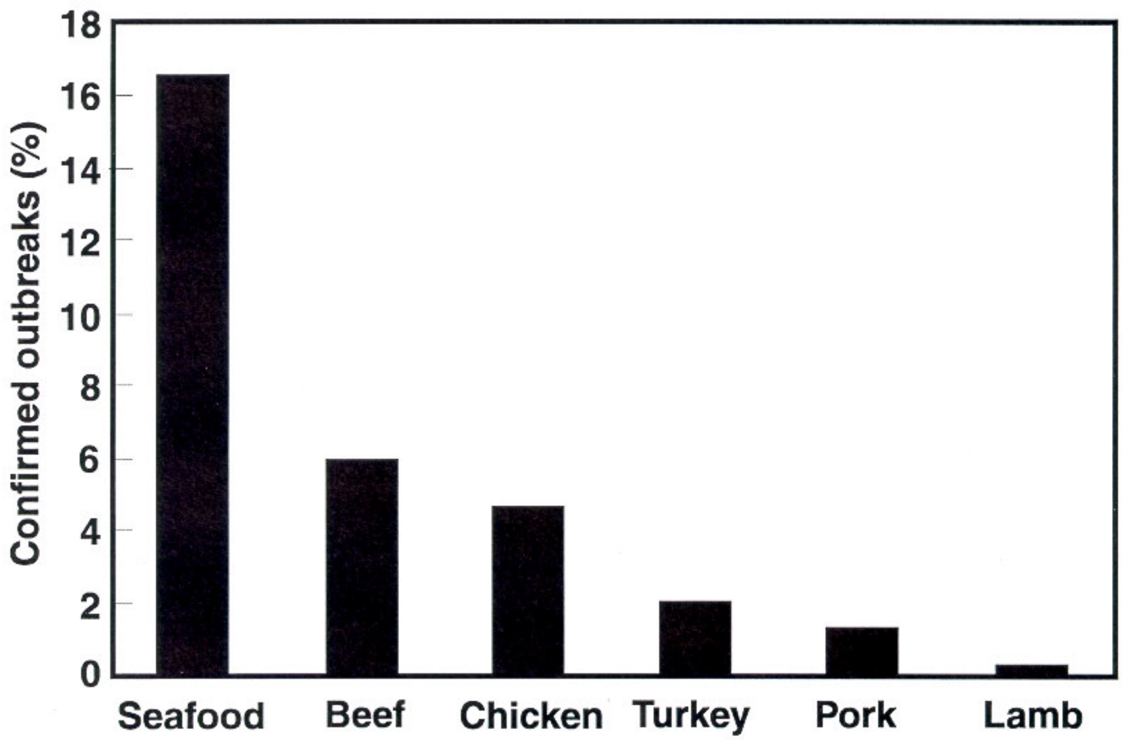

Table 3. Percentage of confirmed outbreaks of food-borne disease from meat, poultry and seafood (From: Nickelson, 1998).

Garrett *et al* (1997) examined public, animal and environmental implications of aquaculture (of which aquaponics is one component), and made the point that although most seafood is safe for human consumption, it is not entirely without risk. For example, of the seafood-borne illnesses reported to the Centers for Disease Control and Prevention (CDC) in the USA, more than 90% of the outbreaks and 75% of the individual cases were associated with ciguatoxin from a few reef species, and scombrotoxin from tuna, mackeral (Family Scombridae)[1], bluefish (*Pomatomus* sp.), and the consumption of mainly raw mollusks etc.. However, none of these outbreaks was associated with aquacultural products (MacMillan, 2001).

Garrett *et al* (1997) noted that in many developing countries, it is a common practice to create numerous small fish ponds, an approach that can have a greater adverse effect on human health than the use of a single large pond. Small ponds increase the overall aggregate shoreline of ponds and produce higher densities of mosquito larvae and cercariae (intermediate stages in the life-cycle of flukes), which in turn, can increase the incidence and prevalence of human diseases such as filariasis and schistomoniasis, respectively.

The improper or illegal use of chemicals such as tributyl tin to control pests such as snails in ponds in some parts of the world can result in hazards to human health (Garrett *et al*, 1997; Howgate, 1998).

A number of reviews related to the safety of fishery products (cited by Howgate, 1998) relate to products harvested from the wild, and are heavily weighted toward the hazards of fishery products consumed in technologically more advanced countries. Most information on these hazards and on incidents

[1] Poisoning by scombrotic fish is caused by the excessive production of histamine in the muscle, and is the result of the breakdown of amino acids by a range of bacterial species (Inglis *et al*, 1993).

of food poisoning is derived from these countries. By contrast, there is only scant literature related to food poisoning from fishery products consumed in developing countries, which are located mainly in tropical climates.

Reviews of bacteriological hazards associated with fish often separate the food-poisoning organisms into two main groups: those that are indigenous to the aquatic environment from which fish/shellfish are harvested, and those that are present on the fish/shellfish as a result of contamination of the water by human or animal feces. A third group is sometimes considered and is comprised of bacteria introduced to the product during handling and processing (Howgate, 1998).

8. Bacterial Diseases that may affect Fish and/or Humans

Like other animals raised for food, fish reared in aquaculture/aquaponic facilities have the potential to be affected by a variety of viral, bacterial, parasitic, and mycotic (fungal) agents, and also may be contaminated by antibiotics, mycotoxins (toxins produced by molds), pesticides, etc..

Several food-borne pathogens (parasites[2], bacteria, viruses, dinoflagellates) and toxins are associated with aquatic species (Harper, 2002). Because aquaponics is one component of the broader field of aquaculture, and food safety is always paramount, a look at some examples of the bacterial diseases that fish and humans may share in common seems to be in order.

Infectious disease does not happen in isolation. In order for disease to begin, the classical configuration of the interaction of the host, the agent and the environment must come into play. This idea suggests that it is the opportunity for exposure (or lack of) to these various agents and the susceptibility of the host that determine whether these specific infections will occur. The interplay of the

[2] It is interesting that the author found a large, coiled unidentified parasite on the surface of some imported frozen red snapper (*Lutjanus* sp.?) purchased at Save On Foods, Lethbridge)

host, the infectious agent and the environmental conditions certainly affects the final outcome of these biological encounters (Hedrick, 1998; Reno, 1998).

Topically (skin) acquired zoonotic diseases (diseases transmitted from animals to humans) including those caused by bacterial species such as *Aeromonas, Edwardsiella, Erysipelothrix, Mycobacterium, Streptococcus (iniae),* and *Vibrio* spp. have also been discussed by Harper (2002b,c). These topical infections usually occur as the result of injuries from the spines of fish or through contamination of open wounds. Although most humans have a strong natural immunity to wounds infected by marine bacteria, more serious infections are often associated with immune-compromised individuals, deep puncture wounds, and highly virulent strains of bacteria (Harper, 2002b).

The association between disease in fish and the health of in-contact humans is dramatically exemplified not only by reports of tuberculosis caused by *Mycobacterium marinum*, but also by Crayfish Handlers' disease caused primarily by *Erysipelothrix* and *Vibrio* spp. bacteria, and by infection with *Anisakis* spp. nematodes (Anon, 1997a; Alderman and Hastings, 1998). Graphic photos of human infections caused by *M. marinum* (fish tank granuloma, swimming pool granuloma) may be seen at http://tray.dermatology.uiowa.edu/MMarin01.htm . Hu and Koberger (1983) reported the isolation of *Vibrio cholerae*, the human intestinal pathogen, from 11 of 19 (58%) non-diseased American eels (*Anguilla rostrata*) from the estuary of the Suwannee river in Florida. Additional references of the occurrence of this pathogen in other identified marine species are listed in the report by Hu and Koberger (1983).

Human infections caused by the bacterium *Listeria monocytogenes* (Anon, 2002a, 2002b, 2003a), which is often associated with the ingestion of mayonnaise-based seafood and other deli salads and smoked seafood, and those caused by *Salmonella* spp. and *E.coli*, are of importance and concern (Penner, 2003).

Ignorance of the microbial profile of aquacultural products can also affect human health and has led to the transmission of streptococcal infections from tilapia to humans (Weinstein *et al*, 1996). As well, a change in marketing strategies to sell live fish in small containers instead of ice packs, has resulted in human infections with *Vibrio* spp. bacteria originating in tilapia in Israel (Garrett *et al*, 1997).

The abuse and misuse of raw chicken manure as fertilizer for ponds may result in the transmission of *Salmonella* spp. to the cultured product, and hence, to humans (Garrett *et al*, 1997). Several species of aquatic animals, including snails, clams, oysters, newts, frogs, crayfish, turtles, alligators, crocodiles and fish, have been known to carry *Salmonella* spp. (several references cited by Bocek *et al,* 1992). Souter *et al* (1976) found enteric (intestinal) bacteria in a study of common carp (*Cyprinus carpio*) and white suckers (*Catostoma commersoni*) from five locations, four in Ontario and one in Quebec. As well, these authors cultured *Salmonella enteritidis* serotype Montevideo from the intestines of fish netted in the St Lawrence river at Montreal. It is notable that in 1975, the International Joint Commission reported that four of the five areas sampled did not meet water quality objectives in 1974 (Souter *et al*, 1976).

Because of the foregoing findings, especially the observations of Garrett *et al* (1997), some concerns about food safety related to the use of quail in aquaponic systems, as proposed by Nuttle (2003a), arise because of the use of these birds in an aquaponics system. However, to date, Nuttle (2003b) has not reported any disease problem in this system. Nevertheless, in the absence of quality-control measures the potential for disease issues to arise continues to exist in this system.

An experimental study by Bocek *et al* (1992) determined that silver carp (*Hypophthalmichthys molitrix*) could retain a streptomycin (antibiotic)-resistant strain of *Salmonella typhimurium* in their intestines. However, other internal organs such as kidney were not affected. Isolations of this organism from the intestine occurred for 14 days after exposed fish were placed in clean water. These findings and those of Souter *et al* (1976) indicate the potential for the contamination of aquatic environments, and the transmission of *Salmonella* spp.

and other enteric pathogens of humans to other species of fish in the same environment, and by extension, to humans consuming these fish. Many species of *Salmonella* may infect humans. However, other biological factors may mitigate the possibility of such transmission (MacMillan, 2001).

Ignorance of the hazards associated with the use of untreated animal or human waste in ponds has huge implications in human health. For centuries, those engaged in food production have cultured species of fish in wastewater-fed ponds and have grown secondary vegetable crops in waste water and sediment material in integrated aquacultural operations. Under these conditions, the potential for the transmission of human pathogens to cultured species of fish is seldom considered (Garrett *et al*, 1997).

Rice *et al* (1984) conducted a controlled study in Malaysia where four species of fish, (silver, bighead [*Aristichthys nobilis*], grass [*Ctenopharyngodon idella*] and common [*Cyprinus carpio*] carp, and shrimp (*Macrobrachium rosenbergii*) were reared in ponds that received swine manure as fertilizer. These workers showed that although the (worm) parasites *Ascaris* and *Trichuris* spp. were present in the manure from hogs, and in pond water and sediments, human parasites were not found in the digestive tract of necropsied fish or shrimp. Ponds enriched with swine manure generally supported more pathogenic bacteria as well as more total bacteria, compared with control ponds.

Different species of bacteria including *Aeromonas*, *Pseudomonas*, *Corynebacterium* spp. and several species of Enterobacteriaceae were isolated in relatively low numbers from the scales of fish grown in swine manure. These authors concluded that since low numbers of pathogenic bacteria and human parasites were seen in/on these carp and shrimp, potential infections of processors or consumers would be reduced by proper handling and processing. In aquaculture, despite the potential for the transfer of pathogenic bacteria from fish to humans, there are several natural barriers to the transfer of resistance factors among bacterial species and the occurrence of enteric bacteria that infect humans. These barriers include temperature, itinerant (transitory) microbial flora, and important physiological and evolutionary differences. Various physical factors may also decrease the probability of the transfer of resistance.

Likely the most obvious natural barrier is that of body temperature. Farmed aquatic species are all poikilothermic, with a labile body temperature that is dependent on environmental temperature. In poikilotherms, body temperature is generally too low to be considered optimal for the proliferation of most intestinal bacteria likely to infect humans. Most human food-borne pathogens prefer the comparatively warm temperatures of homeotherms (MacMillan, 2001). However, the rearing of a species such as tilapia could allow for the proliferation of an introduced human pathogen, since the warm temperatures required for optimal growth of this species approach those that are also suitable for the growth of bacterial pathogens (Rakocy and McGinty, 1989).

a) *Streptococcus* spp.

Streptococcal septicemia (invasion and multiplication of bacteria in the bloodstream) has occurred sporadically and as epizootics (outbreaks) among cultured freshwater and saltwater fish in many parts of the world. For example, Kusuda *et al* (1978) described the isolation of a *Streptococcus* sp. from an epizootic in cultured eels. However, the streptococcal species most commonly involved is *Streptococcus iniae*. It is mainly a disease of tilapia, hybrid striped bass (*Morone saxatalis* x *M. chrysops*) (Stoffregen *et al*, 1996), and rainbow trout. The known cyprinid species that are affected include golden shiner and blue minnow (*Fundulus grandis*) (experimental). Some species that don't appear to be affected by this agent include common carp, big-mouth buffalo (*Ictiobus cyprinellus*), goldfish, and certain species of tilapia (eg., *Sarotherodon mossambicus, Tilapia sparrmanii*) (Inglis *et al*, 1993). However, Johnson (2003) noted that *S. iniae* is a serious problem in some operations rearing Nile tilapia (*T. nilotica*), and equally importantly, it can be an important disease of humans.

Because of these facts, infections caused by *S. iniae* in humans and tilapia will be discussed in some detail, as follows.

Tilapia spp. are common food fish reared in aquacultural/aquaponic settings. It seems that one of the most serious diseases with which producers may be faced in the rearing of tilapia is infection by *S. iniae*, a β-hemolytic bacterial species that was first reported in 1976 as the cause of 'golf ball disease' in Amazon freshwater dolphins (*Inia geoffrensis*) housed in aquaria in the USA. The first streptococcal infection in fish was reported from rainbow trout in Japan, and in tilapia in 1970. Outbreaks of this disease in tilapia were reported later from Japan in 1981, Taiwan in 1985, Israel in 1986, and the USA and Saudi Arabia in 1992. The species was renamed *S. shiloi* in Israel in 1986, but following taxonomic validation in 1995, the name *S. iniae* was retained because it was published before *S. shiloi* (George, 1998). Worldwide, streptococcal infections have been reported from about 22 species of fish. The most seriously affected species include yellowtail (*Seriola quinqueradiata*), eel (*Anguilla* spp.?), tilapia, striped bass (*M. saxitalis*), rainbow trout and turbot (*Scophthalmus maximus*). The countries in which fish are most affected by this disease include Japan, Israel, the USA, South Africa, Australia and Spain (George, 1998).

Infection by *S. iniae* in humans was first recorded in Texas, USA in 1991 (George, 1998), and in Ottawa, Ontario in 1994 (Weinstein *et al*, 1996). In the initial report from Ontario, *S. iniae* was isolated from four individuals who had a history of preparing fresh, whole aquaculturally-reared fish purchased locally. Three of these individuals had a history of injury to their hands during preparation of these fish. While she was preparing tilapia, one individual punctured her hand with a bone, the second had lacerated the skin over her finger with a knife that had just been used to cut and clean an unidentified freshwater fish, and a third punctured her finger with the dorsal fin of a tilapia she was scaling. The period from injury to the onset of symptoms ranged from 16-48 hours. At the time of hospitalization, these patients had fever and cellulitis (inflammation of the connective tissues beneath the skin), with spread of the infection above the point of injury. Blood cultures from all three patients were positive for *S. iniae*. Treatment with beta-lactam antibiotics (penicillins or cephalosporins, etc.) or clindamycin resulted in complete resolution of the illness.

The fourth patient, a male, had a week's history of increasing pain in a knee, intermittent sweating, fever, difficult breathing, and confusion. About 10 days before he was admitted to hospital, he had prepared a fresh tilapia, but there was no indication that he had injured himself at that time. Blood cultures from this patient were positive for *S. iniae*. He was diagnosed with valvular endocarditis (infection of the heart valves) and meningitis caused by *S. iniae*. Treatment with beta-lactam antibiotics and erythromycin resulted in recovery. Later, surface cultures from four fresh tilapia collected from selected fish markets by health authorities yielded *S. iniae* from three fish; however the strains of *S. iniae* isolated were different from those involved in the outbreak.

The source of fresh whole tilapia sold in Ontario was fish farms in the USA. As a result, samples of live aquacultured fish imported into Canada were to be collected and cultured for *S. iniae*. Additional human cases of the disease have been identified both in Canada and the United States, and further isolations have been made from several species of fish (CMPT, 1997).

Streptococcus iniae is also known to cause disease in tilapia. The organism is transmitted horizontally from fish to fish. It may colonize the surface of the fish or it can cause invasive disease that may result in mortalities of 30-50%. Affected fish may swim erratically and display a whirling motion at the surface of the water, as a result of meningitis. Externally there may be darkening of the affected fish, dorsal rigidity, swollen abdomen, bulging eyes (exophthalmos), corneal opacity, rupture of the eyes, as well as hemorrhage of the lower jaw, abdomen, opercula, anus and the base of fins. Internally, bloody fluid (ascites) may be found in the body cavity, along with a pale liver and enlarged spleen; affected fish die within several days of infection (Perera *et al*, 1994; George, 1998). In tilapia, signs of infection may be absent, or the disease may cause losses of 30-50% in affected fish.

b) *Edwardsiella* spp.

Two members of the *Edwardsiella* spp. group of bacterial organisms infect fish: *Edwardsiella tarda* [formerly called *E. anguillimortifera* and *Paracolobactrum anguillimortiferum* (Noga, 1996)] and *E. ictaluri.* These bacteria produce two different diseases.

Edwardsiella tarda causes septicemia (invasion and multiplication of bacteria in the bloodstream) in warmwater fish, particularly in eels and catfish (*Ictaluris punctata*), but is also known to cause disease in tilapia (Alceste and Conroy, 2002). This organism is widely disseminated in aquatic animals, pond water and mud, occurrences that provide ready opportunities to re-infect cultured fish. Infected fish processed for human consumption are a source of this organism, which can cause gastroenteritis in humans.

Edwardsiella ictaluri causes a septicemia in catfish, and is a highly contagious disease with serious effects on the commercial culture of catfish (losses from 10-50%) in the southern USA (Inglis *et al*, 1993; Noga, 1996).

Edwardsiella sp. septicemia is a mild to severe systemic disease of mainly warmwater fish in the USA and Asia. It is caused by *E. tarda* and is also called fish gangrene, emphysematous putrefactive disease of catfish, and Red Disease of eels. Catfish and eels, notably Japanese eels (*Anguilla japonica*) [but not reported from American (*Anguilla rostrata*) or European eels (*Anguilla anguilla*)], and catfish, are the most commonly infected species. However, the organism has been isolated from a variety of species of fish, including goldfish (*Carassius auratus*), common and grass carp, tilapia, etc. (Noga, 1996;).

In the USA, *E. tarda* has been isolated from 75% of water samples holding catfish, 64% of mud samples from ponds holding catfish, and 100% of frogs, turtles and crayfish from ponds containing catfish. The source of this organism is likely intestinal contents of carrier animals. Catfish and eels, as well as amphibians and reptiles, are likely sources of infection. Although environmental stressors don't appear to be essential for infection to occur, high temperature, poor water quality and crowding are likely contributing factors. Infections caused by *E. tarda* are not confined to fish, but are also found in snakes, alligators, sea lions, birds, cattle, swine and humans (Inglis *et al*, 1993; Noga, 1996).

Edwardsiella tarda is an important zoonotic disease of humans in which it is a serious cause of intestinal disease. In humans, it has also been implicated in meningitis, liver abscesses, and wound infections; most commonly, however, this organism causes gastroenteritis. Catfish fillets in processing plants are often contaminated with this organism that may spread to humans by the oral route (Noga, 1996). In an earlier study, Brady and Vinitnantharat (1990) injected live catfish with *E. tarda* or *E. ictaluri*, *Aeromonas hydrophila*, and *Pseudomonas fluorescens*, and when the injected fish died or were moribund, they were frozen at –20^{o}C. These workers found that *E. tarda* could be recovered on culture for 50 days, *E. ictaluri* for 30 days, *A. hydrophila* for 20 days, and *P. fluorescens* for 60 days, in these frozen fish.

c) *Aeromonas* spp.

Aeromonas spp. bacteria occur widely in fresh water and sewage. For example, Henebry *et al* (1988) found that the most common bacterium in the gut of young silver carp fed alternately on manure-silt and algal sources of food was *A. hydrophila*. Some species of *Aeromonas* are pathogenic for fish, and occasionally, to humans.

According to Noga (1996), motile aeromonad infection (MAI) is likely the most common bacterial disease of freshwater fish, all of which are probably susceptible. Motile aeromonads can also inhabit brackish water, but they decrease in prevalence with increasing salinity.

By far the most important bacterial pathogen of fish is *Aeromonas hydrophila* (synonyms: *A. liquefaciens, A. formicans*). This group of organisms is often described as the *A. hydrophila* complex. Motile aeromonads are common on the mucosal surfaces and internal organs of clinically normal fish, and are often secondary invaders in infections such as those caused by *A. salmonicida.*

Kumar and Dey (1985) reported on septicemia (invasion and multiplication of bacteria in the bloodstream) caused by *A. hydrophila* in silver carp.

Davis and Hayasaka (1983) found that during the first nine months of culture, glass eels and elvers of the American eel (*Anguilla rostrata*) were affected by only a small number of bacterial pathogens and diseases. *Aeromonas hydrophila* accounted for 98.3 % of the *Aeromonas* spp isolated from these eels. In the next several months, only *Aeromonas* spp. were found to be associated with disease in these eels.

Aeromonas salmonicida causes a fatal outbreak of disease called furunculosis in salmonids. 'Furunculosis' is a term borrowed from a human condition. However, the changes seen in salmonids affected by this condition do not resemble the pus-filled swellings on the skin of humans affected by classical furunculosis. Despite these differences, the designation persists because it is too well established in scientific literature to be changed (Inglis *et al*, 1993; Cipriano and Bullock, 2001). Further information on this genus can be found in Cipriano *et al* (1996) and Cipriano *et al*. (1996a).

As a point of interest, it is useful to be aware that infections caused by *Aeromonas* spp. in humans have been known since the early 1950s (Mathewson and Dupont, 1992). The most common manifestation of *Aeromonas* spp. infections in humans is bacteremia (the presence of bacteria circulating in the bloodstream). As well, wound infections in humans are becoming more commonly reported in the scientific literature. The importance of these infections in humans is related to the fact that they can have fatal or seriously debilitating results, such as the amputation of affected limbs (Musher, 1980). Accordingly, wound infections should not be washed in river or pond water!

d) *Erysipelothrix rhusiopathiae*

Although this bacterial organism is not a pathogen of fish, it has been isolated from a number of different farmed species of fish such as cod (Family Gadidae and herring (Family Clupiedae), etc.. It can survive for long periods of time in the mucous layer of fish, and is transmitted to humans through skin injuries from scales, teeth, bones or spines. In humans, the organism can cause three different types of lesions. Firstly, it can cause what is known as 'fish rose', a localized red-purple lesion on the hand or fingers. Secondly, it can cause a more diffuse skin lesion. Although rare, the last form is a septicemia that can lead to endocarditis (infection of the heart valves or the inner wall of the heart). Mortality rates for endocarditis can be 50%. Those at highest risk of infection by this organism are fish producers, handlers and fishermen (Harper, 2002b).

e) *Vibrio* spp.

Vibriosis is a common disease in freshwater and marine fish, and can cause localized ulcers of the skin, inappetance, darkening of the fish, abdominal distention, anemia, subdermal cavitation, plus lesions in muscle and eyes. Several different *Vibrio* spp. cause disease in marine fish, but not all of them are human pathogens. The known zoonotic pathogens include *V. cholerae*, *V. damsela, V. vulnificus,* and *V. parahaemolyticus.* Human disease associated with *Vibrio* spp. is most often associated with the ingestion of raw or improperly cooked fish and shellfish. Clinical signs in humans can include mild gastroenteritis, diarrhea, fever, septicemia, and may even lead to death (Harper, 2002b). As an example of the potential seriousness of rare *Vibrio* spp. infections in humans, in late 2003, *V. vulnificus* was confirmed to have caused the death of an individual working with hybrid tilapia (*O. mossambicus, O. nilotica* and *O. aureus*) in Israel, where tilapia are reared in brackish water (600-3000 ppm salt) (Lenoir, 2003).

f) *Mycobacterium* Spp.

Mycobacteria, consisting of a single genus, *Mycobacterium*, are currently represented by at least 54 recognized species of organisms. Most of these agents are free-living in soil and water; some species cause disease in animals and humans. Mycobacterial infections of fish are, in fact, tuberculosis of a number of species. The disease affects a wide range of freshwater and marine species of fish, and particularly aquarium fish, especially the freshwater families Anabantidae (climbing gouramies), Characidae (piranhas, tetras, etc.) and Cyprinidae (Noga, 1996). However, it seems likely that any species of fish may be infected. Mycobacteriosis is a chronic systemic disease, with lesions (granulomas) developing externally and throughout internal organs.

The species of *Mycobacterium* that are pathogenic for fish are *M. marinum, M. fortuitum* and *M. chelonae.* Treatment is not satisfactory, and diseased stock should be destroyed, especially since these agents can infect humans as well as fish (Inglis *et al*., 1993).

Mycobacterium marinum represents the largest proportion of all mycobacteria isolated from fish. Tropical freshwater and tropical marine fish may be infected, but natural infection in a temperate-water species has not been reported (Inglis *et al*, 1993).

The isolation of *M. fortuitum* has been documented less frequently than that of *M. marinum*, but the prevalence of infection by *M. fortuitum* is likely more widespread than is suspected. This organism infects fish from both tropical and temperate waters, but is most common in freshwater fish, although infection is known to occur in marine species (Inglis *et al*, 1993).

So far, infection by *M. chelonae* has been identified only in coldwater salmonid species. This infection has been specifically linked to freshwater hatchery environments, but once established, it seems to persist throughout both fresh and saltwater phases of the life cycle (Inglis *et al*, 1993).

The main signs of this illness depend on the species of fish involved and the existing ecological conditions (Inglis *et al*, 1993). The common findings are listlessness, lack of appetite, emaciation, difficult respirations, exophthalmia, skin discoloration and external lesions that range from loss of scales to nodules, ulcers and necrosis of fins as signs of advancing infection (Inglis *et al*, 1993). In coldwater salmonids, there may be no external sign of the disease other than mortality, or variable degrees of skin coloration. Internally, lesions are similar in tropical and coldwater fish. Visible or microscopic tiny gray-white lesions may be found scattered in any tissue, but especially in spleen, liver and kidney.

Mycobacteria that are pathogenic for fish can infect humans, in which the lesions are usually localized, non-healing ulcers (fish tank granuloma, swimming pool granuloma) that may be difficult to treat because of resistance by the causative organisms to most anti-tuberculosis drugs. For photos of lesions in humans, see http://tray.dermatology.uiowa.edu/MMarin01.htm. Although the risks to healthy humans are low, infections caused by *M. marinum* have been reported from HIV-infected individuals. Accordingly, gloves should be worn by individuals who are at risk when cleaning aquaria or handling fish (Noga, 1996). Johnson (2003) too has warned of the zoonotic dangers of this organism to individuals working with species of carp.

g) *Listeria* Spp.

Listeria spp. are widespread in soil and water. This species has been isolated with high frequency from both fresh and marine waters and from sediments. Several surveys of fishery products (raw and processed fish collected at the retail level or during processing) for *Listeria* spp. have recovered this organism with frequency: often, it has been recovered from one quarter of the samples examined (Howgate, 1998). It is notable that in their review of *Listeria* spp. in seafoods, Dillon and Patel (1992) did not cite any reference to the presence of this species on freshly harvested fish, either from the wild of from aquacultural sources. The organism has been found in a variety of raw foods such as uncooked meats and vegetables, as well as in processed foods that are contaminated after processing – ie, soft cheeses and cold cuts. Vegetables can become contaminated from the soil or from manure used as fertilizer (Anon, 2003h).

In humans, listeriosis is a serious infection caused by the ingestion of food contaminated with the bacterium *Listeria monocytogenes*. This disease affects primarily pregnant women, newborn infants, and adults with weakened immune systems. Pregnant women are about twenty times more likely than other healthy adults to be infected with this bacterium; persons with AIDS are almost three hundred times more likely to be infected compared with those with normal immune systems (Anon, 2003h). It has been estimated by the CDC that up to 2,500 cases of listeriosis resulting in 500 deaths (20% mortality!) occur annually in the USA (Anon, 2002c). In 2002, the CDC reported an outbreak of listeriosis attributed to contaminated poultry from a processing plant in the northeastern USA, and resulted in the recall of 27.4 million tons of ready-to-eat poultry products (Anon, 2002a, b).

Infected pregnant women may have only a mild influenza-like illness that can lead to miscarriage or stillbirth, premature delivery, or infection of the newborn infant.

Although several of these zoonotic diseases are self-limiting or uncommon, accidents can happen while gutting or handling fish. Immunodeficient patients (ie, those on steroid therapy, HIV patients) are at high risk. Good personal hygiene and proper sanitation during work with fish will help to prevent infections. As well, the assistance of proper medical care in treating abrasions or cuts, especially if healing seems delayed, progresses in size, forms a nodule, or if other signs arise (Harper, 2002b).

h) *Clostridium botulinum*

In freshwater environments, a high incidence of the bacterial organism *Clostridium botulinum* has been found in fish and in sediments in trout farms in Britain and Denmark, but given the widespread occurrence of this organism on the land and in water, it is very likely much more widespread in other farms

(Howgate, 1998). The causative organism is not infective but produces potent toxins. There are seven toxigenic types of *Clostridium botulinum* (A to G) that produce potent neurotoxins. Type E has been incriminated in each recorded case in fish. Spores of the organism are very heat-resistant, and can withstand moist heat at 100°C for several hours, but are destroyed at 120°C in five minutes (Inglis *et al*, 1993).

Botulism causes a severe illness in humans and other animals; in humans, headache, disorders of vision, weakness and respiratory distress, vomiting, abdominal pain and diarrhea may be followed by neurological signs within one to six days. Resulting partial paralysis may persist for months; if the outcome is fatal, death usually occurs in the first 10 days of the illness (Inglis *et al*, 1993.)

In fish, the neurotoxin causes progressive muscular paralysis that affects all but the caudal fin. As a result, fish swim erratically and lose equilibrium; affected fish float on the surface, sink to the bottom, apparently recover and repeat the cycle until death intervenes (Inglis *et al*, 1993).

The presence of the organism in farmed trout has caused considerable debate about the risks of botulism in processed fishery products. The risk factors are more associated with aspects of processing, packaging and storage of the product than with the presence of the organism in fish. In any analysis of the public health hazards of farmed fish, it must be assumed that fish will carry spores of *C. botulinum* (Howgate, 1998). According to Inglis *et al* (1993), the risk to human health from botulism associated with seafood is real but not huge. In a review by the CDC in the USA, it was found that fish or fish products were implicated in 4.4% of outbreaks of botulism. Most of these involved canned, smoked or vacuum-packed seafood of the kind usually consumed without further cooking (which ordinarily would have inactivated the toxin).

The incidence of botulism is low and is associated with bad husbandry. The major reservoir of toxin and disease is fish that have died or are dying of the disease. Stock in contaminated systems must be slaughtered, pond debris removed and all buried in quicklime. Ponds holding affected fish can be returned to use within a month, but improvements in flow rates of water and reduced stocking densities must be made (Ingis *et al*, 1993.)

In Hawaii, in tilapia affected by the Hawaii Tilapia Rickettsia-Like Organism (HTRLO), blood vessels become blocked by large aggregates of inflammatory cells that damage the gills and diminish or block the transport of oxygen. The disease seems to be a seasonal event and occurs primarily during the winter, leading to speculation that it is the result of a complex interaction between the organism and one or more environmental factors, particularly low temperatures. Affected fish have pale streaks in the gills or they appear pale in color. Research continues (Anon, 1996).

As exemplified in the information on *Streptococcus iniae*, a few published reports that reviewed safety or health and farm-reared fish, suggest that it is only by puncture wounds associated with tilapia or catfish, or the consumption of raw fish, that any human disease has occurred (MacMillan, 2001).

In a UK study on *Campylobacter* spp. infections, a cause of gastrointestinal illness in humans, Evans *et al* (2003) found three major factors that contributed to these infections: eating chicken, <u>eating salad vegetables such as tomatoes and cucumbers</u>, and drinking bottled water. The study suggested that vegetables could be contaminated either before or after the point of sale. Contamination at the source could occur through contaminated soil or water during harvesting. As an example of the latter, these authors cited a report (Long *et al*, 2002) of imported lettuce as a vehicle for outbreaks of infection with *Salmonella* and *Shigella* spp. bacteria in the UK. However, they made the point that such infections derived from fruit and vegetables are rare. Further, the study showed that the *Campylobacter* spp. infections were mainly the result of cross-contamination in the kitchen, and that the association with tomatoes and cucumbers was the result of the need for extensive handling of these vegetables during preparation, and often the use of a chopping board.

9. Public Health and Bacteria Associated with Fish

Mankind is more comfortable with both laws and sausages when he doesn't know how they're made. Cross-Country Checkup, 2003

Human infections that may be caused by bacteria in fish include food poisoning and gastroenteritis (*Salmonella, Vibrio, Clostridium* spp., *Campylobacter jejuni*, etc.), wound infections and mycobacterial infections (tuberculosis).

Table 5 lists significant human pathogens isolated from fish or their environment (Inglis *et al* , 1993):

Salmonella spp.	Food poisoning
Vibrio spp.	Food poisoning
Campylobacter spp.	Gastroenteritis
Plesiomonas shigelloides	Gastroenteritis
Edwardsiella tarda	Diarrhea
Aeromonas hydrophila	Diarrhea, septicemia
Pseudomonas spp.	Wound infection
Mycobacterium marinum	Tuberculosis
Erysipelothrix rhusiopathiae	Erysipeloid, septicemia
Leptospira interrogans	Leptospirosis
Clostridium botulinum	Botulism

a) Food poisoning caused by pathogens in the aquatic environment

Fish-borne bacterial food poisoning may be caused by the bacteria naturally present in the aquatic environment, those derived from aquatic pollution, or those introduced during handling and processing. Bacteria naturally present in the aquatic environment and implicated in food poisoning include *Vibrio* spp. and *C. botulinum* type E. Twenty-three of 272 samples of seafood and water taken off southwest India contained *Vibrio cholerae* non-01; the pathogenicity of strains of *V.cholerae* non-01*, V. parahaemolyticus, V. vulnificus,* and *V. mimicus* isolated in the same region has been confirmed (Malathi *et al.* 1988, cited by Inglis *et al*, 1993). Sporadic cases of tropical diarrhea have been attributed to consumption of freshwater fish carrying *Edwardsiella tarda* and *Plesiomonas shigelloides*, with species such as *Tilapia* providing the natural habitat of these bacteria.

If fish contaminated with these pathogens are harvested and stored at temperatures conducive to bacterial multiplication and then consumed, gastroenteritis may result. In humans, the symptoms associated with *V. parahaemolyticus* are characterized by abdominal pain, vomiting, watery diarrhea, fever, chills and headache. The incubation period is 12-48h and recovery occurs commonly within 5 days. Usually cooking or heat processing kills *V. parahaemolyticus* but low temperature storage only reduces multiplication, and organisms have been detected after two days storage at $4^{o}C$ from fish contaminated with about 103 cells per gram. *Vibrio vulnificus* causes septicemia, chills, fever, sometimes vomiting and diarrhea, and cutaneous lesions and ulcers may occur at the extremities. The onset of symptoms occurs within 24 hr of exposure. Individuals with impaired function of liver or stomach are particularly vulnerable.

b) Bacterial spoilage of fish

As noted by Inglis *et al* (1993), bacterial spoilage in fish is a complex process involving microbiological and non-microbiological processes. Nonmicrobiological deterioration is caused by endogenous proteolytic enzymes that are concentrated particularly in the head and viscera; such enzymes attack these organs and surrounding tissues after death. Activity is particularly great in fish that recently had been feeding heavily, leading to early rupture of the gut with dissemination of general contents including enzymes and micro-organisms. Enzymatic spoilage may be compounded by deterioration resulting from oxygenation of unsaturated fatty substances that cause loss of flavor and the development of rancidity.

During life, micro-organisms are present on the external surfaces of the fish and in the gut, but the muscle is normally sterile. After the death of fish, microbiological organisms diffuse into the muscle and increase in number, slowly at first, and then more rapidly, and cause a sequence of changes in odor and flavor. The rate of deterioration related to all processes can be slowed by immediate storage at low temperature, and by rapid removal of the viscera, skin and head. In regard to aquaculture, most of the global production occurs in Asia and the Pacific where refrigeration and other processing facilities may be limited. Several methods of preservation are available – icing, canning, chemical preservation, etc. – to delay these effects.

The bacterial flora of fish is derived essentially from the aquatic environment and varies with seasonal and environmental factors. Further, it is affected by the type of storage and processing following capture. Fish from subtropical waters have a high percentage of mesophilic bacteria, whereas in fish caught in cold waters, psychrophiles such as *Pseudomonas*, *Achromobacter* and *Flavobacterium* spp. predominate. During low-temperature storage, numbers of *Pseudomonas* spp. increase substantially and in one study, were found to reach 60-90% of the total count of bacteria in coldwater fish. *Pseudomonas*, *Alteromonas* and related species are considered to comprise the major part of the spoilage flora. They grow actively at low temperatures near $0^{o}C$, and attack thioamino acids and thioamines to produce hydrogen sulfide and other volatile sulfides. Microbiological safety and quality are usually determined using 'marker' organisms to indicate the presence of given pathogens or toxin formers at specified levels.

The bacteria which present a public health risk grow best at 35-37^{o}C, whereas spoilage bacteria have a lower optimum temperature for growth. A total count of bacteria in a sample incubated at the higher temperature gives an indication of the degree of contamination with potentially harmful bacteria. Determination of the incidence of *E. coli* and coliforms indicates fecal contamination, and sometimes it may be useful to investigate the presence of specific pathogens such as coagulase-positive *Staphylococcus* and *Streptococcus* spp.. Elevated levels of histamine are taken to indicate bacterial quality more generally and the risk of scombroid poisoning.

10. Antibiotics and Bacterial Resistance

Abuse of modern technology in aquaculture includes the willful misuse of therapeutic drugs, chemicals, fertilizers, and natural fisheries habitats. The widespread use and misuse of antibiotics to control diseases in agricultural and aquacultural species is worldwide and may well increase with increasing intensive livestock husbandry. For example, the illegal use of the antibiotic chloramphenicol in the culture of shrimp to control disease may result in residues of this antibiotic in the final product (Garrett *et al*, 1997; Rakocy, 2003a). The importance of chloramphenicol in humans is related to the occurrence of two types of depression of the bone marrow: 1) a reversible, dose-related interference with iron metabolism, and 2) an intractable anemia in some individuals (1:25,000 patients) after treatment with this antibiotic – hence the long-established ban on its use in humans and food-producing animals (Anon, 2004a).

In some countries, the availability or use of drugs for aquaculture is very limited, thus decreasing any potential impact on public health. In the USA, only two products are approved by the FDA for use in aquaculture: oxytetracycline (eg. Terramycin) and the potentiated sulfonamide, Romet-30 (a combination of sulfadimethoxine and ormetroprim) (Stoffregen *et al*, 1996). These two products are approved for use in channel catfish and salmonids, but only for certain diseases. In 2000, it was estimated that approximately 2.4 x 10^4 kg of antibiotics/year were delivered by feed mills in the production of over 600 million pounds of catfish held in ponds. It was also estimated that the industry rearing trout used 2-3 x10^3 kg of antibiotics/year in medicated feed (MacMillan, 2001).

In Canada, approved antibiotics/chemotherapeuticals for use in cultured food fish include florfenicol[3], oxytetracycline, erythromycin, and trimethoprim-sulfadiazine (Anon, 2004c). In 1998, in British Columbia, the aquaculture industry used, and continues to use, three basic antibacterial compounds: oxytetracycline, two potentiated sulfonamides, and florfenicol. When each was considered in its use in fish, it was determined that 99.7% were approved for use in fish; the remaining 0.3% applied to fish were licensed for use in food-producing animals and were prescribed for fish under experimental protocols, or for fish not destined for human food (ie, brood stock). The majority of antibiotic-supplemented feed used in BC aquaculture was applied when fish were juveniles (smaller than 2 kg); between 72-94% of antibacterial drugs applied to salmon were fed to small fish. Such treatment of juvenile fish also created a drug-free clearance period of four to 12 months before fish were considered ready for harvest. None of the antibacterial agents used in farmed fish in BC has been used as growth promotants (Sheppard, 2000).

It has been stated that each farming company in BC applies considerable effort to minimize the need for, and use of, medicated feeds. The decision to use antibacterial products is made with care by the owner and attending veterinarian. Some farms are able to produce fish efficiently without the need for antibacterial

[3] Florfenicol is a fluorinated derivative of thiamphenicol, and is a potent antibacterial agent with bacteriostatic properties (Horsberg *et al*, 1996).

agents. Others have a self-imposed 'no medication' period of six or 10 months before harvest. Some owners find that medication is essential to reduce the effects of bacterial diseases (Sheppard, 2000).

The use of antibiotics to treat disease in humans, and in various agricultural practices, has increased the worldwide prevalence of antibiotic-resistant bacteria. One example of many described illness associated with a cephalosporin-resistant *E. coli* among attendees affected as well with salmonellosis at a summer camp (Prats *et al*, 2003). There is concern that all uses of antibiotics select for bacteria that are resistant to antibiotics; the greater the use of antibiotics, the greater the selection pressure, and the more frequently are resistant pathogens encountered (Alderman and Hastings, 1998; MacMillan, 2001). For example, during epidemiological investigations of an epidemic of human cholera in Ecuador, it was discovered that the local shrimp industry might have contributed to an outbreak of antibiotic-resistant *Vibrio cholerae* in humans. It was suggested that there was improper use of antibiotics in the shrimp industry and that this led to the development of resistant *V. cholerae*. The counter argument was that poor public hygiene in affected areas was the major problem (Angulo, 2000).

Contamination in the kitchen or wound infections may be routes by means of which antibiotic-resistant organisms might cause illness that could be difficult to treat in humans. As noted earlier in this report, *Aeromonas hydrophila, Vibrio* and *Mycobacterium* spp. are organisms most likely to be involved (Alderman and Hastings, 1998). Recent information on human infections caused by *Streptococcus iniae* indicated that it too is such an agent (George, 1998). Persons involved could include food handlers, farm staff and fish processors; although these risks might be hazards of handling, it is also possible that the greater risk to humans in this area might arise, not from farmed fish reared for food, but from ornamental fish (Alderman and Hastings, 1998).

Antibiotic resistance is variably defined depending on specific needs. In terms of public health, resistance is often defined in a clinical context as an indicator of the likely outcome of therapy; it can also be defined in terms of bacterial patterns of growth in the presence of antibiotic-impregnated discs on agar media, the presence of certain genes for resistance, or as an epidemiological attribute. Resistance is either chromosomally or extra-chromosomally mediated. Resistance can be natural or a result of genetic mutation, or it can be induced by the transfer of genetic information among bacteria (Alderman and Hastings, 1998; MacMillan, 2001; Harper, 2002a).

Although all types of resistance may be clinically important, the possibility of extra-chromosomal resistance, such as the transfer of plasmids[4], etc. among different bacteria, is of great concern. For example, an *Aeromonas* sp. bacterium in the water or on a fish, and resistant to oxalinic acid, might transfer a resistance factor to an *E. coli* organism on fish or in the water. Such an organism might infect humans, or transfer the resistance to other human pathogens already present in humans (Bruun *et al*, 2003). The use of antibiotics in terrestrial animals may also cause antibiotic resistance in human pathogens, but it is hard to demonstrate cause and effect, and is considerably more controversial. Even so, some scientific reports support the idea that this is possible. Several reports support the contention that the presence of fluoroquinolone-resistant *Campylobacter jejuni* and *C. coli*, both human pathogens, has increased because of the use of these products in chickens and pigs. These worldwide bacteria can cause human gastrointestinal infections and diarrhea. It is known that *Campylobacter* spp. are spread mainly through the consumption of contaminated poultry. Although the significance of the *Campylobacter* data is in dispute, regulations to ban the use of fluoroquinolones from use in poultry have been instituted in the USA. (Incidentally, the CBC National news for January 15/04 showed a concerned Vietnamese farmer <u>spraying</u> antibiotics over his chickens in an attempt to prevent Avian Influenza that swept through Southeast Asia!) It

[4]Plasmids are extra-chromosomal, self-replicating structures found in bacterial cells; they code for genes for a variety of functions that are not essential for the growth of cells. They replicate independently of chromosomes, and transmit through successive cell divisions, functions such as resistance to antibiotics [called the R plasmid]), etc..

seems, however, that there is no current report that proves that antibiotic contamination of the aquatic environment has caused human bacterial pathogens to become resistant (MacMillan, 2001).

Food-borne or zoonotic disease associated with aquacultural products including aquaponics, seems to be rare; for this reason it is assumed that that food-borne bacterial pathogens that are also resistant to antibiotics in these products are even more rare. As noted earlier in this report, most food-borne illnesses associated with fish in the USA were caused by non-bacterial conditions such as ciguatera and scombroid poisonings, but in none of these situations was an aquacultural product involved (MacMillan, 2001).

In aquaculture, there are several natural barriers to the transfer of resistance factors and the occurrence of enteric bacteria that infect humans. These barriers include temperature, itinerant (transitory) microbial flora, and important physiological and evolutionary differences. Various physical factors may also decrease the probability of the transfer of resistance. Likely the most obvious natural barrier is that of body temperature. Farmed aquatic species are all poikilothermic, with a labile body temperature that is dependent on environmental temperature. Generally, the body temperature of poikilotherms is too low to be considered optimal for the proliferation of most enteric bacteria likely to infect humans. Most human food-borne pathogens prefer the comparatively warm temperatures of homeotherms (creatures like humans that have a relatively stable temperature that is independent of the ambient temperature; warm-blooded) (Inglis *et* al, 1993; MacMillan, 2001).

The usual enteric bacteria of concern in debates on public policy are *E.coli, Campylobacter jejuni, C. coli, Shigella* and *Salmonella* spp*, Vibrio cholerae, V. paratyphi, Staphylococcus aureus, Listeria monocytogenes* and *Yersinia enterocolitica* (Inglis *et al*, 1993; MacMillan, 2001). Of these species, only *L. monocytogenes* is known to be capable of reproducing at low temperatures – in fact, usual refrigerator temperatures (4^{o}C) are ideal for the proliferation of these organisms, and are often used to encourage their growth in diagnostic material. However, the optimal temperatures for the proliferation of this organism are nevertheless in the range of 35-37^{o}C. Optimal incubation temperatures for all of the other species mentioned seem to be above 30^{o}C; these organisms are often isolated from clinical specimens that are incubated at temperatures of 35-37^{o}C. Because the preferred temperature range for the optimal growth of tilapia is 28-30^{o}C (82-86^{o}F) (Rakocy and McGinty 1989), it is possible that some of these organisms could proliferate in tilapia.

Cool and coldwater aquacultural production occurs at temperatures that are generally below 18^{o}C. Warmwater aquacultural production occurs at temperatures higher than 18^{o}C, and includes temperatures as high as 30 ^{o}C. In surveys of bacteria present under aquacultural conditions, *Salmonella* spp., *E. coli* and other potential enteric human pathogens, although rare or in very low numbers when present, are most often found in warmwater aquaculture rather than in coldwater environments. *Vibrio cholerae, V. parahaemolyticus* and other *Vibrio* spp. have been detected in estuarine and marine environments, including marine aquaculture. *Vibrio* spp. are generally the predominant bacterial genus in estuarine waters, and tend to have seasonal changes in abundance; their greatest abundance occurs during months of warm weather, which accounts, in part, for the seasonal occurrence of food-borne illness associated with the consumption of raw shellfish (MacMillan, 2001).

Present evidence suggests that psychrophilic and psychrotrophic bacteria (‘psychro’ is a combining form denoting a relationship to cold) naturally present in aquacultural environments have adapted to life at low temperatures, whereas human pathogens, which are mesophilic bacteria (‘mesophilic’ means preferring moderate temperatures), can be severely inhibited (MacMillan, 2001).

Another natural barrier to the transfer of resistance is related to the transitory nature of the microbial flora in fish. The presence of bacteria in the intestinal tract of fish appears to depend on their presence in the feed or in the water (MacMillan and Santucci, 1990). As the environment or the feed changes, so too does the microbial flora change. In contrast to homeotherms, fish don’t appear to have a permanent microflora. In partial support of this observation, Bocek *et al* (1992) found that silver carp living in water inoculated with an antibiotic-resistant strain of *Salmonella typhimurium* at the level of 10,000 bacterial cells per litre, retained the organism in their intestines for 14 days after they were transferred to clean water.

Earlier, Baker *et al* (1983) mixed *S. typhimurium* in waste from swine, and inoculated this mix into water stocked with tilapia (*T. aurea)*. Salmonellae were recovered from the viscera of these fish for up to 16 days after inoculation, but other tissues were free of the organism. Since the flesh did not contain this organism, it was concluded that proper processing of the harvested product should provide an uncontaminated food for human consumption.

It is known that fish starved for a period of time may have an essentially sterile gastrointestinal tract (MacMillan and Santucci, 1990). Hence, contaminating enteric bacteria from terrestrial animals don't necessarily establish residency in exposed fish, although they may occur temporarily in/on aquatic species. As well, depending on the ambient temperature, these organisms are unlikely to reproduce. Occasionally, various human bacterial pathogens may be recovered from fish or their environment, but it is doubtful that these bacteria have colonized or even reproduced under these conditions. Temperature and physiological conditions in poikilotherms likely preclude the ability of most human pathogens from colonizing farmed aquatic animals; in fact, fish may be resistant to such colonization. Another important point is that because the bacterial flora of fish is transitory, the use of antibiotics as growth promotants does not appear to work, and in countries such as the USA, it is also illegal (MacMillan, 2001).

Generally speaking, the probability of contact between fish or their environment, and humans, is low. Fish processed for human consumption in the USA have very low numbers of potential human pathogenic bacteria. However, it is known that catfish fillets in processing plants in the USA are often contaminated with the bacterial organism, *Edwardsiella tarda* that may spread to humans by the oral route (Noga, 1996). In an FDA study in 1998, *Salmonella* spp. were detected in very low numbers from some farmed catfish and imported farmed tilapia; the source of the organism was not determined.

Rawles *et al* (1997) found that the performance of juvenile channel catfish was not improved by the inclusion of Romet-30 or oxytetracycline in the diet. Rather, compared with controls, performance of these fish tended to decrease when antibiotics were supplemented in the diet. Moreover, residues of antibiotics above the legal limit of 0.1 mg/kg were noted in most samples from fish medicated with antibiotics. A withdrawal period of three or four weeks effectively decreased the content of antibiotics in tissues to undetectable levels.

In a comparative study in Puget Sound, Washington, Herwig *et al* (1997) found that most antibiotic-resistant bacteria below pens holding salmon occurred at a site at which the most antibiotic was used, compared with the least resistance in pens where the least amount of antibiotic was used. On the farm at which most antibiotic was used, resistance to oxytetracycline and Romet-30 tended to parallel each other, and suggested either a common mechanism of resistance, or linkage of the genes responsible for the resistance.

The environmental fate of antibiotics used in aquaculture is generally unknown, however, waste management practices may diminish potential effects on public health. As noted, measurements have demonstrated the accumulation of antibiotics below net pens holding salmon (Smith *et al*, 1994; Herwig *et al*, 1997). Presumably, if sediments were removed from the system, less antibiotic would be present. Although more work is needed to determine the amount of antibiotic present in effluent, any impact on human health remains undocumented (MacMillan, 2001).

The lack of credible data provides grounds for speculation about the impact on public health, of pharmaceutical agents used by all animal industries, including aquaculture. It seems important to determine the total volume of antibiotics used in terrestrial and aquatic agricultural animals, and the prevalence of antibiotic-resistant bacteria on aquacultural products for human consumption. The origin of any recovered pathogenic bacteria, whether from the water, the farm, the processing plant or the retailer, would need to be determined. It would also be useful to determine the likelihood of the transfer of resistance factors from aquatic environments, including fish, and aquatic bacteria through to human pathogens. It would also help to determine the fate of antibiotics in the aquatic environment (MacMillan, 2001).

11. ‘Neutraceuticals’ and Bacteriophages – Practical Alternatives to Antibiotics?

a) ‘Neutraceuticals’

Many opportunistic disease-causing bacteria, viruses, fungi and other organisms exist within or on fish, or in the aquatic environment. Although these organisms are a normal component of all life, the immune system of fish can recognize, engulf and destroy pathogenic organisms. Because of the increased effects of crowding and associated stressors encountered in the rearing of fish, these animals are more susceptible to disease than are free-ranging fish. During periods of stress, the immune system may be overwhelmed or less efficient, and overt disease may be the result.

Since infections are usually opportunistic events, it is sometimes possible to control the spread of infection by correcting the management problems that precede an outbreak of disease. In severe situations, control of these infections may require the use of medicated feed. Alternatively, the feed may be a medium for therapy. Instead of the use (and abuse) of antibiotics, it is claimed that ‘functional’ feeds for fish can be given in an attempt to minimize or prevent disease. Claims for these ‘functional’ feeds indicate that they are enriched with specific natural feed ingredients with properties to reinforce the ability of the immune system to control pathogens. These ingredients are called ‘neutraceuticals’ that are purported to have specific protective functions, thereby offering a benefit beyond simple nutrition or basic fortification. According to claims for these products, proven ‘nutraceuticals’ include:

- Bioflavonoids that act as natural antioxidants, i.e. scavengers of active oxygen radicals that may adversely affect the health of fish.
- Probiotics, the first line of defense against intestinal disease, are health-promoting bacterial organisms that improve the microbalance by selectively suppressing harmful bacteria in the intestine.
- Prebiotics refer to a group of natural sugars such as oligosaccharides that are resistant to digestion by fish but can be utilized exclusively by specific probiotic organisms, allowing them to compete with and exclude pathogens in the gut (called 'competitive exclusion'). Thus, prebiotics are nutrients for probiotic organisms.
- Immunostimulants promote the macrophage (= defence) system to eliminate pathogens in the bloodstream.

Additional claims for 'neutraceuticals' indicate that the use of functional feeds will aid in the control of pathogenic bacterial and fungal growth, as well as reducing the digestive problems that occur commonly after antibiotic treatment or prolonged stresses (de Wet, 2002).

From a practical point of view, there appears to be benefit in some of these alternate approaches to the control of disease, not only in fish, but also in other classes of animals, including humans (Fuller, 1989). Over time, such viable techniques could supplant many of the tons of antibiotics used today in the combined aquacultural and other livestock industries. Such replacement could reduce significantly, the number and species of antibiotic-resistant bacteria in the health of humans and livestock. Some examples of 'neutraceuticals' are listed herewith.

i) <u>Probiotics</u>

Alternative approaches to the use of antibiotics in the treatment and/or prevention of diseases affecting a variety of livestock, including fish, as well as those that affect humans, are gaining greater acceptance. One of these approaches involves the use of probiotics.

Several definitions of probiotics have been proposed. Fuller (1989) gave a precise definition, which continues to be widely used, i.e., '*a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance*'. However, Verschuere *et al* (2000) have expanded this definition to allow for a broader application of the term, as follows: '*A probiotic is defined as a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response toward disease, or by improving the quality of its ambient environment.*'
The means by which probiotics produce positive health benefits in aquaculture have been reviewed by Irianto and Austin (2002).

Numerous organisms, including a wide range of microalgae (*Tetraselmis* spp.), yeasts (*Debaryomyces, Phaffia* and *Saccharomyces* spp.), and Gram-positive (*Bacillus, Carnobacterium, Enterococcus, Lactobacillus, Micrococcus, Streptococcus* and *Weissella* spp.) and Gram-negative bacteria (*Aeromonas, Alteromonas, Photorhodobacterium, Pseudomonas* and *Vibrio* spp.), have been evaluated. The mode of action of the probiotic activities of these agents has not really been investigated, but possibilities include competitive exclusion, ie, the probiotic bacteria actively inhibit the colonization of potential pathogens in the digestive tract by antibiosis or by competition, and/or by the stimulation of immunity in the host. These products may stimulate appetite and improve nutrition by the production of vitamins, by the detoxification of compounds in the diet, and by the breakdown of indigestible components. There is accumulating evidence that probiotics are effective in inhibiting a wide range of bacterial and some viral pathogens in fish (Douillet, 2000; Tae-Kwang Oh [publication date not stated]).

A key point in the development of biological agents such as bacteria, for use in probiotic systems, is that they must be nonpathogenic for humans who consume the final product (Nikoskelainen *et al*, 2001).

A review of probiotic bacteria as biological control agents in aquaculture, including a table summarizing reports on this topic, has been provided by Verschuere *et al* (2000). Other selected references/reviews on the use of probiotics in aquaculture include those of Maeda *et al,* (1997), Xiang-Hong *et al* (1998), Gatesoupe (1999), Irianto and Austin (2002), Villamil *et al* (2002) and Abidi (2003), among many others.

ii) Prebiotics

The official definition of prebiotics in humans is: '*Nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one species or a limited number of species of bacteria in the colon*' (Duggan *et al*, 2002). Prebiotics refer to a group of natural sugars such as oligosaccharides [('oligo' means 'little, scanty or few'] (eg., lesser saccharides resulting from the partial hydrolysis of starch and known to contain a definite number of sugar molecules, such as maltose, a disaccharide), that are resistant to digestion by fish but can be utilized exclusively by specific probiotic organisms, allowing them to compete with and exclude pathogens in the gut (called 'competitive exclusion'). Thus, prebiotics are nutrients for probiotic organisms. Other examples of prebiotics include galacto-, fructo-, and isomalto-oligosaccharides used in the promotion of health. The use of prebiotics in aquaculture could add to the beneficial effects of probiotics in the prevention of infectious diseases, particularly bacterial diseases.

iii) Immunostimulants

Immunostimulants are chemical compounds that aid in bolstering the immune system through the activation of white blood cells, and thereby may render animals more resistant to infections by a variety of biological agents (Raa, 2000). Included among these compounds are vitamins, trace elements, fatty acids, glucans, yeasts, nucleotides and others such as lactoferrin, chitin, levamisole, probiotics, etc. (Lall, 2003). It has been noted that vaccination is likely the best-known method of specific immunostimulation, and that activation of macrophages is an example of nonspecific immunostimulation (Zhou Jin, 2004). Reviews of immunostimulants in aquaculture have been provided by Sakai (1999) and Raa (2000).

It seems that the most promising immunostimulants are the β-1,3/1,6-glucans, because they have a well-defined chemical structure and mode of action on the immune system. In addition, these compounds are non-toxic universal ‘alarm signals’ that activate the immune system by the same basic mechanism in all groups of animals from the simplest invertebrates to humans. The β-glucans occur naturally in the bran of grasses (*Gramineae*) such as barley, oats, rye and wheat, generally in amounts of about 7%, 5%, 2%, and less than 1%, respectively (Chaplin, 2003), and in the cell wall of yeasts (Anon, 2003i). The β-1,3/1,6-glucans bind specifically to a receptor molecule on the surface of certain inflammatory cells called macrophages (macro = large; phage = to eat; hence, macrophages are large cells that engulf foreign material). These inflammatory cells play an essential and pivotal role in the initiation and maintenance of the immune response.

From an evolutionary point of view, macrophages are the oldest and most consistently preserved immunologically competent cell known. In order to function immunologically, macrophages must pass through a stage of activation that involves certain morphological changes but also, most importantly, an entire sequence of metabolic changes. Activation can be initiated by a variety of different stimuli, such as endotoxins, bacteria, viruses, or chemicals that can be too toxic or pathogenic to be useful. Beta-glucan is not only effective orally, it is also completely nontoxic and safe, but is one of the most potent stimulators of the immune response (Anon, 2003i).

The receptor for β-1,3/1,6-glucans on macrophages has been retained during evolution and is found in all animal groups from invertebrates to humans. When the receptor is engaged by β-1,3/1,6-glucans, these inflammatory cells become more active in engulfing, killing and digesting bacteria, and at the same time, they secrete signal molecules that stimulate the formation of new white blood cells. In animals that have specific immune mechanisms (fish and animals higher on the evolutionary scale), in addition to non-specific defences, the activated inflammatory cells produce cytokines that, in turn, also activate antibody-producing white blood cells (B [bone marrow-derived] and T [thymus-derived] cells). For this reason, β-1,3/1,6-glucans enhance the efficacy of vaccines. Because of the basic mode of action of β-1,3/1,6-glucans, products in this category affect a number of different biological processes, including not only resistance to disease, but also growth, wound healing, repair of cells damaged by ultraviolet light, skin care, arthritis, etc.. The β-1,3/1,6-glucans are active not only when injected, but also when administered in the feed or on mucosal surfaces (Raa, 2000).

Duncan and Klesius (1996a) administered β-glucan and the yeast *Saccharomyces cerevisiae*, to channel catfish, and found that, although nonspecific immune responses were activated, the use of these producrts did not lead to enhanced nonspecific immunity to the bacterial pathogen, *Edwardsiella ictaluri*. By contast, Sahoo and Mukherjee (2002) compared four immunomodulating substances – β-1,3 glucan, levamisole and vitamins C and E -- in rohu (*Labeo rohita* Ham), a major species of carp in India. Although all four substances had significantly positive effects, β-glucan was found to be the most effective immunomodulator in these fish.

Among vitamins and minerals, vitamin C (ascorbic acid), and vitamins A and E, and the trace mineral selenium (Se) are important. Along with Se, vitamins C and E are key issues for fish; Se levels are often low in farmed fish (Lall, 2003). The biological importance of Se in the development and maintenance of the immune system of humans and other animals has been reviewed by Koller and Exon (1986).

Vitamin C is involved in specific and nonspecific immunity in fish and other animals. In its nonspecific role, vitamin C protects cells from the damaging effects of free radicals (oxygen), it is important in the production and secretion of interferon, it is required for the synthesis of collagen in the skin and skeleton (therefore important in wound healing), and it maintains the basement membrane of the epithelium of the oral cavity and intestines, etc.. Its role in specific immune responses includes the proliferation of B and T lymphocytes, and the antibody response. The requirement for vitamin C in fish is 25-50 mg/kg (Lall, 2003). Several other studies have examined the role of vitamin C in the nutrition of fish (Durve and Lovell, 1982; Li and Lovell, 1985; Liu *et al*, 1989; Hardie *et al,* 1991; Li *et al*, 1993; Li *et al*, 1998).

Blazer and Wolke (1984a) studied the effects of α-tocopherol (vitamin E) on the specific immunity and nonspecific resistance of rainbow trout fed a control and an α-tocopherol-deficient diet. Fish fed the deficient diet had significantly reduced immunological responses compared with those in the control group, although the deficient fish did not have any visible evidence of deficiency. Hardie *et al* (1990) studied parr of Atlantic salmon (*Salmo salar*) fed depleted, intermediate or high levels of vitamin E. Fish fed the depleted and high levels of vitamin E were then challenged with a virulent strain of *Aeromonas salmonicida*. Fish fed the depleted diet had significantly increased mortality compared with fish given the high-level diet. However, in contrast to the results reported by Blazer and Wolke (1984a) in rainbow trout, Hardie *et al* (1990) found that the function of white blood cells (specific humoral factors) in these salmon was not affected, whereas there were effects on certain nonspecific factors associated with immunity. Hardie *et al* (1990) also studied the effects of vitamin E on the immune response of Atlantic salmon (*Salmo salar*).

Blazer *et al* (1989) examined dietary influences on the resistance to disease in channel catfish, by comparing fish fed three different diets. Significant differences among the groups were observed but because the diets varied greatly, it was impossible to explain the observed differences. Deficiencies of vitamins C and/or E and trace minerals, and/or deficiencies of vitamins C or A were suggested as potential causes. A similar study conducted by Blazer and Wolke (1984b) in rainbow trout, resulted in findings generally similar to those observed in channel catfish.

The role of vitamin E in the immune response and resistance to disease is not so clear as that of vitamin C, however a combination of the two has appeared to be effective. The possible mechanisms of the action of Vitamin E on the immune response include 1) protecting the cellular membranes of white blood cells against the peroxidative damage induced by free radicals (such as oxygen) produced during the immune response, and 2) vitamin E aids in limiting the oxidation of arachidonic acid to prostaglandins PGE_2, $PGF_2\alpha$, TXB_2, and 6-keto $F_1\alpha$ in selected tissues. Since prostaglandins can depress the proliferation of lymphocytes (a species of white blood cell) and regulate the immune response, the inhibition of the synthesis of prostaglandins increases the number of lymphocytes, and as a result, increases cellular immunity (Lall, 2003).

iv) *Spirulina* spp.

Spirulina spp. are blue-green algae that are rich in antioxidants, vitamins, minerals and other nutrients. This product has been used as a food supplement for more than 20 years. *Spirulina* spp. grow naturally in lakes with extremely high pH levels, but it is also harvested from large-scale commercial ponds, where purity is monitored before being dried and distributed in tablet and powdered form.

Several studies with animals have shown spirulina to be an effective immunomodulator (an agent that can affect the behavior of immune cells.) In rats, spirulina inhibited allergic reactions by suppressing the release of histamine in a dose-dependent fashion. In cats, spirulina enhanced the ability of macrophages to engulf bacteria, and in chickens, spirulina increased antibody responses and the activity of natural killer cells, which destroy infected and cancerous cells in the body. Research at the University of California, Davis, found that nutrient-rich spirulina is a potent inducer of interferon-γ (gamma) (a 13.6-fold increase) and a moderate stimulator of both interleukin-4 and interleukin-1β (a 3.3-fold increase). Increases in these cytokines suggest that spirulina is a strong proponent for protecting against intracellular pathogens and parasites and potentially, can increase the expression of agents that stimulate inflammation, which also helps to protect the body against infectious and potentially harmful micro-organisms. Additional studies with individuals consuming spirulina are needed to determine whether these dramatic effects extend beyond the laboratory. One study involving channel catfish fed *Spirulina* sp., showed that there were enhanced nonspecific cellular immune responses, but no specific protection against infection with *Edwardsiella ictaluri* (Duncan and Klesius, 1996b).

In the body, the preferential increase in the production of interferon-γ over interleukin-4 shifts the immune system towards mounting a cell-mediated immune response instead of a humoral response (ie, the production of antibodies). A cell-mediated response includes the activation of T-cells and antibodies that combine with macrophages to engulf invading micro-organisms – hence, the value of spirulina in protecting against intracellular pathogens and parasites. The moderate increase in the secretion of interleukin-1β, a cytokine that acts on nearly every cell of the body to promote inflammation, supports the overall immune response (Gan, 2000).

b) Bacteriophages

Since the advent of antibiotics, both the human health care and agricultural sectors have relied heavily (and continue to rely) on these products to control bacterial pathogens. However, increasing levels of resistance to antibiotics by pathogenic bacteria have reduced the efficacy of many current therapies. As a result, researchers have sought alternative methods to deal with these pathogens. One of these alternatives is the use of bacteriophages, a very old idea that continues to be used in human health in countries such as Russia, (reported in the CBC program, 'The Nature of Things') to deal with bacterial pathogens of the intestine (Stone, 2002).

Lytic bacteriophages are viruses that attach to specific receptors on the surface of bacteria, inject their DNA, and express genes that lead to the synthesis of new phages. This process ends with the programmed lysis (death) of the host bacterium, and the release of many more phages.

The therapeutic use of phages as antimicrobial agents has a number of advantages compared with other methods. Firstly, phages are highly specific and allow for the removal of the specifically targeted microorganisms from a mixed population. Secondly, unlike antibiotics that decay over time, numbers of phages actually increase and work their way more deeply into pockets of infection. Furthermore, phages are living entities that adapt and evolve; they can pass from host to host, and have the potential to establish an infectious cure.

Interest in agricultural applications of phages is now expanding rapidly in three major areas:

- phage control of plant diseases such as bacterial spot on tomatoes and *Erwinia* sp. infections of fruit trees (fire blight) and root crops (soft rot).
- phages to treat diseases of animals, eg, respiratory infections caused by *E. coli* in chickens, furunculosis (*A. salmonicida*) in fish, and mastitis in cattle.
- phages to control human food-borne pathogens such as *Salmonella* spp. in chickens, *E. coli* (O157:H7) in cattle, and *Listeria* spp. during the processing of food (Brabban *et al,* 2003).

In reference to humans, it has been reported that some investigators are attempting to use phages to control MRSA (methicillin-resistant *Staphyloccus aureus*), a bacterial organism that is responsible for the vast majority of serious infections that originate in hospitals (von Radowitz, 2003).

As an example in aquaculture, Park *et al* (2000) found two types of phages that were specific to the bacterial organism *Pseudomonas plecoglossicida*, the cause of bacterial hemorrhagic ascites of ayu fish (*Plecoglossus altivelis*). On the basis of their experimental work, the authors suggested that these phages might be used to control this disease; they also provided a number of references on phage control of several diseases in animals. Also, Grabow (2001) provided an update on the application of bacteriophages as models for viruses in water, along with numerous references on the subject.

12. Comments and Conclusions

Facts are hard….understanding is harder….wisdom is hardest. **- Stephen Becker: A Covenant with Death, 1964**

The apparent scarcity of references attuned specifically to the topic of aquaponics and food safety has been a slight problem in this study, a finding with which Douillet (2003) agrees. Conversely, greater numbers of such references are more readily available for the larger topic of aquaculture in general. Hence, to a great degree, it has been necessary to focus on principles and facts applicable to aquaculture over all, and to try to extrapolate from them to aquaponics in particular.

It is significant that food-borne or zoonotic disease associated with aquacultural products, including aquaponics, seems to be rare; for this reason it is assumed that food-borne bacterial pathogens that are also resistant to antibiotics in these products are even more rare. From the perspective of food safety in aquaponic systems, there seems to be much less likelihood of contamination of vegetable and other aquaponic crops, and fish, with pathogenic bacteria of domestic animal origin, and with microscopic parasites such as *Cyclospora* sp. of human origin, and *Cryptosporidium* sp. of domestic animal origin, in aquaponic systems, especially in indoor systems, compared with the potential of such contamination in the traditional field methods of growing such crops.

It is positive and notable that a study by Robison and Byrne (2003), who collected water and various vegetables grown in the aquaponic facility rearing tilapia at the Lethbridge Community College, found that on unwashed produce, bacterial counts from the vegetables were within acceptable limits for ready-to-eat foods. Numbers of fecal coliforms increased between water entering the system and the water exiting the system, however numbers of fecal coliforms in both samples were very low.

By contrast, a report on a test-marketing study conducted by Choban and Frank (2004) showed that high levels of coliforms were found in unwashed bok choi, culantro roots and chives grown in an aquaponic system. These plants are low-growing and because their leaves are close to trays of recycled waste from fish, they are at greater risk of contamination. As a result, in order to market bok choi and culantro, investigators used a strict washing procedure. All other samples of vegetable produce in this study had no or presumptively negative levels of micro-organisms for which bacterial cultures were conducted. However, all aquaponically-grown produce was washed in 100 ppm chlorine and rinsed in potable water prior to sale. The results of this study indicated that all low-growing vegetables, and perhaps all vegetables produced in aquaponic systems may well require this procedure, in order to further the acceptance of such produce by the buying public.

Bearing on the last statement, it is interesting that in this study, although most feedback from customers at different markets in the province was positive, the feedback from those at a test market in Lethbridge tended to be negative. Customers at this site claimed that the tomatoes offered were not as flavorful as those grown in soil; they liked the taste of cucumbers offered; they felt that field-grown vegetables and herbs contained more soil micronutrients and were healthier and more flavorful; they were uncomfortable with the use of water holding fish for growing produce; some disagreed with the idea of using fish produced in a closed environment. As a result of such comments from customers, this market declined to have further aquaponically-grown produce delivered (Choban and Frank, 2004).

In outdoor systems, bacterial contamination could arise from the feces of rodents, birds and those from domestic animals and humans; in indoor systems, rodents are likely to be a potential source of bacteria pathogenic for humans. However, at the University of the Virgin Islands, where open-air aquaponics have been used in plant production for 20 years, it has been claimed that no one has become ill as a result of work in aquaponics (Rakocy, 2003a).

The inclusion in this document of the USDA 'Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables' under the heading of 'Water' (page 15) was to highlight the potential for the contamination of fruits and vegetables by microbial agents in the broader context of agricultural production, not to arouse unreasonable or irrational fears about aquaponic/aquacultural production. To the contrary, as noted, information available to the present time indicates the relative safety of aquaponic/aquacultural production compared with the safety of traditional methods of producing fruits and vegetable, etc..

The protection of plants from insect pests in aquaponic systems may be accomplished through the selection of insect-resistant cultivars, the use of *Bacillus thuringiensis*, and/or the simpler use of insecticidal soaps.

Fish in aquaponic systems may be subject to the same disease conditions found in those reared in traditional aquacultural systems. As noted in the text (page 36), some of the organisms causing these diseases in fish may affect humans as well. For this reason, this writer has some concerns about the use of an aquaponic system such as that involving the use of quail to provide feces as a source of nutrient for algalculture, as proposed by Nuttle (2003a). The potential for the quail used in his proposed system to introduce a pathogen such as a *Salmonella* sp., etc. is possible, and without defined procedures to regulate and control (quality control) this and other potential pathogens, the system appears to have some potential weaknesses. Perhaps because the idea is so new, future refinements in methodology may well deal effectively with these concerns.

Since warm temperatures are required for the rearing of *Tilapia* spp. (page 12) in this proposed aquaponic system, the possibility of introducing human bacterial pathogens, most of which also require warm incubation temperatures, is likely increased somewhat. Despite all too brief assurances from Nuttle (2003b) that food products derived from his proposed system are safe for human consumption, he did not provide current convincing evidence of quality control measures being used to assure safety of the final food products (algae and fish).

Hutchings (2003) has indicated the unsuitability for aquaponics, of some sources of water from deep wells in the province because of their high content of salt. Accordingly, it might be possible for some producers to explore the development salt-water aquaponics as described by Wilson (2003) and by Nuttle (2003a), or in a broader aquacultural, non-aquaponic endeavor, to rear fish such as tilapia in more brackish waters. In Israel, tilapia may be reared in brackish water (600-3000 ppm salt) (Lenoir, 2003).

It would seem practical that fish entering an aquaponic or any aquacultural facility should be obtained from a reliable (certified?) source in which routine (disease) surveillance procedures and diagnostic monitoring of brood stock are practiced. Such procedures are the most cost-effective method of avoiding the economic losses caused by pathogens. Ideally, operators of grow-out facilities should have samples of incoming stock examined, either at the source or within Canada, for evidence of infectious disease before they are admitted to the facility. Routine diagnostic monitoring of young fish is also valuable in detecting potential problems. When it is available, vaccination against specific diseases may be practical and cost-effective (Reddington, 2000).

Undoubtedly, in terms of attempting to prevent infectious diseases, the significant wave of the present and future for many species of animals, including humans (Salminen *et al*, 1996) and fish, may well be the use of so-called 'neutraceuticals' rather than the well-known use and abuse of antibiotics in the production of livestock. These ‘neutraceutical’ products include prebiotics, probiotics, immunostimulants and immunomodulators (β-glucans, selected vitamins and trace minerals, levamisole, etc.). As well, products containing bacteriophages that target specific bacterial pathogens – rather than the traditional and increasingly risky methods of simply ‘throwing’ antibiotics/chemotherapeutics at organisms that cause infectious diseases in livestock – may well be an additional, practical approach to food safety.

The use of ‘neutraceuticals’, either singly or in selected combination, plus vaccination where it is practical, would seem to be a realistic, rational approach to the prevention or amelioration of infectious disease in fish, other livestock, and in humans who consume them. Such a non-antibiotic approach to rearing food fish could be a major factor, both within Canada and internationally, in promoting and instilling consumer confidence in the quality of the edible product. Similarly, such approaches as an attempt to prevent infectious disease could be a positive factor in promoting aquaponically-grown fish and plants from the perspective of intra- and extra-Canadian trade.

For example, given the seriousness of infections caused by *Streptococcus iniae* in humans handling tilapia (page 38), it would be highly advantageous to develop an effective vaccine (more correctly, ‘bacterin’) against this organism in fish, ultimately to avoid human infections. Failing that, or in combination with it, as part of the routine management of aquacultural/aquaponic operations, the selective use of certain ‘neutraceuticals’ could be of immense benefit in terms of the safety and health of both humans and fish.

One issue that does not appear to have been addressed adequately in the literature to which this writer had access, relates to human safety in the use of live biological products such as probiotics and bacteriophages that are proposed for dealing with defined infectious diseases, especially bacterial diseases, of different species of fish and shellfish (Nikoskelainen *et al*, 2001). For example, some probiotics are derived from nonpathogenic strains of bacteria for which there are also pathogenic strains of the same organism. Reversion or mutation of a nonpathogenic organism to a pathogenic strain could have severe consequences not only for fish but also for humans consuming aquacultural/aquaponic products. Obviously, it is an important issue that needs to be examined, especially in the light of concerns about the threat of biological agents to incapacitate human populations. (Realistically, It may well be that this point is a 'given' in any studies on the use of probiotics in food-producing species of plants and animals.)

The excellent review of the safety to public health of aquaculturally-derived foods by Howgate (1998) concluded that the risks to public health from the consumption of aquacultural products are no greater, and in some instances, lower, than the risks from equivalent species caught from the wild. He makes the point that his review is an assessment of the relative risks; absolute risks from some hazards in aquacultural products are as high as they are in their wild counterparts. With the exception of veterinary residues, the nature of the hazards in aquacultural products is the same as those in fish from wild stocks. Epidemiological evidence shows that the major risks to public health from fishery products, both in nature and extent, arise from intrinsic hazards, ie, those present in the fish/shellfish at the time of harvest.

Other important points raised by Howgate (1998) include the following:

Producers of fish from wild stocks have little, if any, positive control over the intrinsic quality of the catch. The best that can be achieved is to be selective of the species caught, by choice of fishing grounds, and season of capture. One of the several advantages of aquaculture, and by extension, aquaponics as well, as a source of food fish is that the producer can exert control over the intrinsic quality, including safety, of the product.

Cultivation of fish in brackish or freshwater seems to present more hazards and greater risks than those reared in mariculture (fish reared in salt water). In either temperate or warmwater aquacultural facilities, there is a risk of contamination with enteric bacteria when waste water is used or when systems are fertilized with organic manure. There is evidence that these organisms can penetrate into the edible tissues of fish when there are high densities of bacteria in the water. As a result, more detailed studies into the bacteriological risks associated with fish reared in waste water and in systems fertilized by human and animal feces are needed. On a practical level, it would seem advisable to avoid the use of waste water from animals other than fish in aquacultural or aquaponic production.

Finally, inorganic chemical contaminants that arise from natural or human sources can have an impact on aquacultural systems involving fresh and brackish water. There are well-based theoretical considerations for the belief that the risk to human health from these contaminants would be very low in these systems. Hence, there is likely no need for guidelines on maximum limits for inorganic contaminants in supplies of water for aquaculture, in terms of safeguarding human health. One exception might be mercury which is likely the only metal of real concern, since it is absorbed from feed – hence there could be a basis for establishing maximum levels in feed.

13. Addendum

a) Hazard Analysis Critical Control Points

Hazard Analysis Critical Control Points (HACCP) is an internationally recognized system for controlling food safety (Graham, 2003). It was developed originally in the USA to guarantee the safety of food for astronauts in space, and has now been adopted worldwide as a scientific, straightforward, effective approach to enhance food safety. Under HACCP, processors implement controls throughout production, which in turn allow the operator to react quickly to prevent safety hazards before they occur. The seven basic principles of HACCP are:

- Determine the critical control points;
- Establish limits at each critical control point;
- Identify the hazards and list preventive measures to control them;
- Establish procedures to monitor the critical control points;
- Establish corrective action to be taken in case of a deviation;
- Establish effective record-keeping.

The five steps of hazard analysis are:

- Review the incoming material, including ingredients and packaging material;
- Evaluate each step of the processing operation;
- Observe the actual operating practices;
- Take accurate measurements;
- Analyze the measurements.

In each case, the analysis must consider all possible biological, chemical and physical hazards. Once the hazards have been identified and analyzed, the next stage of HACCP is to determine the critical control points (CCP) necessary to control the hazards.

In the manufacturing process, CCP are points or steps at which control can be applied, and a food safety hazard can be prevented, eliminated or reduced to an acceptable level. Determining CCP needed to control identified hazards is the second major principle of a HACCP system. In the food processing sequence, CCP are located at any point where biological, physical and chemical hazards can be eliminated or controlled. Thus, CCP can include cooking, chilling, sanitizing, formulation control, prevention of cross-contamination, employee hygiene and environmental hygiene. It is key that CCP are developed and documented carefully, since the success of controlling hazards depends on the care taken in determining the CCP, the critical limits that must be met at each point, the monitoring procedures used to control each CCP, and the corrective action taken when there is a deviation identified at a CCP. Verification of each CCP in a processing plant ensures that monitoring procedures are in place and that they are effective in controlling the potential hazard.

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