HEALTH AND DISEASE IN WILD FRESHWATER FISH

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FEBRUARY 28 - MARCH 1, 1996

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GENERAL FISH ANATOMY AND PHYSIOLOGY

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WCVM, 1996

Some facts to keep in mind:

1. Among the seven vertebrate classes, three comprise fishes: the osteichthyes (bony fish), the chondrichthyes (cartilaginous fish) and agnathans (jawless fish), thus, there are more different species of fish than there are all other vertebrates put together, therefore many differences in anatomy occur between the various fish species. The following information is thus very generalized and restricted mainly to the teleosts (modern fish) among the osteichthyes.

2. External anatomy: (Fig.1) Fish have adapted to living within various extremes in an aquatic environment; therefore there is great diversity in sizes and shapes. Characteristics of head, trunk, tail and fins are generally used as distinguishing features, as well as general coloration, pigmentation pattern, presence of scales and arrangement, position of lateral line and general body shape: short, long, flattened dorsoventrally or laterally.

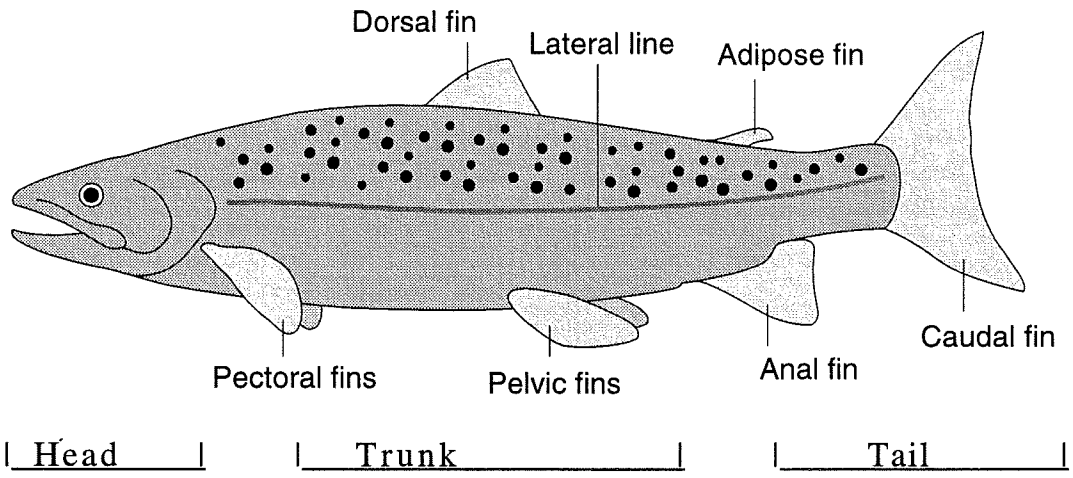

Figure 1. External anatomy of a typical salmonid fish

-**Head**: Most distinguishing features of the head are the eyes, mouth, nostrils and operculum (gill cover). The eyes are usually flattened and lack (or have modified) eyelids. Pupils usually do not contract, and the lens is inelastic. Mouth shapes vary considerably from long snouts to small openings. Barbels and teeth may or may not be present. Two or more nostrils may occur with or without covers. The appearance of the gill covers varies considerably with narrow and wide openings.

-**Trunk**: Main body part, extends to the anal opening. May be short or long depending on fish species. Contains visceral organs. Scales may or may not be present and may cover entire body or small parts. If present, four types of scales have been described based on shape: placoid, ctenoid, cycloid, ganoid (Fig.2). Scale sizes vary tremendously and coverage may be sparse or dense. The appearance of fins is usually descriptive of species and accesory spines or processess aid in their identification. Shape, size, consistency and number of fins varies considerably. The appearance of the lateral line as well as pigmentation pattern which varies with age, sex and species can also aid in identification.

-**Tail**: Shape, size and coloration varies not only between species but also with age, sex and breed. Tail shapes can be protocercal, the body continuing straight back as a middle support between the upper and lower lobes of the tail; heterocercal, with the end of the body turning up and continuing to the tip of the upper lobe; or homocercal, in which the last few vertebrae are fused and joined with other bony elements (hypurals) to support the tail-fin rays. A modification of the heterocercal tail so as to resemble the protocercal type is called diphycercal.

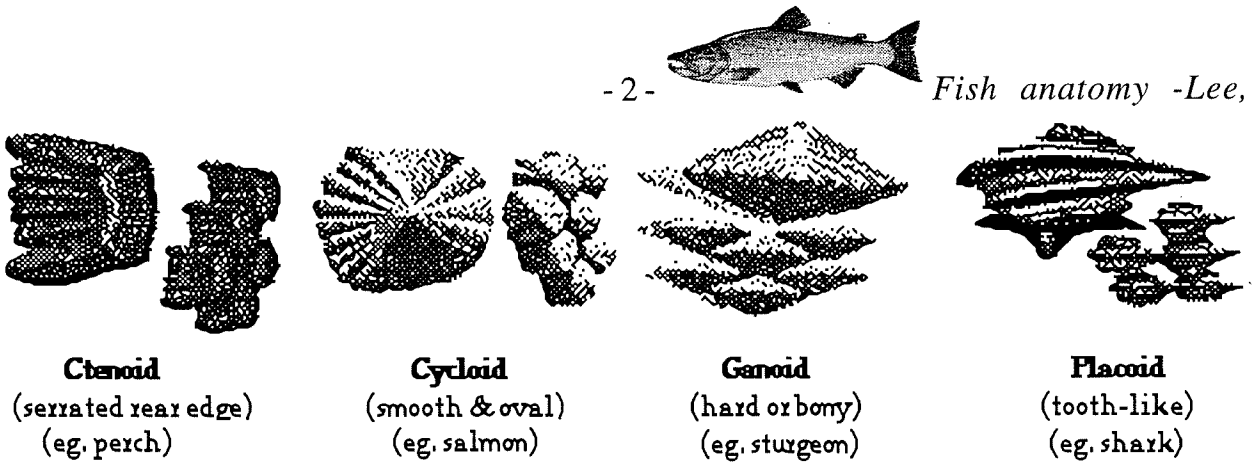

Figure 2. Fish Scales

-Integument: Fish skin is usually covered by a thin layer of mucus under which lie a soft epidermis and a tough dermis. Their thickness and composition vary with species, body site, sex and physiological state. Fish pigmentation depend on the accumulation of pigment cells in the dermis.

-Skeleton and muscle: The skull, vertebral column and fin skeleton are equivalent to mammalian skeleton with modifications (Fig.3). A distinct feature of fishes is the lack of bone marrow. Striated muscle are arranged segmentally: myomeres, divided by the lateral line into epaxial and hypaxial muscles. Hypertrophy as well as hyperplasia of muscle can occur in fish. Dark and light muscle occur in fish, although dark or red muscle is usually restricted to a thin band just beneath the skin.

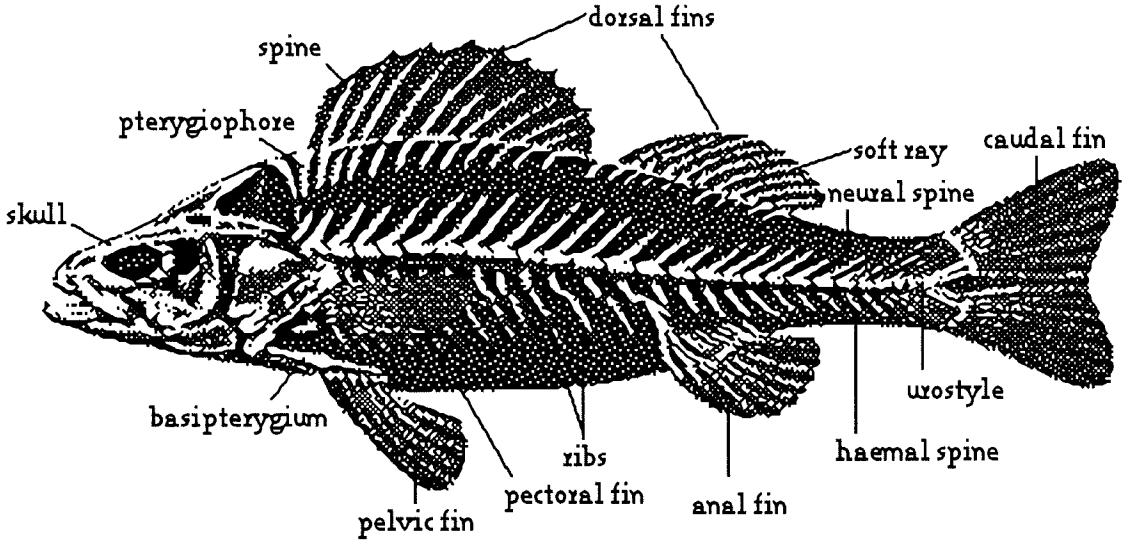

Figure 3. Skeleton of a representative bony fish: yellow perch.

-Gills: Fish obtain oxygen from the water. O_2 levels in water are relatively low compared to air, thus fish have developed an efficient gas exchange mechanism with gills. Gills are among the first organs that should be observed for any pathological conditions in fish. Healthy fresh fish gills show a reddish pink coloration and have a nice sheen without too much mucus. There are 4 pairs of gill arches, each gill arch gives rise to numerous double rows of gill filaments, and each gill filament gives off several gill lamella on both ventral and dorsal surfaces of the filament, forming a vast surface area for exchange of gases. The efficiency of gills is largely due to a counter current mechanism in which water and blood flow in opposite directions.

3. Internal anatomy: (Fig.4)

-**Digestive tract:** diversity of teeth, no salivary glands, rigid tongue, short esophagus, stomachs are absent in 15% of teleosts (ciprinids), intestine varies in length with diet habit.
-**Air bladder**: extension of esophagus. May or may not be present. Can become modified lungs or sound resonators.
-**Liver:** solid or diffuse as an hepatopancreas.
-**Pancreas:** diffuse tissue found within mesenteric fat, may be mixed.

- **Cardiovascular system:** Single circuit except for lungfish. Heart is two chambered but has two accesory chambers: sinus venosus which collects blood and leads into atrium and conus arteriosus, which is an enlargement of the ventral aorta coming out of the ventricle. All are contained within a single pericardial sac. Ventricle has two layers; inner spongiosum and outer compactum. Endocardial cells are phagocytic.

- **Lymphatic system:** paired sinuses and ducts under lateral line return lymph to posterior cardinal vein. No lymph nodes. Lymph makes up 4x circulating volume of blood.
-**Spleen:** non contractile.
-**Thymus:** under operculum.
-**Pronephros:** anterior kidney, equivalent to bone marrow.

- **Urinary system:** Kidney; paired, but may also be fused, found ventral to vertebral column. Divided into anterior hematopoietic tissue, and posterior with nephrons. No cortex or medulla. Each kidney has mesonephric duct or ureter. Bladder, may or may not be present.

- **Nervous system**: Forebrain: greatly reduced. Telencephalon comprises mainly olfactory bulb, and diencephalon comprises small hypothalamus, thalamus, pituitary and pineal gland. Midbrain or mesencephalon is well developed with two optic lobes. Hindbrain consist of metencephalon: cerebellum, and myelencephalon: medulla oblongata.

- **Endocrine system:** similar to mammals. Some are unique to fish: Corpuscles of Stannius, ultimobranchial and urophysis.

- **Reproductive system**: great variation (very fishy!!). Males, females, bisexuals, hermaphrodites, intersexuals. Oviparous, viviparous and ovoviviparous. Fertilization can be internal or external.

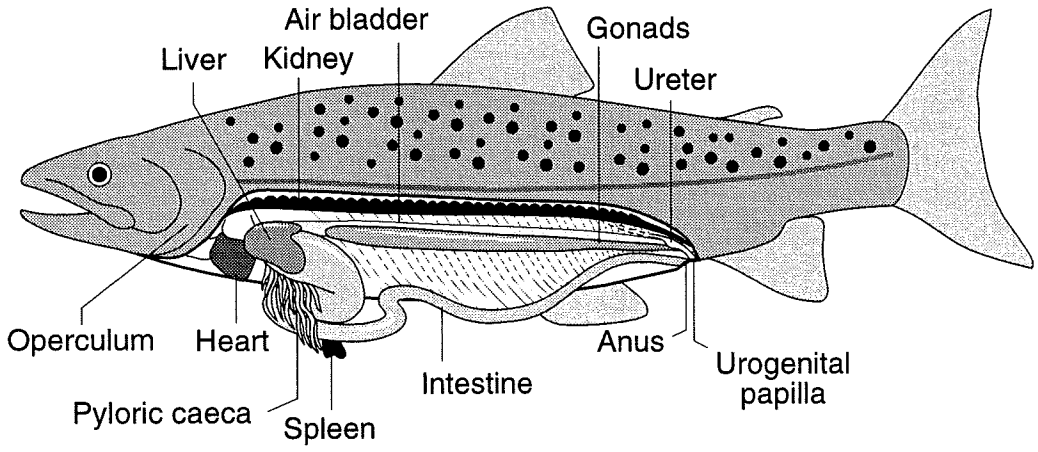

Figure 4. Internal anatomy of a typical salmonid fish.

4. Physiology: (adapted from http://www.floater.com/strength/anatomy.htm)

-CIRCULATION: Fish blood transport oxygen, nutrients, and wastes, much like in other vertebrates. Blood constituents are similar to mammals, but red blood cells are nucleated and platelets are represented by thrombocytes. The typical fish's circulation is a single circuit: heart-gills-body-heart. In contrast, mammals have two circuits: heart-lungs-heart and heart-body-heart.

-RESPIRATION: In order to live, fish must extract oxygen from the water and transfer it to their bloodstream. This is done by gills, lungs, specialized chambers, or skin, any of which must be richly supplied with blood vessels in order to act as a respiratory organ. Extracting O_2 from water is more difficult and requires a greater expenditure of energy than does extracting O_2 from air. Water is a thousand times more dense (heavier per unit volume) than air, and at ambient temperature (20°C) it has 50 times more viscosity (resistance to flow) than air and contains only 3% as much O_2 as an equal volume of air. Fishes, therefore, have necessarily evolved very efficient systems for extracting O_2 from water; some fishes are able to extract as much as 80% of the O_2 contained in the water passing over the gills, whereas humans can extract only about 25% of the O_2 from the air taken into the lungs.

Gills are made efficient in a number of ways:

(1) A large surface area for gaseous exchange means that more O_2 can enter the bloodstream over a given period of time. Gill arches, filaments and lamella constitute about 10 to 60 times more surface area than that of the whole body surface.

(2) A short diffusion, or travel, distance for the O_2 increases the rate of O_2 entry into the blood. The blood traveling in the folds of the filaments is very close to the O_2-containing water, being separated from it by a very thin basement membrane usually 1 to 3 μm thick, and possibly less.

(3) By using countercurrent circulation in the gill, the blood in the lamella travels forward, in the opposite direction to the water flow, so that a constant imbalance is maintained between the lower amount of O_2 in the blood and the higher amount in the water, ensuring passage of O_2 to the blood.

(4) Gills have little physiological dead space. The folds of the filament are close enough together so that most of the water passing between them is involved in the gas-exchange process.

(5) Water flows continuously in only one direction over the gills (Fig. 5), as contrasted with the interrupted, two-way flow of air in and out of lungs of mammals.

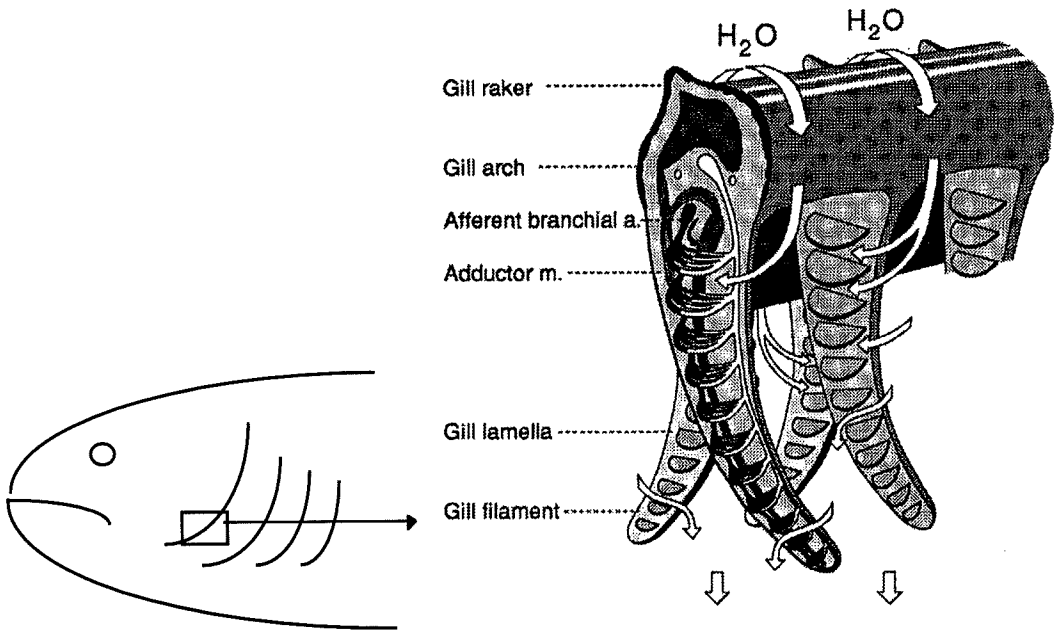

Figure 5. Gill structure.

-WATER BALANCE: The blood of freshwater fishes is typically more salty than the water in which they live. Osmotic pressure, the force that tends to equalize differences in salt concentrations, causes water to diffuse, or enter, into the fish's body, primarily through the gills, mouth membranes, and intestine. To eliminate this excess water, freshwater fishes produce a large amount of very dilute urine. Lampreys, for example, may daily produce an amount of urine equal to as much as 36% of their total body weight; bony fishes commonly produce amounts of urine equaling from 5 to 12% of their body weight per day. As these fishes are gaining water, they are losing salts. Salts contained in their foods are insufficient to maintain the proper salt balance. Freshwater fishes have therefore developed the capacity to absorb salts from water by means of their gills. Marine bony fishes, in contrast, have blood that is less salty than sea water, and consequently they lose water and absorb salts. To offset this loss of fluid, marine fishes drink seawater and produce very little urine. The drinking of seawater, however, adds to the concentration of salts. These salts are eliminated in several ways. Calcium, magnesium, and sulfates are passed out through the anus along with wastes. Sodium, potassium, chloride, and nitrogenous compounds, such as urea, are excreted through the gills.

-BODY TEMPERATURE: Fish are described as cold-blooded, meaning that their body temperature varies with the external temperature. Fish do, however, produce metabolic heat (that is, heat derived from the oxidation, or "burning," of food and from other processes), but much of this heat is lost to the outside at the gills. Blood passing through the gills loses heat to the water quite rapidly, so that a fish's body temperature is usually within a degree or so of the water temperature.

-SWIMMING: Many fishes swim by contracting and relaxing a succession of myomeres, alternately on each side of the body, starting at the head and progressing down toward the tail. The alternate shortening and relaxing of successive muscle blocks, which bends part of the body first toward one side and then toward the other, results in a series of waves traveling down the fish's body. The rear part of each wave thrusts against the water and propels the fish forward. This type of movement is quite clearly seen in the freshwater eel. Because movement of the head back and forth exerts drag, which consumes additional energy and slows travel, many fishes have modified this snakelike motion by keeping the waves very small along most of the length of the body, in some cases showing no obvious movement at all, and then increasing them sharply in the tail region. It is the end of the traveling waves that moves the tail forcefully back and forth, providing the main propulsion for forward motion. A simpler form of tail propulsion is seen in such inflexible-bodied fishes as the trunkfish, which simply alternates contractions of all the muscle blocks on one side of the body with those on the other side, causing the tail to move from side to side like a sculling paddle.Some of the predatory bony fishes are the fastest swimmers; they can cruise at speeds that are between three and six times their body length per second and may be able to reach 9 to 13 body lengths per second in very short bursts. Some fishes, such as the blenny, which has been timed at 0.8 km/hr (0.5 mph), swim very slowly; others, such as the salmon, which may reach a sustained speed of 13 km/hr (8 mph), move much faster; and it has been estimated that tuna may reach speeds of 80 km/hr (50 mph), and swordfish, 97 km/hr (60 mph).

-BUOYANCY: Many fishes have evolved means of reducing their body weight, or density, relative to the density of water, because a weightless, or buoyant, body requires a minimum of energy to keep it at a given depth, and because less energy is required by a lighter body to move at a given speed. Because fat is less dense than water, one method of reducing body density would be to increase the proportion of fat within the body. Fish liver and muscle contain high levels of lipids. Another method of reducing density is to include gases within the body. Many fishes have an air bladder that serves this function. The gases within the bladder are similar to those in air but are present in different and widely varying proportions. The degree of body volume that must be taken up by gas in order to achieve weightlessness depends mainly upon whether the fish is freshwater or marine. Freshwater is less dense than seawater and consequently provides less buoyancy. Freshwater fishes, therefore, require a larger gas bladder (7-11% of body volume) than do marine fishes (4-6% of body volume) to keep them from sinking. The quantity of gas within a fish's bladder must also be adjustable to keep it at a given depth. If, as in the carp, the gas bladder is connected by a duct to the gullet, gas may be expelled through the mouth and gill cavities as the fish rises, and, in a similar manner, gas may be added to the bladder by swallowing air at the water

surface. For most fishes, however, the gas bladder has no connection to the outside. In these fishes, adjusting the quantity of gas within the bladder is done by transferring gases from the gas bladder to adjoining blood vessels and back again.

-**BARORECEPTION**: The lateral line system, found in many fishes and in some aquatic amphibians, is sensitive to differences in water pressure. These differences may be due to changes in depth or to the currentlike waves caused by approaching objects. The basic sensory unit of the lateral line system is the neuromast, which is a bundle of sensory and supporting cells whose projecting hairs are encased in a gelatinous cap. The neuromasts continuously send out trains of nerve impulses. When pressure waves cause the gelatinous caps of the neuromasts to move, bending the enclosed hairs, the frequency of the nerve impulses is either increased or decreased, depending on the direction of bending. Neuromasts may occur singly, in small groups called pit organs, or in rows within grooves or canals, when they are referred to as the lateral line system. The lateral line system runs along the sides of the body onto the head, where it divides into three branches, two to the snout and one to the lower jaw. A swimming fish sets up a pressure wave in the water that is detectable by the lateral line systems of other fishes. It also sets up a bow wave in front of itself, the pressure of which is higher than that of the wave flow along its sides. These near-field differences are registered by its own lateral line system. As the fish approaches an object, such as a rock or the glass wall of an aquarium, the pressure waves around its body are distorted, and these changes are quickly detected by the lateral line system, enabling the fish to swerve or to take other suitable action. Because sound waves are waves of pressure, the lateral line system is also able to detect very low-frequency sounds of 100 Hz or less.

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Health & Disease in Freshwater Fish

An Overview

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February 1996.

WATER QUALITY DISEASES

1. Temperature

This is probably the single most important factor in both the farming situation and in fancy fish keeping. Fish are ectotherms (poikilotherms) and with few exceptions (muscles of fast swimming species such as tuna) mirror the temperature of the surrounding water. Nevertheless, each species has its own *preferred* range. Sustained periods at the extremes of this range, or probably more importantly, rapid changes (even very small ones sometimes) within this range, represent stressful conditions. While metabolic rates alter with varying water temperature (roughly doubling for each 10°C) this does not happen immediately. Advantage is taken of this enhanced metabolic rate in aquaculture where waste heat is available from eg. power stations. Thus the fish will grow faster in warmer water.

In general terms, fish will tolerate a temperature drop better than a rise. Some species are more susceptible to temperature stress than others and this is thought to be due to a poorer ability to osmoregulate at the new temperature (due to failure of the $Na^+\backslash Cl^-$ ATPase enzyme systems to adapt). Higher temperatures cause an increased metabolic rate and hence an increased O_2 demand necessitating increased "irrigation" rates. The increased water flow past the gills causes an increased water influx and thus possible compromise of the osmoregulatory mechanisms.

Fish also appear much more susceptible to bacterial diseases in conditions of rising water temperatures. Even a small change from as low as 5°C can be sufficient to promote overt furunculosis in Atlantic salmon fry. The reason for this susceptibility is unknown in precise terms but it is thought to be the result of an enhanced rate of replication or enzymic production by the pathogen which is not matched by that of the fish's immune mechanisms.

Increasing water temperature in general reduces the survival time of fish in pollutants. With heavy metals, their toxicity is enhanced, but the fish also has an increased respiratory rate and therefore increased exposure - a synergistic effect.

Temperatures in S.E. Asia tend to be higher than in temperate regions, with temperatures of 25-35°C being the norm. Temperature fluctuations are also less common, although these are seen following the monsoons, and some disease problems are associated with these drops in temperature. Temperatures below 17°C are seen, and skeletal abnormalities in fry are associated with these. Rapid temperature fluctuations are encountered in the marine environment, but less commonly in cages.

2. Oxygen

The efficiency of extraction by the gills is high, and it has to be when one considers that at 15°C one litre of freshwater contains only 7 cc O_2, and seawater even less, as compared to almost 21 volumes per cent in air. In warm or polluted waters, where O_2 levels are low, the cost of extraction by the gills may be too high i.e. where the energy required for ventilation exceeds that released by the O_2 so obtained. Under these circumstances, the fish enter *Respiratory Distress Syndrome*. Different species of course have different requirements for O_2: salmonids for example have a high requirement, catfish a lower one. Some species have an accessory breathing apparatus, such as a modified swimbladder, or adaptations to the roof of the mouth, and in some cases rely heavily upon them, although they are nevertheless able to survive in even totally anoxic conditions eg. snakeheads. Small fish have higher requirements than older larger ones: this translates in an aquaculture situation into placing small fish in the best quality water available.

Fish will normally demonstrate an O_2 lack by gathering at inlets or by gasping at the surface and by decreased activity. Although O_2 levels may be higher in surface waters, the gills collapse on contact with the air, so reducing surface area. Increasing water temperatures cause an increased demand for O_2 for the increased metabolic rate so produced, but a decrease in the holding capacity of water for O_2. Appetite also increases, but this extra intake of food may place a burden on the ability of the fish to extract enough O_2 from the reduced levels available to metabolize the food properly. At 28°C (a high temperature in temperate climates, but one that is encountered in some trout farms in Europe for short periods of time) O_2 consumption in salmonids just about matches that which is obtainable and there is thus no O_2 left over to properly metabolize food.

In general terms therefore, with high water temperatures, a reduced food intake is advisable, and a complete cessation of feeding is indicated if furunculosis or other bacterial diseases appear. In some situations it may be necessary to match feeding frequencies with O_2 levels: these are normally highest at mid-day due to photosynthesis, but surprising variations do occur especially in Asian warm-water ponds. Depletion of oxygen is also seen following heavy rainfall or following algal blooms. In most situations, careful monitoring is the best approach. Fish can acclimate to lower O_2 levels to a certain extent by increased hematocrit. Diel variations, however, depress appetite and growth. It has been shown experimentally that low oxygen levels can cause damage to the epidermis, some cells of which derive their supply directly from the water; this is especially important in areas of the body where the epidermis is thickest, such as the non-scaled areas (presumably the non-scaled fish have more general problems).

The LC50 of some toxicants is higher at high O_2 levels, largely due to the reduced irrigation rate over the gills. Similarly, uptake of many toxicants correlates positively with O_2 uptake: thus methyl mercury or endrin uptake increase with increased swimming speed, due to the increased oxygen uptake. The rate of uptake depends on the physiochemical properties of the toxicant: lipid solubility, small molecular size and un-ionized molecules favour uptake. High fecal levels or other organic detritus utilize available O_2 (Biological Oxygen Demand - BOD). Such anaerobic pond situations are common in Asia. A minimum of 5 mg/L is considered a satisfactory level for most stages under most conditions, although higher is certainly preferable for salmonids. Disastrously low oxygen levels are uncommon in the marine environment.

3. Supersaturation

Rapid increases in water temperature or reduced pressure may lead to a situation of supersaturation. Both of these may be seen when water from a tap is used to fill a domestic aquarium. Supersaturation may also be seen in the wild associated with very rapid photosynthesis by plants and algae, or more commonly in an intensive culture operation, associated with leaky valves or pumps, in those farms where pumping is a feature (air is forced under pressure into the water supply - so called Venturi principle). Water which is supersaturated with gas, either O_2 or N_2, may cause the condition "gas-bubble disease". This is the fish equivalent of divers "bends". Fish normally equilibrate quickly with supersaturated water and it should therefore cause little problem.

The reason for the gas in the blood coming out of solution is not therefore completely understood but is thought to be associated with the great drop in pressure experienced by the blood when crossing the gills. Whatever the reason, bubbles of gas cause emboli in the vessels of the gills, pseudobranch, choroid gland and elsewhere. These are often difficult to see, and a "candling" procedure may be found advantageous in diagnosing the condition. An apparent contraindication, vigorous aeration or agitation of the incoming water, or replacement of the leaky valves, are considerations in curing the condition.

"Gas-bubble disease" is also seen following periods of heavy rainfall where the rain moves down through the soil into the ground water or merely falls directly into ponds, warming up in the process, and becoming supersaturated. GBD appears less commonly in the marine environment.

4. Suspended solids

There are various types; organic such as feed fines, fecal waste, pulp mill effluent and inorganic such as clays, which may be inert (kaolin) or non-inert (bentonite). Non-inert inorganics may absorb substances such as heavy metals, pesticides or ammonia, and present these to the gill surface.
In general, there appears to be evidence suggesting that organic particles are more of a problem than inorganics, but whether this is a direct effect, or an indirect one is less clear. Our work with young rainbow showed that fish kept for 3 months at roughly 6,000 ppm kaolin (almost the consistency of whey!) had no significant lesions on the gills. They were smaller than controls because they ate less - they couldn't see the food! They may also have been stressed more because they did succumb to a protozoan infection (*Ichthyobodo*) but they recovered despite continued exposure. In the same set of experiments, 10-12,000 ppm kaolin did kill fish, by clogging the gills. Some species appear to "tolerate" or prefer muddy bottoms than others, and the type of bottom may dictate species found. Definite effects of suspended solids include (1) settling on eggs and young larvae and possibly suffocating them, (2) reducing light penetration and therefore abundance of food, (3) modifying behaviour patterns and natural movements. The evidence for a direct damaging effect on fish is less convincing.

5. Ammonia

Fish excrete this via the gills, and where there is plenty of water to remove it there is no problem. Toxicity arises however, in situations of overcrowding, or where for example, chicken slurry is added to the water. Young fish are relatively quite susceptible. Free ammonia or NH_3 is highly toxic, whereas bound ammonia NH_4^+ is much less so. Free ammonia combines with water as shown in the above equation and dissociates or recombines as shown. In acid water, most ammonia is in the bound or non-toxic form whereas in alkaline water, free ammonia may be more of a problem. Similarly, as water temperature increases, the amount of free NH_3 also increases, whereas elevated calcium levels (seawater, hard water areas) increase the tolerance of fish to ammonia, possibly by decreasing the permeability of the gills to the toxin. The gills also give off CO_2 and due to its combination with water to form carbonic acid, in the local environment of the gills, the lower pH so produced may afford a degree of protection. High ammonia levels have been reported to cause epithelial hyperplasia of the gills, thus effectively increasing the diffusion distance to O_2. At the same time, it is thought that NH_3 affects the ability of haemoglobin to bind O_2, but the precise toxic action of NH_3 is unknown. Chronic levels have been shown to cause meningeal proliferation in young minnows.

Recent evidence suggests that ammonia *per se* may not be directly toxic to the gills (which do after all excrete it), but it still represents a good measure of high stocking densities in which other possibly more damaging excretory products (such as trimethylamine and fecal proteases), may be found in high concentrations. High ammonia levels certainly cause nervous dysfunction and coma, probably due to interference with neurotransmitters in the central nervous system, as is the case in mammals.

6. Nitrite

This is an intermediate in the oxidation of ammonium to nitrate, a process which is carried out naturally and by the bacteria in biological filters. *Nitrosomonas* spp. convert ammonium to nitrite. *Nitrobacter* spp. convert nitrite to nitrate. As we have already seen, alkaline conditions lead to a higher amount of un-ionized ammonia, and this in turn has a greater inhibiting action on *Nitrobacter* than on *Nitrosomonas* spp. Thus alkaline water (seawater) see an increase in nitrite levels. Other parameters which affect the bacteria differently also have an impact. Nitrite levels may be high under some types of aquacultural operations, and in some effluent discharges, notably sewage, metals, dyes and celluloid manufacturing.

Nitrite is actively taken up by the gills (chloride cells probably) and blood levels may be 10 times those of the surrounding water. Nitrite oxidizes the iron in hemoglobin → methemoglobin and this pigment lacks the ability to bind reversibly to oxygen. Grossly, high levels of methemoglobin result in a brown colour to the blood. So-called "chocolate blood disease" of channel catfish farms.

By contrast with mammals where methemoglobin levels should not exceed 1%, fish may normally have levels of 10%. Levels of 50% or more are considered too high. Erythrocytes of fish have a reductase which reconverts methemoglobin to hemoglobin but this takes 24-48 hours if fish moved to normal water. Nitrite also causes a leukopenia, and although in the short term this is probably inconsequential, in the long term with chronic low level exposure, it may increase susceptibility to disease. Under most situations, nitrite levels in freshwater should not exceed 100 ppb in soft water, 200 ppb in hard (EPA).

It is crucial to realize that chloride competes with nitrite for transport across the gills. Thus in saltwater, nitrite levels 50 to 100 times greater than in freshwater can be tolerated. Similarly bromide and to a lesser extent bicarbonate have a similar protective action. Oxygen levels affect nitrite toxicity because of the reduced carrying capacity of the blood. Similarly, temperature has an affect because of the increased demand for oxygen with a decreasing availability.

7. Nitrate

As we have already seen, nitrate is formed by the complete oxidation of ammonia. It is naturally present in sometimes high concentrations in surface waters and in fish farms. It is considered essentially non-toxic although it does enhance the net productivity of aquatic systems, and under some situations, may promote massive algal blooms. High nitrate levels in aquaria impart a yellow colour to the water.

8. Carbon dioxide

CO_2 is of course excreted by the gills, and with water, it forms carbonic acid, which reduces the pH in the microenvironment of the gills. Increasing levels of CO_2 in the blood (or a decreasing pH) decreases the affinity of hemoglobin for O_2 (Bohr effect). Fish hemoglobin is very sensitive to CO_2 (large Bohr effect) by comparison with mammals, which are relatively tolerant of CO_2. In high enough levels, CO_2 will → anesthesia (> 100 ppm). High environmental levels correlate with nephrocalcinosis in intensively cultured salmonids. This is a condition which is common when liquid O_2 is used to boost holding capacities of water. The lesions comprise mineral within renal interstitium and tubules, often leading to severe granulomatous inflammation and cystic dilation of tubules. Muscles dorsal to the kidney are also involved in severe cases. Lamina propria of stomach is usually involved, often before renal lesions. The condition reduces feed conversion efficiency, but causes apparently little else. The major concern is with the esthetics.

CO_2 is utilized by plants during photosynthesis in daylight and in some ponds and surface water systems, this may cause a rise in pH. Similarly, during respiration at night, CO_2 is produced, causing a drop in pH. This diurnal pH change may be as high as 1 pH unit, especially in soft waters.

9. Chlorine

This is commonly added to domestic water supplies to destroy pathogens and improve taste and "slugs" of the chemical frequently found in systems after plumbing disturbances. It is also discharged from industrial processes, especially textile and paper industries and sewage treatment. It is used in experimental conditions to disinfect effluents discharged to surface water. Also used in cooling towers and swimming pools to control bacterial numbers and algal "slime".

At pH 6, 96% of dissolved chlorine is present as the acid HOCl (hypochlorous acid). At pH 9, 97% of this acid is dissociated. HOCl is more toxic than the hypochlorite ion OCl^- but this can dissociate to O and Cl^-, the atomic oxygen so produced being a strong oxidizing agent which rapidly produces gill necrosis. Species such as salmonids are much more sensitive to chlorine than coarse fish. Unlike some other poisons, recovery does not occur, possibly due to necrosis. In the presence of nitrogen compounds such as ammonia, hypochlorous acid forms chloramines which are highly toxic, probably mainly due to the formation of methemoglobin. 0.003 ppm is regarded (EPA, 1973) as the upper limit for continuous exposure.

Removal of chlorine and chloramines is accomplished by (1) activated carbon although in the early stages, with fresh charcoal, some ammonia will be produced, (2) sodium thiosulphite and hydrogen sulphide will also remove chlorine although once again, ammonia is produced: this is often removed by the addition of natural clays (zeolites), (3) merely aerating the water vigorously will often remove most chlorine and a large quantity of chloramines. These methods (1) - (3) are most commonly employed in recirculating systems, or in those experimental systems where domestic water is used.

10. Alkalinity, hardness, salinity and pH

Alkalinity, or the buffering ability of water, is due to carbonates, bicarbonates and hydroxides, and freshwater from limestone areas typically is well buffered, as is seawater. Waters with a good buffering capacity generally have a stable pH, and are able to resist agricultural or domestic pollution.

Hardness is a measure of the quantities mainly of calcium and magnesium ions, and is expressed as the equivalent of calcium carbonate. Different countries and areas within countries have different ideas as to what constitutes "soft" water, but in general:

0-50 ppm - soft
50-150 ppm - medium hard
150-300 ppm - hard

Soft water is usually acidic, and hard alkaline. Some species such as the S. American freshwater tropicals seem to prefer soft water whereas salmonids generally do better in harder water (associated with the coupled pump mechanisms at the gill surface). Another benefit of hard water is that heavy metals tend to precipitate out of solution. Some diseases seem to occur more frequently in soft water eg. bacterial kidney disease.

Salinity measures the total salts dissolved in water and is expressed as parts per thousand (0/00). Full-strength seawater is approximately 30-40 0/00. Some species naturally can adapt from fresh to seawater eg. salmon. Other species such as rainbow trout can also be made to adapt, but in some situations, such fish may struggle to maintain their osmotic balance. An example of this is seen at low water temperatures in full-strength seawater in which rainbow trout become dehydrated so that their muscle is dry, but their stomach full of seawater. Towing the cages to an estuarine area, or running freshwater onto the surface of the water is the only remedy, (increasing the freshwater content of the pellets helps a bit).

A pH range of 5-9 is generally considered to be the "safe" range for fish, although maximum productivity is seen from 6.5-8.5. Fish populations are found naturally however from 4-10. The addition of acid waste to hard water results in the liberation of CO_2 from bicarbonate, and this may be sufficient to be acutely toxic. Heavy rain may flush out peat bogs (humic acids) or strip-mining areas leading to such an acid flush. "Acid-rain" is of course a major problem in various parts of the world and is the result of sulphuric and nitric acid aerosols produced by fossil fuel combustion, metal smelting and other industrial processes. In areas where the buffering capacity of water is low, the pH of lakes and streams has decreased and all levels of aquatic life affected, decomposers, primary producers, primary and secondary consumers. Some sensitive species have disappeared altogether. The effects have been acute mortality, reduced growth, skeletal deformities but especially reproductive failure. Spring run-off from melted snow may cause a flush of acid and metals eg. in S. Ontario, Canada, from 5.5 to 4.5 and aluminum levels from roughly 0.2 mg/L to 1 mg/L. Aluminum toxicity (in the hydroxyl form) is highest at pH 5.0 and causes increased branchial mucus production and hyperplasia. The first run-off may contain most of the acid or metal, thought to be due to a process of ion separation or freeze concentration.

Acute toxicity of acid results from gill damage, with lamellar epithelial edema and necrosis, plus possibly plasma electrolyte imbalance due to a failure of ionoregulation.

11. Light intensity

This is very important but is poorly understood. High light levels have been shown to lead to lymphocytopenia and thrombocytopenia in Atlantic salmon, but not in brown trout or rainbow trout. Eggs and fry of salmonids are usually kept shaded, leading to increased hatchability and improved smoltification. Shaded fish seem to have lower incidence of disease, both bacterial and protozoal. Deep sea species displayed in high intensity aquaria may lead to retinal degeneration, and sunburn lesions are seen in several species, even in tilapia kept in cages at high altitudes, where ultraviolet levels can be dangerously high.

12. Heavy Metals

Can occur in high concentrations in natural waters near deposits, but usually present only in trace amounts. The salts are soluble but precipitate out in hard water and in addition may be absorbed to some of the non-inert clay particles.

Cadmium and copper are extremely toxic to fish, especially in soft water of low oxygen content. Damage to the gills is prominent with lamellar fusion, and edema as early as 24 hours after exposure. Zinc has a similar action but in addition, scoliosis is seen, possibly due to a myelopathy, which causes darkening as well. Mercury causes branchial changes but also renal tubular changes due to increased permeability of cell membrane and impaired mitochondrial ATP production → cell death. Cadmium has a similar action.

13. Pesticides and Herbicides

An in-depth discussion of this topic is not presented here. Many herbicides eg. 2,4-D often find their way into waters containing fish, and should always be considered in cases of large fish kills. Water chemistry should always be borne in mind whenever assessing significance of analytical results, as factors such as hardness, temperature and pH have a profound affect on toxicity of chemicals. Age and size of fish also have a marked influence as increased surface area/body size increases uptake, as does increased respiration (smaller fish have a higher respiratory rate). Similarly very young fish may have poorly developed organ/enzyme systems to handle toxicants - presumably this could be beneficial if a degradation product is the specific toxicant.

Species of organism is also important. In general, fish are more resistant than invertebrates, although there are exceptions (the herbicide trifluralin).

14. Algal blooms (include dinoflagellates, diatoms, and blue-green algae).

These may proliferate in large numbers in warm weather ("spring rise") so that shading occurs and there is a decrease in photosynthesis in deeper water and hence lower O_2 levels. Anaerobic conditions may eventually develop especially at night, and fish die, algae die, and various anaerobic products accumulate such as H_2S or methane which are themselves toxic to fish.

Diatoms are non-swimming and have silica shells. Thus in calm waters they may sink, leaving the swimming dinoflagellates to dominate (some species have lipid droplets which can help floatation). The chain-forming diatom *Chaetoceros convolutus* is causing problems in British Columbia, Canada. The backward-pointing spines act rather like a burr, burying into the gills and causing severe acute branchitis, and eventually a foreign-body type reaction to the silicate spines.

Certain algal species however, also produce ***toxins*** which are lethal to fish and other vertebrates. Implicated species include *Anabaena*, *Microcystis* (a blue-green alga), *Gymnodinium* and *Prymnesium*. Many deaths of fish under field conditions due to toxin release, occur during sunlight (as opposed to darkness for O_2 utilization) suggesting that toxin production accompanies photosynthesis.

Gymnodinium brevis is one of the "red-tide" dinoflagellates, as is *Ptychodiscus brevis*, which caused major human health problems on Florida and Carolina coasts in 1988.
Prymnesium parvum (another dinoflagellate) has been responsible for pond mortalities of tilapia in brackish waters. The toxin (prymnesin) is thought to alter gill membrane permeability and hence cause loss of ionoregulation.

Ciguatera toxin accumulates in high trophic species such as groupers and barracuda probably due to their eating coral species which graze on coral algae. While not toxic to fish, this toxin can kill man, although vomiting, cramps and peripheral tingling are more common results (atropine helps control symptoms).

Paralytic shellfish poisoning of man (and other animals) is also a dinoflagellate-produced neurotoxin (by *Protogonyaulax*) which causes muscular paralysis and respiratory failure. Diarrheic shellfish poisoning is another syndrome.

15. Electrocution
This is occasionally encountered with badly insulated pumps, or aerators, or due to improper use of electro-shocking equipment. Lightning may cause massive fish kills: the amount of current needed to kill fish depends on several factors, but water hardness is one of the most important. Soft waters are poorer conductors than hard, and thus the fish's body with its higher conductance will be a selective path. Thus, lethal voltages need to be higher in hard than soft waters. Outside ponds can be affected, but indoor facilities are not immune, as lightning can enter via pipes or drains. Fish so affected may be killed outright, but survivors may show hyperesthesia or spinal damage due to overcontraction of muscles. A common location is the fulcrum just beneath the dorsal fin in salmonids.

BACTERIAL DISEASES

These are extremely important, and along with protozoa and water quality problems, rank as major causes of loss to aquacultural enterprises.

1. <u>Furunculosis</u> *Aeromonas salmonicida*
This is one of the most economically important diseases of fish, affecting cultured and wild populations worldwide, cold and warmwater species. It is essentially a disease of freshwater fish, although a few outbreaks are reported in saltwater, especially in fish such as salmonids which are transferred from fresh to salt.

Rising water temperature or other stress such as transport will break down carriers → overt disease (corticosteroid injections will do the same).

McCarthy splits the organism into 3:

A. salmonicida subsp. *salmonicida*
A. salmonicida subsp. *achromogenes*
A. salmonicida subsp. *nova*

A. salmonicida subsp. *salmonicida* consists of typical pigmented strains isolated from salmonid fish, but also non-salmonids. The subsp. *achromogenes* consists of atypical strains (including non-pigmented) isolated from salmonids. This includes Schubert's subsp. *masoucida*. The subsp. *nova* was proposed for a distinct grouping of atypical strains responsible for diseases in non-salmonids such as carp erythrodermatitis. There is good evidence to suggest that atypical *A. salmonicda* infections have a different distribution within salmonid and other populations, than typical strains.

The bacterium is a Gram-negative short bacillus. It is non-motile and a glucose fermenter. Pigment may be produced, although this is variable or late; contaminants may suppress growth of organism itself, or pigment production. No selective medium is yet available. Several serological tests are available for diagnosis eg. latex beads.

It can be transmitted horizontally through water, and although reproductive products are infected, organisms are probably not present within the egg. Thus disinfection works, although so too does incubation in clean water. The organism survives well in dead fish (often 10^8 orgs/ml tissue: hence removal of carcasses is crucial to disease control measures) even when frozen at -10°C for up to 50 days. It also survives well on sides of tanks, equipment etc.

Pathology. Furuncles are an inconstant finding, and usually seen only in more chronically affected fish. In young fish and highly susceptible species such as brook trout or brown trout (these species are sometimes used as sentinels on rainbow trout farms) the disease is essentially an acute bacteremia. Gross lesions are often few and may be little more than peripheral vasocongestion, especially at base of fins and round anus, although these lesions seen with most bacteremias. Splenomegaly and an enlarged softened kidney may be seen. Histologically, bacteria may be seen almost anywhere, but especially in spleen, kidney and heart. In the spleen there is often ellipsoidal necrosis → vascular collapse and splenomegaly. In the kidney, look in the peritubular capillaries and sinusoids of anterior portion - vascular walls are a favoured location, with or without fixed macrophage involvement. If severely involved, the kidney may be enlarged. In the heart, the myocardium is a favoured site, and the lesion is so characteristic as to be almost pathognomonic. Subdermal microcolonies (within the dermal lymphatics) are quite frequent, as is branchial colonization.

In older fish and more resistant species, necrotic and hemorrhagic lesions in the muscle may be seen → ulceration. The lesion resembles an infarct, which would not be too surprising, given the propensity of the organism for vessel walls. A more proliferative response is seen in chronic infections, especially in the epicardium. Necrosis is not a major feature of this disease, except the ellipsoids in acute infections, and of the muscle and blood vessel walls. Thus large numbers of organisms may be found in several tissues eg. heart, not as a terminal event, with virtually no response in the surrounding cells. Nevertheless, production of a leukotoxin is postulated for this organism, in an attempt to explain the supposed lack of an inflammatory response. The response is not really all that impoverished, and the evidence for the presence of such a toxin is questionable.

Carp erythrodermatitis is caused by an atypical strain of *A. salmonicida* and is part of the "carp dropsy" syndrome (along with *Rhabdovirus carpio* and swimbladder inflammation). Skin lesions are superficial (i.e. not systemic) and "punched out". If severe, osmoregulation may be impaired. Secondary infections with fungi are common. It is especially a problem in E. European countries.

Haemophilus piscium reported in older literature is an atypical strain of *A. salmonicida*. The same is true for Norwegian isolates of *Pasteurella piscicida*.

2. ***Aeromonas hydrophila*** (syn. *A. liquefaciens*).
This is a Gram-negative rod which is commonly found in freshwater habitats, especially if there is plentiful organic matter present. It is the cause of a "hemorrhagic septicemia" in freshwater fish, associated with rising water temperatures. *A. hydrophila* is not considered by some to be a primary pathogen and usually requires fish to be stressed by environmental or infectious agents before it will cause disease; others believe that indeed it is a primary pathogen. It has zoonotic potential. Predisposing factors include elevated water temperatures, overcrowding, poor water oxygenation and high organic load in the water. It commonly affects carp suffering from spring viraemia (*Rhabdovirus carpio*). It may also be found in association with other pathogens, even other aeromonads. It is one of

the most common pathogens associated with the recently recognised and devastating syndrome "Epizootic ulcerative syndrome" (EUS) sweeping the Asian region, affecting and causing major losses in snakeheads, catfish, cyprinids and gourami (other agents associated with this disease include a rhabdovirus, and an IPN-like virus).

As with other Gram-negative bacteremias, affected fish are usually dark and have large haemorrhages on the body, especially at the base of the fins and around the vent. There is frequently ascites, and hemorrhages over the internal viscera. Frequently, the kidneys and spleen are swollen and soft. Histological lesions are those of necrosis especially of the hematopoietic tissues and intestinal mucosa. In carp, which may survive for some time, haematoidin and haemosiderin are deposited in various organs. The lesions of EUS are non-diagnostic, and include dermal erosions and ulcerations. Whereas, *A. hydrophila* is easily isolated from heart blood and kidneys of most affected fish, this is not the case with cultured eels where the organism is mainly restricted to the ingested food and intestines. In this instance it is suggested that the condition is more a toxemia than a septicemia.

3. ***Citrobacter freundii***

This organism has caused epizootics amongst sunfish (*Mola mola*) in seawater in a public aquarium. It has also been associated with dermal ulceration in turtles, and proliferative enteropathy in lab animals. Lesions in fish include dermal and subdermal vesicles with hemorrhage, plus multifocal granulomas in kidney (may help differentiate from *Vibrio*). It is a Gram-negative member of *Enterobacteriaceae*, and is often recovered on media selective for *Salmonella*.

4. ***Pseudomonas*** spp.

Pseudomonas fluorescens is a Gram-negative rod which produces septicemia in fish in the freshwater environment, usually at high water temperatures. The disease has been mainly reported in carp and tilapia, but tropical aquarium fish and salmonids may be affected. Pathologically it is difficult to distinguish from other Gram-negative septicemias in freshwater, such as *Aeromonas hydrophila*. Thus hemorrhagic skin lesions and congestion at the fin bases are common.

Pseudomonas anguilliseptica. This is the cause of "red-spot disease" ("Sekiten-byo") in Japanese eels and, to a lesser extent, in European eels. It has also been recorded in other species in Japan, and recently was reported as a pathogen of salmonids (rainbow trout and whitefish) in Finland. The disease occurs mainly in the spring in brackish water at water temperatures between 20-27°C. Mortality can be very high, up to 95%. Once again, petechial hemorrhages seem to be the major gross lesion.

It is believed that initially there is systemic infection leading to bacterial multiplication and macrophage infiltration in vascular walls, the heart, the spleen and dermal connective tissues. As well as the presence of bacterial colonies, haemorrhages and macrophage infiltrates in a number of tissues, especially the skin, muscle and heart, there is a marked atrophy of liver parenchymal cells.

5. ***Yersinia* sp**

Yersinia ruckeri is a Gram-negative variably-motile rod, the cause of "enteric redmouth disease" (ERM) of freshwater fish. It mainly affects salmonids, but can infect goldfish and possibly other fish species. There are three recognized serotypes: Serotype I is the "Hagerman" strain which is the cause of "Hagerman redmouth". Serotype II is the "Big Creek" strain which is less virulent than serotype I. Serotype III is the "Australian" strain isolated from diseased and normal rainbow trout and salmon in Australia; it is considered relatively avirulent. In classical ERM the fish show subcutaneous/submucous haemorrhages especially around the mouth, but also involving the fins (especially the bases of the fins) and the vent. Other signs include lethargy and anorexia. In less severe case the lesions are typical of any septicaemia. Chronic cases frequently have exophthalmos panopthalmitis and loss of orbital tissues with concurrent (resultant) darkening of the body. Histologically, bacterial colonies are found in many

tissues. Pathological differentiation from other freshwater Gram-negative bacteremias is not easy. A number of effective vaccines, applied as bath to young fish, are helpful in controlling the disease, especially classical "redmouth".

When a population of fish is surveyed for the presence of *Y. ruckeri*, cultures should be made of the contents of the lower intestinal tract as carriers may only have significant bacterial levels at this site. The organism can be recovered from the GI tract of water rodents, which can therefore act as carriers.

6. *Edwardsiella*

a) *E. ictaluri* - the cause of enteric-septicemia of catfish (ESC) It has also been isolated from aquarium species (green knife fish *Eigemannia virescens* and danios *Danio devario*). It is a cause of substantial losses to the catfish industry in the southern USA, especially at moderate water temperatures (20-30°C). It is a Gram-negative member of the family Enterobacteriaceae. A characteristic "hole in the head" lesion is seen in fingerling catfish, between frontal bones of head. Multifocal dermatitis of lateral body wall also seen, as in a hemorrhagic enteritis. Other lesions are typical of a hemorrhagic septicemia. Anemia and hypoproteinemia consistently found. Tilapia are slightly susceptible to I/P inoculation. Bighead carp, golden shiners, largemouth bass are not susceptible.

b) *E. tarda*. Another warmwater disease of catfish in the southern USA, but also reported from other cultured species (eels in particular, plus cyprinids) in other parts of the world (S.E. Asia, Japan and Taiwan). It causes "paracolo disease" of cultured eels, and outbreaks in saltwater have been seen in mullet and crimson sea-bream. It is common at higher water temperatures (>30°C). Dermal and muscular lesions are the hallmark; these are often gas-filled. Fibrinous peritonitis, hepatic and renal necrosis, are other common lesions. The organism can be isolated from urine and feces of man, and indeed it may be a human pathogen. Siamese fighting fish with multifocal dermal ulcerations, plus splenic, hepatic and renal granulomata, were shown to be infected with *E. tarda*; thus aquarium species also susceptible. It can also be isolated from snakes and turtles.

7. <u>"Myxobacterial Diseases"</u>

There are 2 Orders:

- Myxobacterales - Gram -ve long gliding rods, fruiting bodies
- Cytophagales:

Family Cytophagaceae

Cytophaga no fruiting bodies
Flexibacter

Most of the organisms we deal with have no fruiting bodies and are not "Myxobacteria". Therefore this is rather a misnomer.
Flavobacterium is very similar to *Cytophaga/Flexibacter*, but do not glide.

a. <u>Columnaris disease</u> *Cytophaga* (formerly *Flexibacter) columnaris*.

This is a common problem in most species of freshwater fish at higher water temperatures (usually > 15°C), although similar diseases are also seen in seawater. Disease is most notable in cultured species, although we see it in wild fish too. The disease is more common in hard water with high levels organic matter; divalent cations and other competing bacteria are important factors. *Clarias* and other spp. are very susceptible, with mortality approaching 90-95%

Etiology. *Cytophaga columnaris* is a Gram-negative long gliding rod which swarm on top of one another to produce "columns". Pigmented colonies are produced on cytophaga agar (Flexirubin pigments).

Pathology. This is a necrotizing disease affecting mainly skin, mouth ("cotton-wool mouth") and less commonly gills. Grossly there is a pale "saddlepatch" round the dorsal fin. The pallor is due to total sloughing of the epidermis, removing underlying pigment cells in the process, probably combined with some neurological upset. There is spongiform degeneration of epidermis, with the bacteria lining up between the collagen fibres, making them often hard to detect; whole-mounts of fresh tissue are very useful. Lesions may target the skin round the anus. "Black patch necrosis" is the name of the disease affecting Dover sole in seawater - improper substrate for the fish is considered a predisposing factor.

If the gills are affected, the lamellae and even filaments may be necrotic. This helps to differentiate this condition from the other "myxobacterial" disease of gills, bacterial gill disease, in which necrosis is relatively minimal at the l.m. level. The organism may occasionally become bacteremic, which is hardly surprising considering its extreme drgree of invasiveness.

b. Bacterial Gill Disease (BGD) *Flavobacterium branchiophila*

This is one of the most important diseases of cultured fish on a worldwide basis, although it is also responsible for sometimes extensive losses in wild fish populations e.g. chinook salmon in the Great Lakes. Affects a variety of species, but common in salmonids. It is *the* most significant disease in Ontario, Canada hatcheries, where it can cause high mortality unless carefully controlled. The disease often coincides with Spring thaw.

It is essentially a disease of young fish, although market-size fish are also susceptible. Earlier views were that declining or altered water quality is the major predisposing factor leading to gill damage, the bacteria then coming in as secondary invaders and colonizing this damaged tissue eg. excess mucus production due to irritation. The present view is that the causative organism is capable of causing disease even in good quality water conditions, although doubtless high ammonia levels, high suspended solids, and low oxygen, would all exacerbate the situation.

A decreased appetite is an early sign, and helps to differentiate from other gill diseases. Mortality can be high, and horizontal transmission will lead to disease within 24 hours.
Etiology. A variety of "myxobacteria" (yellow-pigmented bacteria) are usually isolated from gills, although *Flavobacterium* has been used to experimentally reproduce the disease. In many cases in S. Ontario, and elsewhere in the world, this group is the dominant isolate.

Pathology. Flared opercula and erosion of trailing edges with mucoid gills beneath are a typical appearance. Fish become lethargic and crowd at sides of tanks, sometimes at water inlets. Increased mucus production often leads to foaming of water. The increased mucus and hyperplasia decrease O_2 exchange → respiratory distress. Early histopathological changes are characterized by filamentous bacteria sitting on the lamellar surface, producing necrosis. With time, there is lamellar fusion with pavement cell hypertrophy, and hyperplasia of epithelial cells becomes pronounced. There may be accumulation of cellular debris in the inter-lamellar spaces, and mucous metaplasia of severely fused lamellae. The hyperplasia may be more severe at the distal ends of the filaments → a "clubbed" appearance. Grossly evident necrosis is not a feature of this disease, although the recognition of this process at the ultrastructural level helped explain many of the questions about the pathogenesis and clinical presentation of affected fish.

Treatment. This can lead to total restoration of normal architecture in a short time (7 days). Chloramine-T or formalin treatment, combined with supportive therapy aimed at reducing the rate of ion loss, are both indicated.

c) Bacterial cold-water disease "Peduncle disease" (*Cytophaga psychrophila*)
This disease is associated with severe erosions of the dermis at the peduncle (from the adipose fin back). It is essentially a disease of salmonids at low temperatures (2-10°C), which does however occur at higher temperatures up to 15°C, and which does become systemic.

8. <u>Bacterial kidney disease</u> (synonyms Dee Disease, Kidney Disease). *Renibacterium salmoninarum*.

This is an economically extremely important disease of salmonids (although other species are susceptible to experimental infection). Because of its insidious nature, fish are usually quite well grown (and therefore valuable). It is mainly a problem of farmed fish although wild populations may also develop disease.
Etiology. A small gram-positive diplobacillus, which is found intra-cellularly, although also seen extra-cellularly. It will not grow on normal media and needs cysteine. Thus KDM-1, 2 and 3 specialized media have been developed. It is slow growing and grows best at 15°C, although still takes roughly 2-3 weeks to produce white, round raised colonies. Gram-stain of kidney or FAT used for diagnosis, as is ELISA. The organism is transmitted within eggs and thus disinfection may be useless. It may also induce tolerance as a result of this.

Pathology. The lesions are multifocal pyogranulomas, in almost any tissue, with or without cavitation. The kidney is a target organ, and may be severely affected appearing grey and nodular, with cavities extending sometimes into the dorsal musculature.
Subdermal blisters are not uncommon, especially in larger fish. The spleen, liver, gills and myocardium frequently have multifocal granulomas. The epicardium is a favoured location, especially in younger fish, causing a fibrinous epicarditis, and often severe necrosis of myocardium, particularly the compact layer. If lesions are widespread, there may be a meningitis. The choroid gland of the eye is another favoured site and may → exophthalmia.

If renal granulomas severe, there may be compression atrophy of nephrons, endocrine elements or juxtaglomerular (JG) cells (causes the fluid retention seen in some fish due to decreased renin production and hence decreased blood pressure). Anemia and low serum protein also seen. Rupture of the renal granulomas into the peritoneal cavity leads to a locally severe peritonitis, with exudation, compression of the swim-bladder, and increased passive uptake of water from enhanced respiation needed to maintain location in the water column, and in the face of a greatly reduced ability to excrete water, this ultimately leads to transudation as well.

The cellular response comprises macrophages (stuffed with bacteria) neutrophils (occasionally contain the odd one or two) and fibroblasts later. Fibrin outpouring seen in some early infections.

9. *Clostridia*
Anaerobes are probably much more common than are presently realized owing to the fact that few diagnosticians routinely culture for anaerobes.

a. *Cl. botulinum* type E is carried in the GI tract and gills as a contaminant on many farms. Only rarely will it cause disease in fish, usually at high water temperatures, and when trash fish are fed instead of commercially-prepared pelleted feeds. It occurs in Denmark as "bankruptcy disease" in rainbow trout farms. Symptoms are sluggishness, with intermittent periods of recovery. No other lesions.

b. *Cl. hastiforme* has been associated with acute necrotizing gastritis in aquarium cichlids with "cichlid bloat". Other associated organisms included a flagellate, possibly *Cryptobia*.

Lesions included granulomas in lamina propria (likely due to the protozoa) ranging through necrosis of mucosa, full thickness necrosis of gastric wall, perforation, peritonitis, and eventually necrosis of body wall musculature. Spore-forming *Clostridia* are in the peritoneal exudate. They fluoresce with Wellcome *Cl. novyi* antiserum. Fish were being fed frozen lumps of brine shrimp (a braxy-like syndrome?). Surviving fish treated with Ampicillin and diet changed to reflect more natural feeding (these fish are ectophyte grazers).

c. Other Clostridia variously reported eg. a *Clostridium* sp. associated with an ankylosing spondylosis in giant perch.

10. *Streptococcus*
A variety of syndromes has been associated with this group:

a) a post-stripping peritonitis in salmonids and other species, probably due to rough handling. *Strep. faecalis* has been isolated, and it was considered to be a human contaminant. Other organisms are involved in such a syndrome including *Lactobacillus piscicola*.

b) a hemorrhagic septicemia in trout (seen in S. Africa and Australia), with retrobulbar hemorrhage and exophthalmia, lethargy and skin darkening. Lancefield's group D, weakly B-hemolytic; unclassified. The organism experimentally reproduced the disease in trout, but not in tilapia or carp. Muscle necrosis, meningitis, focal hepatic, splenic and renal necrosis, and panophthalmitis, are some of the lesions.

c) a non-hemolytic group B *Streptococcus* from golden shiners in freshwater and several species of saltwater fish in N. America. Similar to *Strep. agalactiae*. The disease was a hemorrhagic septicemia, but the eye appeared to be the target organ. A non-hemolytic Group B *Strep.* also associated with mortality in various species in the Chesapeake Bay (U.S.A.) - meningitis was a notable finding.

d) disease of cultured fish in Japan, especially yellow tails and eels, associated with non-hemolytic *Streps*.

e) epizootics described from tilapia.

11. *Lactobacillus piscicola* (Now called *Carnobacterium*).
A Gram-positive bacillus as a cause of disease in older salmonids, especially those subjected to the stresses of handling and stripping. Lesions associated include peritonitis, visceral hemorrhages, muscular abscesses and sub-dermal blisters, often hemorrhagic. (NB. These latter lesions, associated with this Gram-positive organism make it an important differential from *Renibacterium salmoninarum*.

12. Pasteurellosis - *Pasteurella piscicida* (not in Bergey's).
This is an important disease of cultured yellowtail in Japan ("pseudotuberculosis"). It was also reported as a cause of disease in N. America in white perch and striped bass, with epizootics in Chesapeake Bay in 1963. This organism was however, subsequently re-identified as a non-pigmented strain of *A. salmonicida*. *P. piscicida* is a Gram-negative bipolar staining, non-motile bacillus, which requires at least 0.3% NaCl to grow. Clinical symptoms include sluggishness, tachybranchia, and pigmentary change. Lesions are either a hemorrhagic septicemia with bacteria-laden macrophages in kidney and spleen, or multifocal granulomas ("pseudotubercles") in kidney, spleen and liver. This latter syndrome is seen mainly in yellowtails.
Ampicillin appears to be the drug of choice at approximately 40 mg/kg body weight. Formalin-killed organisms appear to protect even when given orally.

13. *Vibrio* spp

The *Vibrio* spp. are primarily marine inhabitants, but may be found (and cause disease) in other environments as well. Fish in freshwater may acquire vibriosis if they are fed fresh (raw, frozen) marine trash fish or if small amounts of seawater is added to the freshwater supply (e.g. during smoltification or as a treatment). Several species may be pathogenic to marine fish, amphibia, shellfish and humans. A few of the most important species will be listed briefly below:

Vibrio cholerae. The causative agent of cholera in man
Vibrio parahaemolyticus: Causes gastroenteritis in humans eating contaminated seafood.
Vibrio parahaemolyticus, Vibrio alginolyticus, Vibrio vulnificus. All these 3 species have been isolated from infected skin lesions and from patients with otitis in humans frequently exposed to seawater (divers, swimmers, fishermen, fish-farmers). All 3, as well as *V. anguillarum*, are associated with disease in sea-caged grouper, sea-bass and snapper in Asia. The disease is common in fry or fingerlings during the first few weeks after introduction to the cages, but they are also responsible for disease during the grow-out period.
Vibrio damsela. Isolated from skin lesions in damselfish (Pacific Ocean).
Vibrio carchariae is a pathogen of sharks in which it causes vasculitis, especially of the reticuloendothelial system. It has also been isolated from dermal lesions of sharks.
Vibrio fishcheri. This species emits light when grown on special media. The *Photobacterium* spp. are closely related to the *Vibrio* spp. All of them are luminescent and emit light. They are very common in decaying material in the marine environment and may be found in the light-organs of deepwater fish. Some of them may be associated with diseases in Atlantic salmon and rainbow trout.

Vibrio anguillarum. The causative agent of "classical" vibriosis in most marine fish, and the commonest cause of bacterial disease in the marine environment. Vibriosis may have several manifestations in different species of marine organisms. The name derives from the disease "red pest" in Baltic eels *Anguilla anguilla*, which this organism causes. *Vibrio ordalii* (= atypical strains of *Vibrio anguillarum*, or biotype II). The cause of vibriosis in salmonids on the Pacific coast of North America.
Vibrio anguillarum and Vibrio ordalii cause vibriosis in a wide range of marine organisms, both farmed and wild: the marine equivalent of *Aeromonas salmonicida* in pathological terms. The most severe outbreaks will usually occur in the summertime at temperatures above 12°C. Major predisposing factors include overcrowding, and dermal abrasions. Scavenger fish often carry the organisms which in any case are extremely common in the marine environment. Classical lesions of vibriosis in almost any species are: petechial haemorrhages on the abdomen, haemorrhage and congestion at the base of the pectoral, pelvic and anal fins, plus dermal ulceration, sometimes very deep, with perforation to abdominal cavity. Haemorrhage and "furuncles" (really a necrotizing myositis, and often in a wedge-shaped pattern, suggesting infarction) in body musculature. Dark, swollen (sometimes "liquid") spleen. Anemia is a common finding. Serous exudate in body cavities. Yellowish, granulated gut contents. In cod (*Gadus morhua* L.) fry and fingerlings, the most common manifestations are fin rot and tail rot, while in adult fish: panophthalmitis. In flatfish the most common lesions are haemorrhages on the white underside ("blind side") of the fish, ophthalmic lesions and excessive amounts of serous exudates in body cavities. With *Vibrio ordalii*, the lesions are usually grossly similar to those caused by *V. anguillarum*, but histologically, tissue localization (as opposed to septicemia) is more obvious.

Vibrio salmonicida sp. nov. is one of several species associated with "Haemorrhagic syndrome" ("Hitra disease" or "coldwater vibriosis") in Atlantic salmon in Norway. This one however is now accepted as being the cause, regardless of any predisposing nutritional factors (such as vitamin E/selenium deficiency), and vaccines to control the disease are available. Diseased fish may be without gross lesions, but quite often the anus is bloody and swollen, there are haemorrhages at the base of the ventral fins and on the abdomen. The body cavities may be filled with large amounts of sero-haemorrhagic fluid. Liver and spleen are usually light in colour and may be swollen. Petecchia are on

most visceral surfaces. Occurrence of the disease at low temperatures (as low as 2-4°C) is a useful feature from a diagnostic viewpoint. Myopathy in heart and skeletal muscle is seen in early stages, with large numbers of bacteria in many organs later.

Most marine *Vibrio* spp. will grow well on marine agar (MA) and Blood agar plates with 2% NaCl added (or made with artificial seawater or natural, sterilized seawater). Optimum temperature is 20 - 22°C for *V. anguillarum*, but 12 - 15°C for *Vibrio* spp. associated with HS. *V. anguillarum* is haemolytic. Luminescent strains and *Photobacterium* spp. are usually cultivated on Sea Water Yeast Peptone Agar (SWYP agar). Luminescence should be checked every 6 hr.

Several vaccines are available. Many of them are made from "local" strains of *V. anguillarum* and *V. ordalii*. The fish are usually vaccinated in the spring as pre-smolts or as smolts. Several methods are in use: Bath (long-time immersion in dilute vaccine solution), Dip (short-time immersion in concentrate vaccine solution) and injection (intraperitoneal injection of a small amount (0.1 ml.) of vaccine). The latter method is considered to be the most efficacious. Water temperature should be above 6 - 8°C to achieve good immunity within 2- 3 weeks.

14. *Mycobacterium* spp.

Mycobacterium marinum is the main mycobacterium infecting fish, although *M. fortuitum* and *M. chelonei* and others have also been reported. These organisms are acid-fast and relatively fast growing, compared to mammalian spp. They have zoonotic potential, and will cause usually self-limiting infections of the skin in man. Infections have been reported in fresh and marine species, cold and warm waters and cultured and wild fish. There is evidence that infections are spread by carrier fish. Feeding of infected fish offal is a potent means of enhancing infection rates. Usually (but not always) the disease is chronic and affected fish are emaciated, have ascites and may have exophthalmos, lordosis, scoliosis or pigment changes (tropicals lose colour, salmonids become brighter). Dermal ulceration, and fin erosions, often with secondary bacterial or mycotic infections, are commonly seen.

Pathological findings include miliary lesions especially in the liver, spleen and kidney. Histologically, these are found to be typical tubercles, but rarely contain Langhan's giant cells. The organisms are usually carried to the melano-macrophage centres, which should not be mistaken for granulomas, although once there, granulomas will develop. Calcification does not occur. Usually, acid-fast organisms are very numerous in the tubercles.

M. neoaurum has been associated with panophthalmitis in chinook salmon (in association with a *Rhodococcus*).

15. *Nocardia*

N. asteroides is a Gram-positive, weakly acid-fast, branching organism which sometimes causes granulomatous disease in fresh-water and marine fish, including salmonids. Affected fish become emaciated and frequently show abdominal distention. The disease is similar to mycobacteriosis, which is the main differential.
Nocardia kampachi infection of cultured yellowtail in Japanese mariculture. Abscesses and/or nodules may be found in some or all of the spleen, liver, kidney, heart, muscle, gills and swim bladder. Histologically, the lesions show a progression from abscess (neutrophils, macrophages and necrotic material) to "actinomycotic" granulomata.
Other *Nocardia* spp. isolated from farmed giant gourami in Japan.

VIRUS DISEASES

1. Infectious Pancreatic Necrosis (IPN)

This and similar bi-stranded RNA (BIRNA) viruses are widely distributed in salmonid, non-salmonids (tropical, freshwater and marine species) and shellfish populations. In young salmonids however, we occasionally see clinical disease, and even then managemental conditions are usually extreme. Thus in salmonids infection is common (more than 90% Norwegian fish have titres to IPN) but clinical disease is rare. The same applies to non-salmonids with the exception of eel virus kidney disease and spinning disease of menhaden which are both probably caused by IPN-like viruses. The disease IPN has not been reported in wild fish, only young cultured salmonids.

Virus - a bi-stranded RNA virus with 2 segments. Similar therefore to infectious bursal disease of chickens. It is an icosahedron 60-65 nm in diameter, with no envelope. The virus is quite robust although it is susceptible to commonly used disinfectants such as formalin, chlorine and iodophores. Ab serotype largely non-virulent in Europe while Sp serotype virulent. VR-299 (ATCC) is the reference N. American strain.

Pathology - as the name suggests, necrosis of pancreatic acinar cells is the major lesion although multifocal necrosis of hemopoietic tissue and intestinal mucosa is also reported (catarrhal enteritis was the name originally given to the condition). Non-specific symptoms and lesions include anorexia, darkening and spinning with trailing fecal casts. Increased numbers of intestinal mucosal apoptotic cells accompany the anorexia, and the stomach may contain a mucoid gel. Release of pancreatic enzymes does not lead to auto-digestion as the enzymes only activated when they reach the gut.

Diagnosis - the widespread nature of the virus, combined with the non-specific nature of the lesions (pancreatic necrosis may be seen in many conditions including poor fixation) make this a tough diagnosis. Correlation of lesions with the presence of the virus (IFAT) is probably the safest, although $>10^5$ PFU (plague forming units) per gram of fish are usually found in diseased fish. RTG-2 or FHM cell lines are often used to isolate virulent serotypes although BF-2 cells are considered ten times more sensitive, especially for non-virulent isolates.

Control - mortality is usually not very high in this disease, and it occurs in very young fish, so it does not represent a severe financial loss. Buying in more eggs than necessary, to offset anticipated losses is commonly practised. Brook trout are probably the most sensitive, with rainbow trout more hardy. Low temperatures i.e. 4-6°C not only prolong incubation time but also reduce mortality. Similarly higher temperatures i.e. 16°C reduce mortality which is highest at 10°C. Adult fish may remain carriers and even careful egg disinfection may fail, suggesting the virus within the egg. The virus survives passage through the gut of fish-eating birds and mammals. Older fish are susceptible to becoming infected but do not develop clinical disease.

2. Eel virus disease (eel virus European, EEV, EVE)

First recognized in Japan following importation of eels from Europe, this IPN-like virus causes significant mortality in cultured eels. Only described in Japan. It is a BIRNA virus most closely related antigenically to IPN Ab serotype (low virulence for trout). The major pathological lesions include branchial lamellar hyperplasia and congestion plus a proliferative glomerulonephritis. Multifocal interstitial necrosis and some nephrosis may also be present causing gross renal hypertrophy and some ascites. It grows on regular salmonid cell lines, plus BF-2.

3. Menhaden Spinning Disease Virus

Atlantic menhaden in Chesapeake Bay area have shown a peculiar spinning disease in the spring which ultimately results in death. Peripheral hemorrhages are also seen. Some limited work has recovered an IPN-like virus from brain and pancreas, and has experimentally reproduced the disease. The virus will not grow on regular cell lines used for IPN.

4. Golden shiner virus

A bi-stranded RNA virus recovered from outbreaks of low mortality in this bait fish on farms in USA. Serologically distinct from IPN and unlike IPNV, will replicate at 30°C. Some hemorrhage reported from affected fish, but experimentally, it produced little clinical or pathological disease.

5. Infectious hematopoietic necrosis (IHN)

An acute rhabdoviral disease of salmonids, especially rainbow trout, chinook and sockeye salmon. Different disease syndromes in early 1970's (sockeye salmon disease etc.) now shown to be caused by the same viral agent. Distribution restricted mainly to Pacific-Northwest (enzootic) and also Japan, although there was a recent isolation of the virus in Europe.

Virus. A typical rhabdovirus measuring approximately 160x90 nm and resembles rabies virus. It will replicate at low temperatures (4-20°C), and will grow on common cell lines such as RTG-2 and FHM. Viral hemorrhagic septicemia (VHS) is the only other rhabdovirus of salmonids - this is not neutralized by IHN antiserum, and until recently it was restricted to Europe.

Pathology. Young fish are most susceptible although it will cause disease in older fish (2 y.o.). It is primarily a disease of restocking hatcheries. As the name suggests, hematopoietic tissue is the target, with acute necrosis of all cell types in the renal interstitium and spleen. Renal portal endothelium and in later stages the tubules too, are also affected. Splenic ellipsoids may be involved as with the pancreas and liver in which there is drop-out of acinar cells and individual hepatocytes respectively. The classical lesion, and one which is pathognomonic when present, is necrosis of the eosinophilic granule cell layer in the submucosa of the gut. This latter lesion is not invariable however, even though it is common. Melanomacrophages in the kidney are totally necrotic and there is granular debris throughout the tissue. Grossly, affected fish are dark and anemic with petechial hemorrhages internally, and over the body surface.

Control. Mortality is seen at a wide range of temperatures and can be as high as 80%. It used to be thought that in some locations, mortality was lower at temperature greater than 15-18°C, but recent papers suggest this may not be so. Survivors may become carriers, excreting the virus especially with eggs which are difficult to disinfect (could even be within the egg). Incubation time is usually 5-14 days. Reduction of virus level in parent stock helps reduce mortality, as may incubation in higher temperature (>18°C) water.

6. Viral hemorrhagic septicemia (VHS, Egtved disease)

A major rhabdoviral disease of European rainbow trout first described from Egtved in Denmark. The disease is the subject of stringent control measures. There have been several isolations of the virus in the Pacific Northwest (U.S.A.) in the last 2 years (1988, 89), but only in returning salmon, and never associated with any disease. Natural disease also described in brown trout and pike fry, although Atlantic salmon and goldfish experimentally susceptible. Not seen in N. Zealand, Australia, UK. This is a relatively low temperature disease, and can lead to 100% mortality in young trout at 4°C. Temperatures of 16°C and higher leads to disappearance of signs. Continuous temperature changes between 4 and 12°C may be best way to promote disease. This temperature effect means that fish often develop disease in winter months and fish may be 1 year old.

Virus. There are 2 or 3 serotypes (uncertain in literature) which all grow well in routine cell cultures, and are all pathogenic. It is a typical rhabdovirus with an envelope and a single stranded RNA core.

Pathology. The older literature confused "chronic VHS" with PKD and this must always be borne in mind whenever reading such reports - even the report of VHS in brown trout may indeed have been PKD. The virus is endotheliotropic and gross lesions reflect this with hemorrhage in muscle and elsewhere, including the choroid gland of the eye (causing exophthalmia) and gills. The virus is in fact taken up by the lamellar pillar cells. Hematopoietic necrosis with hemorrhage, leading to hemosiderin accumulation are constant findings. More chronically infected fish have segmental thickening of

glomerular basement membranes. Cardiac muscle may also be necrotic. Survivors often become latent carriers with intermittent shedding of virus, probably a temperature or stress-related event.
Control. Vaccines give equivocal results. Avoidance is best policy. Egg-associated virus and therefore disinfection helps although mainly probably horizontally transmitted with uptake through gills. Raising water temperature above 15°C is not usually feasible.

7. Spring Viremia of Carp (SVC)

Caused by *Rhabdovirus carpio*, the viral condition was split out from the "infectious dropsy of carp" complex which also includes (1) swimbladder inflammation (SBI) now known to be caused by the pre-spore stage of the myxosporean *Sphaerospora*, and (2) "carp erythrodermatitis" caused by an atypical *Aeromonas salmonicida*. The condition appears to be restricted to Europe, and can cause high mortality.
Virus. A typical bullet-shaped rhabdovirus which replicates in FHM, BF-2 and at high virus titres will also cause CPE in RTG-2 cells.
Pathology. The lesions suggest an endotheliotropic virus, with the gross and histological picture dominated by hemorrhagic and fibrinous exudates. A decent pathological description of the disease is badly needed. Exophthalmia from retrobulbar hemorrhage, darkening and possibly necrotic enteritis may be other lesions.
Epidemiology. The species most commonly affected is the common carp, but probably other species are susceptible. Springtime (low water temperatures) is the peak occurrence although some workers feel it can occur at other times of year. Uptake of virus from the water occurs in the gills, with kidney, liver, spleen, heart and gut being targets. Transmission with parasites such as *Argulus* or *Piscicola* (leech) is also thought to occur.

8. Eel rhabdovirus disease

A group of 4 serologically similar isolates from young *Anguilla* spp. (*A. anguilla* and *A. rostrata*) in Japan and France (EVA, EVEX, B12 and C30). Only EVA was associated with disease; none produces experimental disease in eels although EVEX may be pathogenic for young rainbow trout. Thus the significance of even EVA remains to be established. The disease in the EVA eels was largely multifocal necrosis of liver, spleen, renal tubules, and skeletal muscle. RTG-2 and BF-2 replicate all but the B12 isolate.

9. Perch Rhabdovirus

Isolated from *Perca fluviatilis* in France, with nervous signs and 30% mortality. The virus caused marked mortality in experimental inoculation, especially after intra-cranial injection. No information on pathology, but the literature suggests the brain as the target. Serologically, this virus is not neutralized by anti-VHS or IHN sera.

10. Pike fry rhabdovirus

Possibly a single isolate from an outbreak of disease in N. Europe amongst young cultured pike. Grossly the fish had cranial "blips". NB. Other agents capable of causing cranial defects include over-treating with malachite green.
Other lesions included exophthalmia and multifocal hemorrhages in muscle, gills and skin. Some renal tubular and glomerular necrosis also reported. Similar viruses have been isolated from other species in Europe, including brown trout in N. Ireland.

11. A cichlid rhabdovirus

Isolated in N. America from a group of *Cichlasoma cyanoguttatum* intended for behavioral studies. Mortality in the group of 50 was 100%. Isolated on FHM and BF-2 cell lines at 23°C. The isolate had pathogenicity for convict cichlids inoculation I/P (80% mortality).

12. Cod ulcus syndrome

Two separate viruses have been isolated from dermal ulcers in wild cod off the Danish coast. One is a rhabdovirus, the other probably an iridovirus. Neither virus gave a CPE in original inoculated cultures. The rhabdovirus grew best in pike sarcoma cell line. Papules and vesicles 2-8 mm in diameter were the early lesions, prior to ulceration. Scale pockets are involved with inflammation of the superficial dermis. Underlying muscle is involved in older lesions following ulceration. Although bacteria, notably *Vibrio*, are also involved, they are systemic in only 9% of fish. Intra-cardiac inoculation of cod with the presumed iridovirus does cause skin lesions. Precise involvement of viruses not fully worked out

13. Snakehead rhabdovirus

Associated with severe dermal ulceration ("Epizootic ulcerative syndrome - EUS") in striped snakehead (*Ophicephalus striatus*) and eels in S.E. Asia. The significance of the isolate is not yet determined, but it is not found in many instances of the disease, whereas *A. hydrophila* is). The outbreaks of disease, which may cause severe losses amongst the farmed fish, occur in the cooler months of the year. A birna virus has also been associated with the disease outbreaks.

14. Finish rhabdovirus.

Little information as yet (only reported in 1988), but seen in salmonids causing multi-focal hepatic and renal necrosis.

15. Hirame rhabdovirus (*Rhabdovirus olivaceus*).

Named after the Japanese flounder (yamame), although other species of marine fish (black and red sea-bream, plus *Sebastes* and rainbow trout), are susceptible. Natural outbreaks occur when water temps. exceed 15°C. Again, hemopoietic tissue is targeted.

16. *Herpesvirus salmonis*

Only isolated once in the Pacific northwest of the USA although a similar virus has been reported from Japan, causing epizootics in sockeye salmon and called the Ne VTA virus. Most of the information therefore comes from experimental infections caused by inoculation. Only trout less than 6 m.o. are susceptible although mortality is 50-100%. Other salmonids appear to be refractory.

Virus. A typical herpesvirus which replicates best at 5-10°C. Syncytia and intranuclear inclusions develop in RTG-2 and CHSE-214 cell lines, followed by necrosis.

Pathology. First signs are seen 3 weeks post injection and are non-specific lethargy, anorexia and exophthalmia, with congested fin bases and trailing fecal casts. Kidney and heart are target organs. Focal necrosis in liver kidney, gut and myocardium is seen, with myocarditis. Some hyperplasia of hematopoietic tissue is evident with congestion and edema of pseudobranchs, spleen and gills. Syncytia in pancreatic acinar tissue are considered pathognomonic for this disease.

17. Oncorhynchus Masou Virus (OMV) - Yamame Tumour Virus

A herpesvirus found so far only in masu salmon populations (yamame) in Japan causing mortality in young fish and papillomas or basal cell tumours in survivors.

Virus. A herpesvirus which replicates in salmonid cell lines, eg. RTG-2, but not non-salmonid cell lines such as BB or FHM. Optimal growth is at 15°C, and cells have small eosinophilic I/C inclusions. It is serologically distinct from *Herpesvirus salmonis*.

Pathology. Other young salmon eg. chum, coho, kokanee and rainbow trout are all susceptible to experimental infection by immersion. Older fish (>240 days) are almost totally resistant whereas younger ones show mortality of 60% by 65 days p.i. with grades inbetween. There is a 60% incidence of papillomas or basal cell tumours in survivors. Acute lesions include multifocal hepatic necrosis with syncytia plus splenic necrosis. By contrast with *H. salmonis*, kidney and pancreas are normal. Tumors are found most frequently round the mouth, then caudal fin, operculum and eyes.

18. Channel Catfish Virus Disease (CCVD)

A severe disease of young cultured channel catfish, almost always less than 4 m.o., occurring in warmer water (>23°C). Only reported from Southern USA and Honduras, and although it is likely that other species of catfish are susceptible, this may only be true for experimental infections (*Clarias batrachus* is exp. susceptible to injection). In addition, certain strains of channel catfish are more susceptible than others.

Virus. A typical herpesvirus which grows in BB (brown bullhead) lines at 10-33°C with an optimum of 25°C. Channel catfish ovary cell line may give a higher titre more quickly. The virus is rapidly inactivated in pond mud and stability in dead fish is also poor. Infectivity in pond water containing organic matter at 25°C is totally abolished in 7 days. The carrier state is uncommon and virus is usually only recovered from clinically affected fish. Occasional cases of adult carriers do occur however. Vertical transmission is strongly suspected, but not proven.

Pathology. Hanging vertically (head up) in the water, with exophthalmia, ascites, and pale gills, with petechial hemorrhages on ventral body wall, are typical signs. Necrosis dominates the histopathological lesions, especially in liver (hepatocytes round pancreas especially), kidney (interstitium as well as nephrons) and spleen with marked necrosis of all elements and splenomegaly. Intestinal mucosa is necrotic and may slough and there may be multifocal necrosis of skeletal muscle. Neurones in the brain may show vacuolation and there may be myocardial necrosis and myocarditis.

19. Walleye Herpesvirus

Isolated once from a Canadian walleye with epidermal hyperplasia, this herpesvirus is distinct from the retrovirus associated with dermal fibrosarcoma or lymphocystis virus both found in the same species. Whether the cause of the hyperplasia or not, is uncertain.

Virus. Originally isolated on walleye ovarian cell line, and will not grow on salmonid cells.

20. Carp pox

Again a plaque-like epidermal hyperplasia with more or less dermal invasion. Virus-like particles (not poxvirus) associated, and lesions can be transmitted by scarifying healthy skin and rubbing in lesions. *Herpesvirus* was suspected from the EM pictures in late 1960's and a *Herpesvirus* was recently isolated in Japan from tumours in fancy carp. Tench plus carp and other cyprinids (golden ide in N. America) may be affected.

21. Other herpesviruses

A herpesvirus has been described and associated with dermal and branchial cytomegaly in young turbot off the Scottish and Welsh coast. The virus was not isolated. Another has been described (but not isolated) from multifocal dermal lesions in Pacific cod, again with cytomegaly of epidermal cells.

Epizootic epitheliotropic disease (EED) of lake trout (Great Lakes). This is a recently emerging disease of young lake trout, which is considered to be due to a herpesvirus.

22. Chum salmon reovirus (CSV)

A virus "discovered" during routine examinations of normal chum salmon returning to Japan. No pathological changes are reported either in naturally infected or experimentally inoculated fish, with the exception of mild multifocal necrosis (no virus present in these lesions) in exp. infected fish.

23. Picorna viruses.

These have been recovered from mortalities in Atlantic salmon in British Columbia, from smelt in New Brunswick, Canada (a rotavirus was subsequently recovered from the same fish), and from smelt with papilloma-like skin lesions in the Elbe River, Germany. The significance of these isolates in relation to the lesions and/or mortality is unknown. Picorna-like viruses have also been recovered from larval barramundi in Australia, suffering from mass mortality, and with central nervous lesions, although again, the precise significance of the virus remains to be determined.

24. Opaleye Calicivirus (San Miguel Sea Lion Virus)
This calicivirus causes similar lesions in the skin of both sea lions and pigs (vesicular exanthema) and it also replicates in opaleye (*Cirella nigricans*) at 15°C, but without apparently causing any harm to the fish. A link between the fish and the sea lions is to be found in a lungworm (nematode) which uses the fish as the intermediate host. The isolates from pigs, marine mammals and fish are serologically related but even the pig isolates will replicate in the opaleye.

25. Lymphocystis.
A largely epithelial (mainly skin) infection of a great variety of teleosts. Occurs mostly in higher orders and has not been reported in salmonids. Found in both freshwater and marine environment, in waters of all temperatures. Young fish are more susceptible than old.
Virus. An iridovirus which replicates in any fibroblast cell line causing changes which mimic those seen in the host i.e. cytomegaly.
Pathology. The virus replicates in skin fibroblasts causing extreme cytomegaly so that the individual cells can be recognized grossly - "cysts", and often this occurs in clusters. Individual cells measure 100-1,000 um. The cell membrane becomes thick and hyaline, the nucleus and nucleolus enlarge and the cytoplasm contains basophilic strands of virus inclusion material. Inclusion material is arranged in 2 ways: a) "flounder-type" with cords or b) "plaice-type" which is lacy and distributed throughout the cytoplasm. Fins are a predisposition site although body surface, gills, and rarely the serosal surfaces of peritoneum and heart may become infected. The fish usually recover coinciding with an invasion of lymphocytes, although they may become re-infected, presumably by direct contact with other infected fish. Abrasions from substrate or fighting enhances infection.

26. Goldfish iridovirus.
Two isolates recovered from normal goldfish during a routine examination.
Cell cultures of primary swimbladder cells had intra-cytoplasmic inclusions. The viruses are enveloped double-stranded DNA particles with an icosahedral capsid.

27. Orange chromide cichlid iridovirus.
Associated with severe mortality in imported (to Canada) chromide cichlids, this sole Asian representative of the cichlid family, exhibited pronounced nervous dysfunction due to lesions surrounding the cranial nerves, hemorrhage and almost total mortality. The identity of affected cells, which had I/N inclusions on L.M., was not established, but epithelial cells were not affected. The virus has not yet been isolated.

28. White Sturgeon iridovirus.
Associated with gill and skin infections (again causing cytomegaly) in young cultured white sturgeon. Associated with mortality.

29. Piscine erythrocytic necrosis virus (PEN)
An erythrocyte infection of almost exclusively marine fish which causes mild erythrocyte lysis, with nuclear degeneration plus I/C inclusions. These changes are easily seen on blood smears and may affect a few or many rbc's. Young fish are more susceptible than old, and in Pacific salmonids, anemia and mortality may occur. Epizootics also reported in Pacific herring from Alaska. The virus, probably an iridovirus, has not been cultured. As might be expected, if infection rate of rbc's is high, hematopoietic tissue is "geared-up". Transmission has been accomplished by co-cultivation of fish and by injection.

30. Epizootic haematopoietic necrosis (EHN)
First recorded fish virus isolation from Australia. An iridovirus isolated from moribund redfish perch (*Perca fluviatilis*) with focal hepatocellular and hematopoietic necrosis (spleen and kidney). Pancreas may be involved. Isolated on RTG-2 cells. Causes high mortality in O+ fish in early summer. Atlantic

salmon experimentally inoculated with the isolate showed blindness and vacuolar degeneration of optic lobe plus focal hepatocellular necrosis. It was recently diagnosed in rainbow trout showing same lesions as perch plus encephalopathy.

31. Carp Gill Necrosis Virus

An iridovirus-like disease of Russian carp with pathological changes of gill necrosis. Little other information is available.

32. Cauliflower disease of eels

Possibly an orthomyxovirus isolated from eels with oral papillomas mainly in Baltic population (1 isolate from N. America also) of *Anguilla anguilla*. Precise relationship of this virus (eel virus-2) to the tumours is unknown.

Cauliflower disease growths are temperature and salinity sensitive. Increasing salinity decreases incidence, and the tumours de-differentiate. Incidence highest in summer months. Mouth is commonest site (may interfere with feeding) but can be found anywhere on body.

33. Possible viral diseases - agents seen in EM, but not grown.

a) Atlantic salmon swimbladder leiomyosarcoma. Associated with cultured fish in Scotland. Possibly C-type particles seen budding. The tumour often occupied a large part of the abdominal cavity.

b) Lymphosarcoma of pike and muskellunge. Leukemic lymphoma associated with a retrovirus in N. America and Scandinavia. Tumours may be found anywhere in body - often ulcerate if superficial.

c) Walleye dermal fibrosarcoma. Severe disfiguring fibrosarcomas in wild walleye populations associated possibly with a retrovirus. Lesions found on head and elsewhere over body comprising extremely hard often nodular growths which may ulcerate. Lymphocystis disease and a herpesviral epidermal hyperplasia may be associated.

d) Northern pike epidermal hyperplasia. Herpesvirus-like particles associated with cytomegalic cells in I/C inclusions within "plaques". Retroviruses seen to be associated with more focal lesions in some fish.

e) Atlantic salmon "papillomatosis". Diffuse plaques of epidermal hyperplasia seen in young fish, causing no problems, and soon clearing up. Seen in August/ September in fresh or saltwater. Malpighian cells proliferate but there is no or little dermal pegging.

f) Brown bullhead papillomas. Virus-like particles seen within papillomas in N. America.

g) White sucker papillomas. In Great Lakes, these lesions are common on lips and elsewhere on body surface. May be plaque-like epidermal hyperplasia or true papillomas. Some may invade basal lamina → squamous cell carcinomas. Retroviral association has been suggested.

h) Sea-bream papilloma. Virus-like particles seen in gilthead sea bream in Spanish waters with epidermal papillomas.

i) Perch papilloma. Crystalline arrays of virus-like particles associated with perch in Scottish waters.

j) Ulcerative dermal necrosis of Atlantic salmon. Cause of this disease, which has been of major economic importance in the past, is unknown. A viral etiology has been suggested (as has virtually everything else) but only a few virus-like particles have ever been seen associated with the often severe dermal lesions which rapidly become secondarily (?) infected with fungi and bacteria. Some work seems to suggest that fungi may indeed be the primary agent.

k) Adenovirus-like disease of cultured sturgeon. Affected mucosal lining of intestine and spiral valve in cultured fish in California. Affected cells had enlarged nuclei, often 5 times larger than normal, with adenovirus-like particles inside. The disease was transmitted to young fish, but the virus was not isolated.

l) Winter flounder papilloma. Papova-like virus seen in papillomatosis type lesions.

m) Adenovirus-like I/N particles seen in dabs, associated with epidermal hyperplasia.

34. Chlamydia/Rickettsia

Intermediate between viruses and bacteria. "Epitheliocystis" or "mucophilosis" only example in fish. Originally described by Plehn (as was so much else!) in 1920 from carp, and considered to be an alga. Affects a variety of fish both freshwater and marine causing usually benign infections of epithelium (often gill). The enlarged epithelial cells (mucus and chloride cells may be infected also) contain a large number of small 1 µm ovoid particles. If very extensive hyperplasia and possibly death may occur, especially during winter in carp. Exact classification uncertain, and although probably chlamydia, there are some differences from other species. It is possible that there are species-specific strains of organisms, and cross-infection between species of fish may therefore be unlikely.

PROTOZOAN DISEASES

These are extremely important causes of loss in wild, cultured and ornamental species. "New" diseases appear as more species of fish cultured. They will be covered from a system viewpoint.

SKIN AND GILLS

a. ***Ichthyophthirius multifiliis*** (syn. "Ich" or "white spot").

A holotrichous ciliate (uniformly covered with cilia) commonly found in freshwater fish, farmed, wild, temperate and tropical species. *Cryptocaryon irritans* is the marine equivalent, although it is a smaller parasite. Disease can reach epizootic proportions in marine aquaria. Ich. is very large, often up to 1 mm in diameter and can be seen with naked eye. It is essentially spherical and has a characteristic horseshoe-shaped macronucleus seen on scrapings of affected tissue, plus a dark brown granular cytoplasm on phase contrast. The juvenile stage or tomite (20 µm in diameter) invades the epidermis → trophozoite and grows within it. When mature, it breaks out and encysts in the substrate, dividing internally to produce up to 1000 tomites which then hunt for new hosts. The time this cycle takes is a temperature dependent process.

Pathology. It is important to appreciate that this parasite is within the epithelium of skin or gills and that emergence therefore causes a break in the osmotic integrity of the tissue. This can be especially damaging if large numbers of parasites emerge at the same time, and can kill the fish. Gills and skin are common sites, but eyes, nares and mouth are all susceptible. The parasites penetrate epithelium usually down to the basal lamina, although they may be prevented from doing so in some cases (immunity). The severity of the host response depends on whether the basement membrane is reached: if yes, response is minimal but if no, epithelial proliferation, necrosis, and inflammation occur. Occasionally parasites may penetrate to meninges of brain. Severe infections may result in dermo-epidermal separation. Affected fish show irritation and flick themselves off the sides of tanks, ponds ("flashing" - a new connotation!) or even jump out of the water. Lymphopenia and neutrophilia with a left shift, are systemic responses reported in carp. Increased mucus production → foaming of water surface, is a frequent accompaniment to epithelial irritation of any cause.

b. ***Chilodonella*** (*Brooklynella* is marine equivalent).

Sometimes confused with Ich. as it is found in similar location, but although this parasite is also a holotrichous ciliate, it is smaller (approx. 70 µm long), flattened and heart-shaped. Thus in scrapings or sections, you will see it turning over, or fail to see a circular shape respectively. There are 2 major species (others are described) differentiated on the number of rows of cilia, which are present on ventral surface only:

C. cyprini. A cold water species (5-10°C) with 8-15 rows cilia and found on skin and gills.

C. hexasticha. Warmer water species (optimum ± 22°C) with 5-10 rows cilia, and possibly favours gills alone.

Pathology. *C. cyprini* causes most problems in overwintering carp in eastern Europe. Unlike Ich., this parasite is not found within tissues, but browses over and feeds from them, by means of an extrudable pharynx. This causes hyperplasia and mucous metaplasia of skin, and in the gills, fusion and hyperplasia of lamellae. Inflammatory responses include an influx of eosinophilic granular cells into the filaments, easily seen in the central venous sinus, plus lymphocytes, plasma cells and macrophages. Dermal lymphocyte numbers also increase.

c. *Tetrahymena* (*Uronema* marine equivalent).
A holotrichous ciliate which on occasions, and with no apparent predisposing cause, can cause severe disease. *T. corlissi* is reported as an epizootic cause of mortality in guppies. Lesions are not specific but the parasite invades most organ systems in large numbers leading to rapid mortality. Muscle swelling is usually the only grossly visible lesion. *T. corlissi* has also been reported as a cause of ulcerative dermatitis of the cranium in young cultured Atlantic salmon.

d. *Epistylis, Heteropolaria, Vorticella, Scyphidia.*
All belong to the subclass Peritrichia, and are common ectocommensals. These peritrichous ciliates are occasionally pathogenic. They are attached to the host by means of a holdfast organelle (scopula) and some have stalks. Also found parasitizing eggs, as well as gills and skin. *Heteropolaria colisarum* is associated with "red-sore disease" in North American fish in association with *A. hydrophila*. Lesions are multifocal ulcerative dermatitis. Such parasites may be present at a variety of temperatures in both fresh and saltwater, and their numbers may be large enough to cause a grey mat on the epithelial surface.

e. *Trichodina, Trichodinella, Tripartiella, Paratrichodina.*
Shaped like flying-saucers, these peritrichs are not anchored, but move freely over the body surface. In stressed fish, numbers may build up and cause substantial damage usually to the gills where they are mainly located. Some spp. may be found in the urinary tract, including the urinary bladder. The adhesive disc can cause direct damage to branchial epithelium resulting in hyperplasia, fusion and metaplasia of lamellae.

f. *Ichthyobodo necator* (formerly *Costia necatrix*).
This, or probably a closely related species, found in saltwater, but essentially it is a freshwater parasite. It is a small, bean-shaped dorso-ventrally flattened flagellate, which becomes pyriform when attached to host. It is approximately 10-15 µm long i.e. approximately the same size and shape as an erythrocyte, and it is easily missed therefore in sections. Its jerky spiralling motion on wet smears however, is very characteristic. It is found on skin and gills, often in large numbers, attached by means of a cytostome which is injected into host cell rather like a hypodermic needle. The irritation causes increased mucus production, then hyperplasia with ↑ goblet cell numbers → grey sheen over the surface of the fish. Then spongiosis and dermo-epidermal separation with sloughing are seen. Favoured sites include the skin immediately beneath the operculum and round the dorsal fin. Opercular colonization may be first with subsequent spread elsewhere. It causes severe hyperplasia and fusion of gills. Mortality can be high in caged salmonids (up to 40%). Gradual acclimation of salmonids to seawater may allow adaption of parasite.

g. Dinoflagellates. (*Amyloodinium, Crepidoodinium, Ichthyodinium*).
Parasites of freshwater and marine fish, they are also responsible for "red-tides". The life cycle involves a free-swimming dinospore which moves by means of 2 flagellae. It attaches to the host and transforms to a sac-like trophont which has elaborate attachment mechanism. It feeds and grows and detaches from host, sinks to substrate where it encysts to form palmella which produces dinospores. Some trophonts have chloroplasts. *A. ocellatum* is the cause of "velvet disease" which infects mainly gills, although skin too in heavy infections. See hyperplasia, branchitis plus necrosis. Invasion beneath epithelium → disseminated is reported by some, denied by others. Infects many species of fish. A major problem in private and public marine aquaria, and marine fish farms too. It is difficult to control - $CuSO_4$ at 0.5 mg, ionic Cu/l, or UV. 25°C is optimum temperature for growth. *C. cyprinodontum* trophont

opposes itself to host cell surface, rather than penetrating it. This organism has chloroplasts and produces less damage than *A. ocellatum*. *I. chabelardi* parasites ova and larval egg sacs of sardines. "Red-tides" are produced by blooms of non-parasitic dinoflagellates such as *Gymnodinium breve*. As well as consuming oxygen, some spp. produce toxins. Other algae are occasional parasites of fish skin.

h. Amebae.
Occasional outbreaks of proliferative gill disease are associated with protozoa with ameboid features. "Nodular gill disease" is one such condition affecting salmonids and other species in Ontario. The lesions are very severe epithelial proliferations often obliterating entire arches while adjacent ones remain absolutely normal. The parasites, identified by one worker as members of family Cochliopodiidae, form syncytial-like lining, covering the lesions. Fish do not stop feeding, but die in smaller numbers than BGD over a longer period of time. Treatment is very difficult - formalin at high concentrations for extended periods. Other species identified as *Thecamoeba* occasionally described from other species, in both freshwater and marine environments (Australia and west coast of N. America). Systemic infections are seen; one notable example is an infection of gouramis - all organ systems are involved, and there is moderate necrosis. These types of infections can be difficult to separate from pre-spore myxosporean infections on purely morphological grounds. Response to treatment may help, as the ameba should respond quite well.

BLOOD PARASITES

a. Trypanosomes.
With typical trypanosome morphology, these parasites are common in fresh and saltwater species. May not be strictly host specific. Transmitted by leeches, in the gut of which they undergo development to produce small round amastigotes without a flagellum. These reproduce enormously. The amastigotes develop eventually into trypomastigotes which are then injected into a new host, when the leech next feeds. In the bloodstream of the fish, the trypanosomes may again change shape from small to large forms, the latter indicating a chronic infection, the former an acute one. Originally considered to be non-pathogenic, but now known to be capable of causing anemia, with damage to hemopoietic organs and death under experimental conditions (eg. *T. danilewskyi* in goldfish). There is often a seasonal incidence in infection - associated with temperature dependency of host immune response?

b. *Trypanoplasma* and *Cryptobia*.
These are bi-flagellates, but otherwise resemble trypanosomes. Until recently, these two genera were used interchangeably but *Trypanoplasma* have a better developed undulating membrane and they need a leech to complete their life cycle in the gut, as with *Trypanosoma*. Very recent evidence however, has shown that in some species (eg. *Trypanoplasma salmositica*) a leech vector is not needed. Thus the taxonomy of the 2 genera is up in the air again and it is thought by some that there may indeed by no difference.

Trypanoplasms cause disease themselves, and also immunosuppress their hosts (probably only the blood forms?). *Cryptobia* are found on skin, gills and GI tract. Some evidence that *Cryptobia* may be capable of invading stomach of cichlids → submucosal granulomas, and granulomas elsewhere, and possibly in combination with other pathogens → "cichlid bloat". *Trypanoplasma (Cryptobia) salmositica* is a cause of extensive losses in hatcheries in British Columbia and is endemic in the wild fish populations. Lesions include anemia, exophthalmia, multifocal necrosis in spleen, kidney and liver, and arteritis. *Cryptobia branchialis* reported by some to be highly damaging to cyprinid gills in China and elsewhere, despite an apparent lack of invasion of epithelial cytoplasm by attachment machinery.

c. Piroplasma.
Typically they are parasites of erythrocytes and the RES. Examples include *Haemohormidium, Haematractidium and Babesioma*. Little is known about life cycle or pathogenicity, but they probably increase red cell fragility → lysis

d. Haemogregarines.
These are apicomplexan protozoa within the subclass Coccidia. The life cycle is characterized by proliferation (merogony) in blood cells of host, initially leucocytes and then rbc's. Sexual development probably takes place in a vector such as a leech. Life cycles incompletely known. *Haemogregarina sachai* in farmed turbot (marine) produced severe multifocal nodular granulomas and anemia with a marked monocytosis comprised mainly of immature cells many of which contained parasites. Some species eg. *H. bigemina*, are not host specific.

GASTRO-INTESTINAL TRACT

a. Hexamitids (*Hexamita, Spironucleus, Octomitus).*
These pyriform flagellates are common inhabitants of GI tract, and occasionally are associated with severe disease, mainly so far in freshwater species. Life cycles are poorly understood. Cyst forms may be present, but probably not within mucosa, rather within lumen. Round forms also found in lumen - on whole mount, this is how the parasites appear.
H. salmonis infects pyloric caeca and intestine of young farmed salmonids and is associated with severe disease and often heavy mortality. Grossly fish have exophthalmia and distended abdomen: they are lethargic, inappetant and spiral in water (most young sick salmonids do this!). Proteinaceous casts are frequently seen, often accumulating on the outflow screens, or trailing from the fish. Whole mounts are crucial to a diagnosis, as they can easily "disappear" in fixed tissue. Parasites closely apposed to brush border elicit little more than "blebbing" of mucosa plus an increased number of apoptotic bodies (interpreted by early workers as cyst forms?). Multifocal hepatic necrosis also present, but in the absence of parasites.

Spironucleus (possibly *Hexamita*) is a common cause of disease in aquarium species. In Siamese fighting fish, it has been associated with necrotic and ulcerative gastritis, the parasites invading blood vessels and disseminating to other tissues such as the kidney. The granulomatous peritonitis which results from gastric perforation is inevitably compounded by the bacterial contaminants. In angel fish, *Spironucleus* is often found, as an incidental, but together with *Capillaria* nematodes, may cause severe enteritis. In oscars, discus and similar fish, *Spironucleus* is associated with "hole-in-the-head disease". Lesions include severe multifocal ulcerative dermatitis, the cavities extending often deeply into the cranium. The route of access is unknown, but the ability of these flagellates to disseminate within the bloodstream from the GI tract, suggests a hematogenous route as a possibility.

b. Balantidium.
As with mammals, these ciliates are not infrequently found in the posterior intestine, and in carp, *C. ctenopharyngodonis* has been associated with an ulcerative enteritis, although their primary pathogenicity is questioned. They penetrate the epithelium and adhere to cells with cytostomes.

c. Coccidia.
Includes the families Eimeriidae and Cryptosporidiidae. 3 separate phases to life cycle include:

i. massive replication (merogony)
ii. formation of separate gametes, flagellated microgametes and oocyte-like macrogametes, plus their fusion (gamogony)
iii. development of zygote into oocysts with sporocysts containing sporozoites (sporogony)

5 Eimerian genera found in fish:

i. *Eimeria*. Sporocysts have a Stieda body and have a completely intracellular life cycle.
ii. *Epieimeria*. Sporocysts similar to *Eimeria* but are epicellular (eg. within brush border of gut) schizogony and merogony.
iii. *Gaussia*. Sporocysts wall in 2 halves.
iv. *Crystallospora*. Crystal-shaped sporocyst.
v. *Calyptospora*. Sporocyst wall has knobby projections covered by a thin veil.

All oocysts have 4 sporocysts each with 2 sporozoites, and unlike mammals, the oocyst wall is fragile. Sporogony is completed in host, and oocysts are shed sporulated. Many species have extra-intestinal locations, and some species eg. *Calyptospora*, may have an intermediate host such as a crustacean. Intestinal diseases are the same in fish as in birds, although severe fibrinous reactions are not seen. Some infestations are diffuse, others produce "nodular coccidiosis". Extra-intestinal infections are common, and oocyst numbers can be so large as to eventually obliterate spleen, liver and pancreas, which are common locations. In some instances, pronounced granulomas are found, but in others, inflammation is limited. *Calyptospora funduli* infects the estuarine killifish in the southern USA and its effects on liver and pancreas can be so severe as to affect population numbers. Monensin administered orally controlled parasite numbers. The swimbladder is a favoured extra-intestinal site in some species. Heavy infections impair buoyancy and may be fatal. There is only one report of *Cryptosporidium* in fish (a tropical marine species) but it was associated with disease. (Thus Cryptosporidia reported in all vertebrates except amphibia).

SYSTEMIC INFECTIONS

a. Myxosporea.

A huge group of parasites infecting mainly fish although some are found in amphibia, reptiles and invertebrates. Major features include the presence of polar capsules within spores, and endogenous cell division (endodyogeny or internal budding) so that mother cell contains daughter cell. Parasites are usually inter-cellular, although a few species have some stages within cells, while a few more are intracellular within myocytes. Spores are very characteristic, and their morphology is the basis for taxonomy, as relatively little is known about life cycles. They comprise a shell which is refractile in H&E sections with 2-6 valves which are joined by suture lines. The shell may have ridges or projections leading to distinctive shapes, or a mucus coat which aids in flotation. Spores in sections are best demonstrated with Giemsa or toluidine blue - polar capsules stain intensely. "Best guess" at life cycle is as follows:

Spores are ingested and coiled polar filaments are "shot" out from the polar capsules, the valves of the shell break open to release the ameboid sporoplasm which penetrates gut epithelium into b.s. or l.s. Sexual process now involves fusion of the 2 nuclei to form a trophozoite. This then proliferates by internal budding to form many parasites before sporogony begins or a single large plasmodium is formed containing generative cells in pairs, one of which "envelopes" the other (envelope and generative cell) to produce a sporoblast. These undergo maturity to form typical spores within a pansporoblast, different cells forming valves and polar capsules (valvogenic and capsulogenic).

Pre-spore stages probably found in sites other than final resting place of typical spores, and in some cases are responsible for severe disease eg. proliferative kidney disease, swimbladder inflammation. Gills and skin are other sites - suggests other than gut infection? Some pre-spore stages may be found in bloodstream. An intermediate host (the oligochaete worm *Tubifex*) is reported to be an essential part of the life-cycle for *Myxosoma cerebralis*, the cause of whirling disease. Previously it was known only that a degree of "maturation" of the spores in earthen bottoms of ponds was necessary, in order to "activate" the spores. Whether an intermediate host is important for other myxosporean diseases remains to be determined. Even the origin of the plasmodium is questioned by some who consider the possibility that it may be a host cell eg. macrophage.

Parasites are of 2 basic types - histozoic within tissue, or coelozoic within cavities and lumena eg. urinary tract or bile ducts. Histozoic species are typically much more damaging. Typical locations include muscle, often causing serious economic loss to commercially important wild species. Examples include various species of Kudoa, important in marine species such as herring, hake, blue whiting. Not only are the muscle fibres themselves replaced by spores, but when the fish are killed, some parasites are thought to release proteolytic enzymes which → liquefaction of even uninfected muscle. Inflammatory responses to early parasitic stages are few. Some spore aggregates ("cysts") may be

superficial and → ulceration, with liberation of spores. Other histozoic species found in brain, cartilage, round heart, involving viscera eg. *Henneguya*. Spinal curvatures seen in Australia in redfin perch associated with the histozoic myxosporean *Triangula percae.* Examples of important myxosporean diseases in farmed fish include: whirling disease, ceratomyxosis, proliferative kidney disease and swimbladder inflammation .

i. **whirling disease** - *Myxosoma cerebralis*. A virtually worldwide disease of young salmonids (except Ireland?). Cartilage of head and spine invaded by parasites → labyrinth involvement and "whirling" plus spinal deformities. Can cause heavy mortality. Initial lysis → granulomatous inflammation. Older fish i.e. > 6 months, are less susceptible due to ossification of cartilage. They can become infected, but do not develop disease. Intermediate host is *Tubifex* which is required for some development of spores. Hence raising fish in concrete ponds until older than 6 months works as a control. Intermediate stage of *Tubifex* originally regarded as a separate parasite - *Triactinomyxon*. Very controversial. Diagnosis is based on presence of typical spores within cranial cartilage. May need special digest techniques of heads and differential centrifugation plus staining to demonstrate. FAT also feasible. Ultra-violet treatment of inflow water does control infective stage, but usually impracticable.

ii. **Ceratomyxosis** - *Ceratomyxa shasta*.
Geographically restricted infection of young salmonids. Mainly found in Columbia River basin of western USA but also British Columbia (in chum salmon). Causes mortality in both farmed and wild fish populations. The parasite multiplies in many tissues causing multifocal granulomas in spleen, liver, kidney, muscle and intestine leading to abdominal distension due to an increase in fluid which contains the spores. *C. shasta* is the only freshwater histozoic species of the genus: all others are marine and coelozoic. Strains of chinook salmon from the Columbia River basin show a low prevalence of the disease, while "exotic" strains of chinook are highly susceptible (compare this with PKD - see later). The geographical restriction again suggests the possibility of an intermediate host.

iii. **Proliferative kidney disease** - cause unknown, but probably a pre-spore stage of a *Sphaerospora*-like myxosporean. A major disease of most salmonids, particularly in Europe although also N. America. It is a temperature-related disease, and lower than approximately 15°C inhibits development of clinical disease. This fact forms the basis of control measures i.e. allow exposure to the infectious agent at a time just prior to falling water temperatures. Fish the following season seem mainly immune. Mortality may easily reach 50% in summer months - July and August in Europe are the peaks. Only fish not previously exposed are susceptible - "O+" fish. Gross symptoms include swollen abdomens, exophthalmia and anemia. Appetite remains high right to the end. Gross lesions include an often massively swollen kidney with grey bulbous ridges involving anterior and posterior. Spleen is also frequently involved. The reaction in all tissues is a granulomatous one, centered round the typical "mother parasites" which contain daughters. Macrophages and lymphocytes surround the spherical "mother parasite" which has an eosinophilic stain and is roughly 20 μm in diameter. Clusters may often be seen apposed to blood vessel walls. Early depletion of melano-macrophages within an affected kidney is typical of renal granulomatous responses, but very useful in this case where the parasites may be very hard to find. The interstitial tissue proliferates - esp. neutrophil series. Granulomatous foci wherever parasites found. Tissues involved are all, except nervous tissue. The parasites have a typical appearance ultrastructurally with small dense spherical 100 nm diameter bodies (some with tails - Golgi or RER derived) within the cytoplasm of the mother cell, never the daughters. These aggregate under the plasmalemma, and have an electron lucent bar oriented at right angles to it - significance or function unknown. Recovering fish in N. America see *Sphaerospora*-like stages within renal tubules. Not a feature of European fish. Nevertheless, brown trout from a river supplying water to a badly affected rainbow trout farm did have *Sphaerospora*-like parasites within renal tubules supporting the idea that the disease may be due to the pre-spore stage of this myxosporean parasite.
There are several possible approaches to control:

a. **genetic selection**. Avoid importing "naive" strains of fish. It is well known from practical experience that import of a strain from another country may lead to heavy mortality in the imported stock despite zero mortality or even evidence of infection in indigenous fish.
b. **temperature control**. Usually impracticable, but it is unlikely that PKD will develop into a severe clinical disease at temperatures < 15°C. If disease does develop, a decline in temperature (naturally or artificially) will speed recovery. Recovery will occur however, regardless of temperature.

c. **management stocking**. As mentioned earlier, once it is known that a water source is infected, stocking to utilize "natural vaccination" just prior to declining water temperatures.
d. **ultra-violet treatment of inflow water**. As with whirling disease, there is some evidence to suggest that the infectious stage is susceptible to u.v. treatment.
e. **vaccination**. Not available yet, although the solid immunity produced in recovered fish suggests that it is feasible, if sufficient ag. were available.
f. once fish are infected, reduce feeding to a minimum and supply best quality water available. Anemia is likely cause of death.
g. malachite green has been shown to reduce the incidence and severity of the disease. Presumably this indicates a reduction in the infective dose which the fish receives.

iv. **Swimbladder inflammation of carp** - cause unknown, but again probably a pre-spore stage of a *Sphaerospora*-like myxosporean (*S. angulata*). Once considered to be caused by a rhabdovirus indistinguishable from that causing spring viremia of carp (SVC). Recent evidence suggests that the severe hemorrhage and necrosis of epithelial lining is at least in some cases, due to pre-spore myxosporeans. Blood stages of the parasite seen in blood whole mounts - active movement. Mainly a disease of young cyprinids in eastern Europe in their first or second summer, in the first half of July (same as PKD). Along with swimbladder inflammation (anterior sac first) swelling of kidneys and peritonitis are seen. The renal changes correlate with the appearance of the early stages of *S. angulata*. In these cases, the disease is unassociated with SVC virus or any bacteria.

b. <u>Microspora</u>.
Strictly intra-cellular parasites of many groups of animals including fish. *Nosema (Encephalitozoon)* is example from mammals. *N. bombyas* is cause of silk-worm disease. Spores in sections are refractile (as with myxosporea) but they are usually much smaller - roughly 2 μm as opposed to 10 μm of myxosporea. They are Gm +ve. They appear to have a central "girdle" in sections - this in fact represents the coiled polar filament. The polar filament is injected into the host and the sporoplasm inoculated (with nucleus) through it into the cell. It then undergoes schizogony, some of the schizonts undergoing surface changes and cytoplasmic organization → sporonts.
<u>Sporogony</u>. Sporonts can do 1 of 2 things:-

1. Pansporoblast develops in which sporont divides into sporoblasts with a persisting surface membrane.
2. A pansporoblastic development in which entire sporont splits into sporoblasts.

Pleistophora is an example of 1). The sporont → a multinucleate plasmodium and additional layers are added to the plasmalemma. The plasmodium splits into numerous sporoblasts while the additional layers persist as a pansporoblastic membrane. The number of sporoblasts is characteristic for different genera. The surface membrane may invaginate to divide up the pansporoblast. *Glugea* is an example of 2). After thickening of sporont membranes, the whole sporont divides by binary fission → 2 sporoblasts. In some genera, the sporonts may lie in close association with host cell cytoplasm, or in others be isolated by a host cell derived membrane. *Encephalitozoon* has a disporoblastic sporogony similar to *Glugea*. The sporoblasts mature into spores.
Pleistophora hyphessobryconis is the cause of "neon tetra disease", affecting neons plus other tetras. The pre-spore and spore stages are found within muscle fibres often replacing large numbers of them. Inflammatory response is very minimal and develops only when the cell ruptures, macrophages

removing the liberated spores. Other *Pleistophora* spp. cause similar lesions in muscles of commercially important species eg. "Beko disease" in yellowtail.
Glugea anomala in sticklebacks however, induces marked cytomegaly of host cell which develops into a xenoma with a thick laminated wall, a peripheral layer of cytoplasm containing schizonts and strands of host cell nucleus, a middle layer containing sporonts, and a centre of the "cyst" packed with spores. The enlarged cell induces pressure atrophy of surrounding cells and fibroplasia; the xenoma wall often degenerates with eventual disappearance. The remaining granuloma with ingested spores resolves over time. *Glugea hertwigi* causes epizootic disease (often 100% infection) in smelt and salmonids. It attacks gut and all other organs causing large numbers of cysts (up to 9 mm diameter) which exert a space occupying effect and can → parasitic castration. Losses in Canada are often high. *Spraguea* (syn. *Nosema*) *lophii* infects ganglia of the angler fish, but despite appearances and location, exerts little detrimental effect. Spores may be dimorphic in this species. *Loma* causes cyst formation in the gills of Pacific Northwest salmonids, but also vasculitis as a systemic disease - the precise mplications of this are not fully understood, but they do cause mortality.

Fumagillin (the antibiotic is from the fungus *Aspergillus fumigatus*) has been used to successfully treat infected fish. Early results show that it may also be effective against myxosporeans.

METAZOAN PARASITES OF FISH

A. Freshwater

Phylum: Platyhelminthes

Class: MONOGENEA

Almost all monogeneans are ectoparasites on the skin, gills or fins of fish. They feed on mucus, epithelium and sometimes blood. Most species are very host specific. In freshwater fish the most common and important genera belong to the orders Gyrodactylida and Dactylogyrida. These groups possess a simple undivided haptor without paired suckers or clamps on the haptor. These two groups, most notably the two families, Gyrodactylidae and Dactylogyridae are the most important monogenean pathogens of fish both in culture and in the wild. Species from another family, Ancyrocephalidae (O. Dactylogyridae) are commonly found on gills of centrarchids, and to a lesser extent cyprinids and ictalurids. Nothing has been reported regarding their pathogenicity.
The gyrodactylids include the genera *Gyrodactylus* (fw,bw,m) and *Gyrodactyloides* (m) which are of significance in all types of fish culture. They are small worms about 0.3-1.0 mm in length found on the skin, gills and fins of fish (cf. *Dactylogyrus*). Most species are extremely host specific and may even be specific to a certain site on the host.

The most significant feature of gyrodactylids is that they are viviparous which enables them to reproduce extremely quickly under favourable conditions. The occurrence of gyrodactylids in epizootic proportions in a fish population is generally a symptom of poor water quality for the fish. Gyrodactylids are often found in conjunction with one or more ectoparasitic protozoan species,and often carry bacteria on their surface, giving rise therefore to secondary bacterial infections. Heavily infected fish may have a bluish film of mucus on the skin, the fins become frayed, the skin ulcerated and the gills damaged. The fish is damaged by feeding activity (anterior end) and by the attachment process (hooks at the posterior end). *Gyrodactylus salaris* in Atlantic salmon is a controlled and reportable parasite in European marine cage

culture operations. Control of gyrodactylosis can be achieved in the short term by formalin baths and in the long term by improving husbandry conditions. Organo-phosphates are also used.

The dactylogyrids include the genera *Dactylogyrus, Pseudodactylogyrus, Neodactylogyrus, Cichlidogyrus and Cleidodiscus.* Although superficially rather similar to the gyrodactylids they are oviparous and a little larger, up to 2 mm in length. They are usually found on the gills and are of major significance in Europe in the farming of cyprinids, especially carp. The life cycle is simple and direct. Eggs laid by the adult worm embryonate and hatch in a period which varies greatly with temperature. Optimum temperatures vary depending on species. This is significant since in some species eggs laid in the fall may over-winter and hatch upon the return of more favourable conditions in the spring. At this time newly hatched fry of the host species are available for the parasite to invade. When the egg hatches, a free swimming ciliated larva (oncomiracidium) is released. This survives from a few hours to a few days, again depending on temperature and species. Once attached to a newly found host, development to the adult stage takes only a few days.

Dactylogyrids can be very damaging, especially to very young fry where relatively few parasites can cause great harm. Severe gill destruction can result from the activities of the parasites resulting in haemorrhage and metaplasia of gill tissue. Secondary infection may also occur. *Dactylogyrus* has caused very severe mortalities of young carp. The most dangerous species involved is *D. vastator* which affects fry of 2 to 5 cm in length during their first summer. It has a relatively high optimal temperature (20-25°C) and is therefore more important in warmer countries. Reported from goldfish in California and southern Ontario. *D. extensus* from Europe and recently from southern Ontario is another serious parasite of carp, affecting both young and older fish. It has an optimum temperature of 16-17° and thus has a more northernly distribution the *D. vastator.* Recently, pseudodactylogyrosis in a freshwater eel culture in Denmark was reported. In this case temperatures were approximately 25°. *D. puntii* and D. *cyprinii* are recorded from many fish species in S. E. Asia. *Cichlidogyrus sclerosus* was recently reported (previously found only in Africa and the Middle East) from the Philippines causing gill damage in tilapia. Control of dactylogyrids is usually by dips or baths of chemicals such as formalin, or organophosphates (Dylox, Dipterex, Neguvon, Chlorophos), Bromex-50 and potassium permanganate. The use of (or rather the abuse of) these chemicals in marine fish-cages has recently been a rallying point for environmentally-aware groups, forcing the serious consideration of alternative methods of control such as cleaner-wrasse, or pyrethrums in oil which is floated on the surface of the water so that the fish only come into contact with it when they breach the surface (these latter measures apply particularly to the salmon louse). Husbandry measures can also be utilized such as separating fry from older fish which might act as carriers. In the case of *D. vastator* a good growth rate of the fry helps ensure that the parasite never reaches epizootic proportions.

CLASS DIGENEA

Digeneans are found as both adult and larval (metacercarial) stages in fish. As adults they are mainly confined to the gut of the host but as metacercariae they can be found in most parts of the body (depending on species). Metacercariae are usually much more dangerous to the host than the adults. The life cycles of fish digeneans are extremely varied and may take one of several possible courses.

Adult Digenea

O. Strigeatida
Sup. F. Schistosomatoidea
F. Sanguinicolidae - *Sanguinicola* spp.
These are small spindle-shaped worms, 1-2 mm in length, which live in the vascular system of the fish host. They are important parasites in the culture of many fish species world-wide including: (1) carp in Europe and the USSR (2) salmonids on the west coast of North America and (3) some marine fish

cultured near the coast of Japan. The life cycle of these parasites is simple, involving only a molluscan intermediate host. In carp an acute and chronic infection from *S. inermis* is recognized. In 0+ and 1+ fish the acute disease manifests itself by infarction of the gills due to blockage of capillaries by fluke eggs and subsequent necrosis of gill tissue. The eggs do not lodge in the gills of older fish but are carried to the kidneys where they cause renal dysfunction resulting in a chronic condition with ascites, exophthalmos and erection of scales. *S. fontinalis* and *S. klamathensis* have resulted in heavy mortalities of salmonids in N. American hatcheries. Severe haemorrhage occurred when the eggs hatched and the miracidia burst out of the gills. Kidney damage has also been observed. We see pancreatic damamge in white suckers in the Great Lakes associated with sanguinicolids blocking mesenteric blood vessels.

Sub. O. Allocreadiata
Sup. F. Allocreadioidea
F. Allocreadiidae - *Crepidostomum* spp.
This is a common parasite of many freshwater fish, particularly in rivers, throughout the northern hemisphere. The life cycle involves sphaerid clams or the gastropod *Lymnea pereger* as the first intermediate host and mayfly nymphs are the usual second intermediate host. Gammarids, crayfish, caddisflies and cladocerans are also used as second intermediate hosts by members of this genus and by other genera within the family. This parasite has been known to cause inflammation in the gut of trout in N. America following heavy infection (few hundred parasites). Other commonly encountered members of this family include *Bunoderina, Bunodera* and *Allocreadium*.

There are hundreds more species from over a dozen more families of adult digeneans found in fishes. The life cycles of many representative genera or species from these families have been at least partially elucidated but the pathogenicity of the vast majority of these groups is still largely unknown. The following is a list of some of the more common **adult** digeneans found in N. American fishes:
O. Strigeatida
Sup. F. Bucephaloidea
F. Bucephalidae *Bucephalus* (fw), *Prosorhynchoides* (m)
Sub. O. Hemiurata
Sup. F. Hemiuroidea
F. Hemiuridae *Brachyphallus* (m), *Derogenes* (m), *Lecithaster* (m)
Sup. F. Didymozooidea
F. Didymozooidae (m)
Sub. O. Plagiorchiata
Sup. F. Plagiorchioidea
F. Macroderoididae *Macroderoides* (fw)
Sub. O. Opecoelata
Sup. F. Opecoeloidea
F. Opecoelidae *Nezpercella* (fw), *Plagioporus* (fw), *Podocotyle* (m)
O. Opisthorchiida
Sub. O. Opisthorchiata
Sup. F. Opisthorchioidea
F. Cryptogonimidae *Centrovarium* (fw), *Cryptogonimus* (fw), *Caecincola* (fw)

Larval Digeneans

By far, the most important group of larval digeneans invading fish are the metacercariae of members of the superfamily Strigeoidea. The life cycle involves gastropod molluscs as first intermediate host, and fish as second intermediate hosts. Piscivorous birds and mammals are the final hosts. A considerable number of genera from several families are involved including:

Diplostomum, Diplostomulum, Posthodiplostomum, Cotylurus, Ichthyocotylurus, Uvulifer, Apatemon, Tylodelphys, Neascus and *Tetracotyle.*

Taxonomically this is a confusing group constantly under revision. It is very hard to distinguish metacercarial stages beyond the generic level. Almost all parts of the fish can be invaded by strigeoids, although site and host-specificity varies greatly between parasite species. The metacercariae may or may not be encapsulated, and may or may not result in melanin deposition.

F. Diplostomidae

One of the most important species is *Diplostomum spathaceum* (syn.= *D. flexicaudatum* in N. America), the "eye fluke". This parasite is found in many species of freshwater fish, including cyprinids and rainbow trout. The first intermediate hosts are *Lymnea* spp. and the final hosts are primarily gulls and terns. The metacercariae are found in the outer part (cortex) of the fish lens and if present in large enough numbers can cause opacity of the lens and blindness. In extreme cases the outer part of the lens may be completely destroyed, and occasionally will rupture causing a very severe inflammatory response. Infected fish are not usually killed but visual impairment does interfere with their growth rate. Infected fish in farms are very wasteful of food and will feed only off the bottom. In a "put and take" trout fishery blind fish will not take a fly, thus fishing is ruined. Blind fish will be more susceptible to predation. Cercariae are released from the molluscan host in water temperatures above about 10° and thus infection of fish occurs from late spring to early autumn. Brown trout are less susceptible. The cercariae penetrate the skin and migrate to the lens within a remarkably short space of time (15 minutes). Maturation to the metacercarial stage is dependent upon the parasites reaching this location. Other spp. metacercariae may be found within different sites in the eye in the same fish. Other species of *Diplostomum* in N. America include *D. gasterostei, D. scudderi* and *D. adamsi.* All of these species infect the retinal layer.

D. scudderi (= *Diplostomulum baeri eucaliae*) is also known to infect the brain of the brook stickleback causing a tumor-like lesion, and leading to the fish altering its position within the water column, and hence leading to easier predation. In Europe, *Posthodiplostomum cuticola* encapsulates in the skin of cyprinids causing an unsightly "blackspot" condition. *P. minimum* (white grub) is a N. American species that is found thinly encapsulated throughout the viscera of centrarchid and cyprinid fish. It can cause death of the host in experimental conditions. A number of species of *Cotylurus* and *Ichthyocotylurus* are found, sometimes in very large numbers, encapsulated in the abdominal and pericardial cavities of many fish species. Usually they appear to cause little harm although *I. erraticus* has been known to cause severe loss of condition in wild populations of coregonids in the U.S.S.R. There is some information that cardiac output can be reduced as a result of impaired stroke volume due to the restricted epicardium.

O. Azygiida

Sub. O. Troglotremata

Sup. F. Troglotrematoidea

F. Nanophyetidae - *Nanophyetus salmincola*

This parasite is important because of its role in the transmission of the rickettsial organism *Neorickettsia helmintheca*, the microbe which causes "salmon poisoning" in dogs and other canids after eating salmon infected with the metacercariae. Metacercariae encapsulate in the fish intermediate host, primarily salmonids.

Sup. F. Opisthorchioidea

F. Heterophyidae

Members of this family are found as adults in mammals and birds. Two commonly reported genera in N. America are *Apophallus* (fw) and *Cryptocotyle* (m). Both *A. brevis* and *C. lingua* cause "black spot" in their respective fish intermediate hosts. *A. brevis* can cause bony cyst formation within the tissues of perch - "sand-grain grub". These bony capsules are found within the axial musculature and the extrinsic

musculature of the eye, and can be used to age the infection as they lay down concentric rings with each season, rather like scales.

O. Strigeatida
Sup. F. Clinostomoidea
F. Clinostomidae - *Clinostomum marginatum* (= *C. complanatum*)
The metacercariae of this N. American species are known as "yellow grub" and are apparently non-specific as they are found in large number of fish species. As adults they are found in the pharyngeal region of herons. The parasite is very unsightly to anglers and, if numerous, can do considerable damage to fish in both hatchery and wild populations. The metacercariae of *Euclinostomum* have been reported to cause mortality in farmed tilapia in Asia. Metacercariae of *Clinostomum complanatum*, also found in Asia, are capable of producing laryngo-tracheitis in man.

The *control* of digenean parasites of fish can be achieved by a number of methods. Parasites in the gut of farmed fish can be controlled by the use of anthelminthics. For those species present as larval stages or adults in sites other than the gut, control is best achieved by breaking the life cycle. Molluscicides such as copper sulphate, Frescon or Bayluscicide can be used in fish farms or small lake or reservoirs. They can be extremely toxic to fish and therefore must be used with extreme care. Applications of molluscicides are best made just before release of cercariae from the molluscs occurs, if this is known. Draining of fish ponds and treating with quicklime can be used to control mulluscan and other invertebrate intermediate hosts. A number of systems have been advocated to remove cercariae from farm water supplies. These include an electrical grid (which has been used successfully against *D. spathaceum*, although it is expensive), an ultra-sonic system (experimental) and U.V. sterilization. In Denmark, trout farmers are obliged to set strings over their ponds to keep away piscivorous birds thus preventing them from trying to catch their fish prey while at the same time defaecating parasite eggs into the water. Destruction of piscivorous birds may also be possible in some cases.

CESTODA

A number of genera of cestodes are economically important parasites of freshwater fish. These cestodes may be found as adults, in which case they occur in the gut of the fish, or larvae in the abdominal cavity or musculature. Due to their habitat in the fish, larval cestodes are, in general more dangerous than adult worms which are usually harmful only to younger fish. Larval cestodes can often have severe effects on wild populations of fish maintained as sport or commercial fisheries.

The life cycles of fish cestodes involve at least one invertebrate host. Those species found as larvae in fish have another vertebrate species in the life cycle acting as the final host. This host is usually a piscivorous bird or fish. Fish cestodes come from 5 main groups (Orders): Caryophyllidea, Pseudophyllidea, Proteocephaloidea, Tetraphyllidea, Trypanorhyncha.

Adult Cestodes

O. Caryophyllidea
The caryophyllaeids are unsegmented (monozoic) worms with a simple scolex and only one set of reproductive organs. The life cycle involves only one intermediate host, a benthic tubificid oligochaete. Larvae, infective to the fish final host take about 2 to 3 months to develop in the intermediate host. Two species are of economic importance in carp farming in Europe, *Caryophyllacens fimbriceps* and *Khawia sinensis*. This latter species has been introduced from Asia. In these species there is a seasonal cycle of occurrence in the fish host, the adult worm living only one year. New infections of fish occur in late

spring and early summer when outbreaks of disease may be found in carp up to 2 years old. Older fish act as carriers.

In N. America, *Glaridacris* and *Hunterella* are two of the more commonly reported genera. Most caryophyllaeids reported in N. America are reported almost exclusively from catastomids. One exception to this rule is *Khawia iowensis*, which to my knowledge has only been reported in carp from N. America.

O. Pseudophyllidea

Bothriocephalus gowkongensis

This is large segmented worm up to 20 cm in length. A parasite of carp, it has been introduced to Europe from Asia. The life cycle involves only one intermediate host, a cyclopoid copepod. Disease occurs in 0+ carp which are still feeding on plankton during their first summer. Heavily infected fish have a swollen abdomen, they become sluggish, emaciated and cease to feed. In both *B. gowkongensis* and caryophyllaeid infections (in carp), fish develop a haemorrhagic enteritis with destruction of the intestinal epithelium.

Cyathocephalus truncatus (syn. = *C. americanus)*

This is a parasite of salmonids and is common in wild fish in the U.K. and N. America. The life cycle involves a gammarid crustacean intermediate host. The scolex of the cestode becomes embedded in the mucosa of the pyloric caeca which eventually become fibrous and thickened.

Eubothrium spp.

Members of this genus are also parasitic in salmonids (mainly). The life cycle may involve only a copepod intermediate host although in some situations an additional fish host, such as a stickleback, may be included. *Eubothrium* is typically a parasite of fish in lakes or reservoirs where there are often large populations of copepod intermediate hosts. Individual cestodes may be very large (> 50 cm), and although their scolices are attached in the pyloric caeca of the fish host, their strobila may stretch for a considerable distance down the intestine. Very large infections may occur so that the intestine appears to be blocked but the effect of the parasites on the fish is difficult to determine. There is no evidence of severe physical damage but Canadian workers have suggested that infected juvenile fish may show slower growth, poor swimming performance and aberrant behavior which, in wild populations might render them more susceptible to predation.

Control of adult cestodes in farmed fish is possible by the use of anthelminthics such as di-n-butyl tin oxide or di butyl tin dilaurate. Alternatively, husbandry methods can be used such as the draining and chemical treatment of ponds to kill the intermediate hosts and parasite eggs, filtration of water supplies to remove intermediate hosts and separation of young susceptible fish from older carrier individuals when appropriate.

Larval Cestodes

O. Pseudophyllidea

F. Ligulidae

The ligulids have a complex life cycle involving copepods as the first intermediate hosts, fish as the second intermediate hosts and piscivorous birds as the final hosts. In the case of *Ligula*, the most widespread genus, grebes are probably the most important avian host. Ligulids are important parasites of cyprinid fish in lakes and reservoirs. The parasites are large fleshy undifferentiated worms which can be 10-20 cm in length, and the total weight of the parasites in a fish can exceed the latter's body weight! The presence of such a large amount of parasite tissue within the host has far reaching effects.

Compression and distortion of the viscera occurs, gonadal maturation is inhibited and associated with this are disturbances in behaviour. Infected fish do not enter spawning shoals, often swim poorly and are more susceptible to predation. In N. America only *L. intestinalis* has been described.

As mentioned previously with regards to larval digeneans, larval helminth parasites in the viscera or musculature of a fish cannot be treated with anthelminthics and thus control is made extremely difficult, especially in wild populations. Destruction of the piscivorous bird final host is one possibility. In smaller waters it might be feasible to remove infected fish which are often easily recognizable by their swollen abdomen.

F. Diphyllobothrium

Diphyllobothrium spp. in fish have a similar life cycle to the ligulids, but are mainly parasites of salmonids and coregonids. An exception to this is *D. latum*, the broad tapeworm of man, of which the plerocercoid occurs in a large variety of fish. Birds and mammals are the final hosts for all species. Various species occur in N. America, including *D. sebago* which can kill trout fingerlings. *D. dentriticum* occurs in the U.K. and has been responsible for serious epizootics in the past. Diphyllobothriid plerocercoids may become encapsulated amongst the viscera and musculature of the fish causing adhesions, sterility and sometimes death. More often however, the worms remain free in the fish coelom or viscera. Because of this and the fact the worm is almost transparent, it can easily be missed and possibly eaten unknowingly. Worms remain viable when the fish they're in is eaten by another fish and in fact this is quite common for diphyllobothriid and ligulid plerocercoids. The parasite is long lived in the fish host which therefore accumulates infection throughout its life. As a result of these facts, the most severe effects are often seen in older fish. Control problems and solutions are similar to the ligulids.

Triaenophorus spp.

Adults of the two main *Triaenophorus* species in N. America (*T. crassus* and *T. nodulosus*) are found primarily in pike (*Esox* spp.) but the plerocercoids are found encapsulated within the viscera and musculature of other fish. *T. nodulosus* occurs in Europe and N. America and the plerocercoids are found predominantly in yellow perch but also in burbot and white sucker. The liver can become swollen and inflamed and death can result. Fry, and 0+ fish, which feed on the copepod first intermediate host, are particularily susceptible. The plerocercoids of *T. crassus*, a N. American species, encapsulate in the musculature of mainly coregonid fishes thus seriously reducing their market value. Control of *Triaenophorus* spp. is sometimes possible by eradication or reduction of the pike final host.

O. Proteocephaloidea

Proteocephalus spp.

Proteocephalids are very common cestode parasites of fish throughout the world. Normally the life cycle involves only a copepod intermediate host and the adult worms do no great harm to the fish final host. In N. America there is a species, *P. ambloplites* - the bass tapeworm, which is found mainly in the small and large mouth bass. A fish intermediate host can be involved in the life cycle (though it is not necessary). When the plerocercoid is eaten by the final fish host (bass) it does not necessarily remain in the gut and mature to the adult stage, but rather undergoes a tissue phase migrating through and damaging the viscera. The gonads especially are affected so that spawning is inhibited. The only possible control measure that can be adopted is to ensure by the use of anthelminthics that bass used for stocking parasite free waters are not harbouring *P. ambloplites* in their guts.

NEMATODA

Although many species of nematodes are found either as adults or larvae in fish, very few have been implicated as serious pathogens of their hosts. The following species are adults or larvae which are

found in tissues and organs apart from the gut. A nematode life cycle involves 4 larval stages (L1, L2, L3 and L4) prior to becoming adult. Each stage is separated by a molt - 4 molts in total.

Sup. F. Dracunculoidea
Members of this super family are found as adults in all classes of vertebrates. One usually only sees the gravid female worms which are considerably larger than their male counterparts. The female worms are viviparous and the intermediate host is a copepod. Most representatives found in fish from N. America are freshwater species and most of these have annual cycles.

Philometra spp.
Members of this genus are found in various anatomic locations in the fish (depending on species) including; skin (especially around fins), serosa of the air bladder, free in the abdominal cavity and, rarely in blood vessels. Several species are reported to be a problem in carp farming in the U.S.S.R. and Europe. The genus also has representatives in many wild fish populations in the northern hemisphere. The mature adult female worms appear thin and red and can get up to 16 cm in length. *P. lusiana* from carp are found in the scale sockets particularly on the anterior part of the body. The adult females protrude the posterior part of their bodies into the water, these then burst releasing their larvae (L1). The latter are ingested by the copepod intermediate host and if this is in turn eaten by a fish the larvae (now L3) penetrate into and migrate around the body cavity, mature, mate and then the females migrate to the scale pockets. Carp fry are killed by the activities of 5-9 larvae in their body cavities. Older fish may develop unsightly skin ulcers and become carriers of the parasites. For this reason strict separation of older fish and fry should be observed as a control measure.

Several species are found in N. American fish including *P. kobuleji* in the abdominal cavity of catastomids and *P. cylindracea* in the abdominal cavity of yellow perch. Adult females from another unidentified species are known to occur in the orbit of freshwater drum.

Philometroides spp.
A commonly reported species from eastern N. America is *P. huronensis* from catostomids (esp. white sucker). Subgravid and gravid female worms are found mainly in the pectoral fins during the spring. Another species, *P. nodulosa* is found in the cheek galleries of catostomids and in the subcutaneous tissues of the head of freshwater drum.

Philonema spp.
The adults of this genus occur in the abdominal cavity of salmonids from N. America and Europe. The intermediate host is again a copepod. *Philonema* cause adhesions of the viscera which in severe cases prevents normal functions, including reproduction. *P. oncorhynchi* is found in *O. nerka* (mainly) and is interesting because the worm develops as the fish matures out at sea (approx. 3 y). The worms only become mature once the fish host has returned to freshwater to spawn.

Sup. F. Ascaridoidea
Raphidascaris acus
Adults are found in the gut of northern pike. The intermediate host is primarily yellow perch but also cyprinids. L4 larvae are found encapsulated in the liver of the fish intermediate hosts. Perch acquire L2 from juvenile aquatic insect paratenic hosts.

Contracaecum spp. and *Hysterothylacium* spp.
Larvae from these genera can be found both encapsulated and unencapsulated within the stomach wall, musculature, viscera and mesenteries of several dozen freshwater fish species in N. America. As adults, *Contracaecum* spp. are found in piscivorous birds and *Hysterothylacium* spp. are found in the gut of piscivorous fish species.

Sup. F. Seuratoidea

Dichelyne cotylophora

Adults are found mainly in the gut of yellow perch. Experimentally it has been shown that a cyprinid can act as the intermediate host whereby L3 larvae are ingested and eventually migrate to the liver where they molt to L4 and become encapsulated.

Truttaedacnitis stelmioides

Adults are found in the gut of brook lamprey. The ammocoetes larvae ingest hatched L1 nematode larvae which then develop to L3 in the ammocoetes' gut during the summer. L3 then migrate to the liver and remain there for up to 4 y (as L3). During transformation (3-4 y later) the L3 molt once then migrate to the gut and mature in the gut of the transformer or adult lamprey.

Sup. F. Habronematoidea

Cystidicola spp. and *Salvelinema* spp.

Adults, which are very long lived, live in the swim bladder of salmonids. Excess mucus and epithelial metaplasia have been reported from the swim bladders of arctic char and lake trout harbouring *C. stigmatura*.

Sup. F. Gnathostomatoidea

This super family uses copepod intermediate hosts (L1-L3) and the adults are found in all vertebrate classes except birds. The use of vertebrate paratenic hosts is very common by members of this group, which includes freshwater fish. *Spiroxys* spp. larvae (L3) (adults in turtles) are found encapsulated within the gut mucosa, viscera and mesenteries of freshwater fish. *Gnathostoma* spp. are of zoonotic importance in Asian countries causing visceral and cutaneous larval migrans and are also found as L3 in freshwater fish.

Sup. F. Dioctophymatoidea

Dioctophyma renale L3 and *Eustrongylides tubifex* L3 and L4 are found encapsulated within the viscera and musculature of freshwater fish. Adults of *E. tubifex* are found in piscivorous birds and tubificids act as the intermediate host (L1 to L3). Fish act as an evolutionarily recent intermediate host (c.f. paratenic host) for *E. tubifex* whereas they are strictly a paratenic hosts for the mink kidney worm, (*D. renale*). *Eustrongylides* spp. larvae in fish are a rather unsightly blood red in colour and up to 10 cm in length. If encapsulated in the gonads, particularly the ovary, it can cause severe damage and even complete degeneration of these organs.

ACANTHOCEPHALA

Despite there rather fearsome proboscis with its rows of hooks, acanthocephalans have not yet been observed to be serious pathogens of fish. This is surprising in view of the sometimes severe local damage which can be caused to the intestine of the fish by the proboscis. The great majority of acanthocephalans in fish are found as adults in the gut. The life cycle involves only one intermediate host, a crustacean. Control of acanthocephalans might be achieved by the methods already described for cestodes. *Acanthocephalus jacksoni* may cause damage to the intestinal mucosa of *Salvelinus* and *Salmo*. Similarly, *Echinorhynchus salmonis* in *Coregonus*.

Pomphorhynchus laevis

This is perhaps the most spectacular species that occurs in British fish. It is fairly non-specific but seems to favour chub and grayling. In some waters, particularily the Hampshire Avon, it can be present in very large numbers. The life cycle involves a gammarid intermediate host. The proboscis of *P. laevis* has a bulb as well as hooks and when embedded in the intestinal wall it elicits a severe host response so that a fibrous capsule is formed around it. Sometimes the parasite penetrates right through the

intestinal wall. Despite the large scale destruction of mucosa that can occur, no effect of growth rate or survival of infected fish has been observed.

P. bulbocoli is reported from many N. American fish. Several authors have reported similar histological findings to that of *P. laevis* with the exception that *P. bulbocoli* in most instances penetrated right through the intestinal wall into the coelom.

MOLLUSCA

Glochidia Larvae

The larvae of many freshwater bivalves must go through a parasitic stage on the gills of fish. The larvae are shed from the parent mollusc and attach to the skin, fins or gills of the fish host. Host specificity of the mollusc varies between species. Once attached, the larvae become surrounded by host tissue reaction. They remain attached for up to several months during which they metamorphose before leaving the fish. In Britain, infection occurs during the winter months. In the U.S., mortalities of trout fingerlings have been caused by heavy infections of the gills. Locally in Ontario, infection in yellow perch peaked during the summer corresponding to the reproductive behavior of the mollusc. Secondary infection may occur when the glochidia slough off the host. Filtration of fish farm water supllies is the only possible means of control of these parasites. Obviously, fish ponds should be kept clear od adult molluscs.

Recently, there was a report of an epizootic of post-veliger mussel larvae on the gills of caged Atlantic salmon causing considerable gill damage, mortality to the fish and decreased growth rate in survivors. The mussels became encapsulated within the gill epithelium similar to glochidial encapsulation.

ARTHROPODA

C. CRUSTACEA

O. Branchiura

Argulus spp. (fish louse)

This is a dorso-ventrally flattened crustacean about 4 - 8 mm in length and is found on the skin and fins (rarely the buccal or opercular cavities) of most species of freshwater fish. *Argulus* is easily the largest genus (in terms of numbers) in this order and of the approximately 120 *Argulus* species worldwide, 75% are freshwater. On the ventral surface of the parasite is a pair of curved hooks and suckers which serve as organs of attachment. There is also a proboscis or feeding organ which is inserted into the epidermis and underlying tissues of the host. The life cycle of *Argulus* spp. is direct, eggs are laid into the water and hatch to produce a free-swimming larva which must find a suitable host within 2 - 3 d. Heavy infections can cause mass mortalities, especially of young fish. The wounds inflicted by the parasites may become necrotic and secondary infection may occur.

O. COPEPODA

The parasitic copepods are among the most devastating of freshwater fish parasites. The stage that one sees attached to the fish is usually the mature female. Copepodid life cycles involve a number of different nauplius and copepodite stages and sometimes chalimus stages. Some of these stages are free swimming. Intermediate hosts are not usually required. After copulation the male dies and the female matures and produces egg sacs.

Ergasilus spp.
This genus is found on the gills of many fish species. Many species are present in N. America. Reproduction occurs during the summer corresponding to the times of disease outbreaks. Severe gill damage can be caused by the feeding activity of the copepods. The gills become pale and secondary infections may occur.

Lernaea spp. (anchor worm)
This is a very dangerous parasite, especially to young fish when only a few parasites can cause death. Older brood stock can also become severely affected. The most important species is *L. elegans* which is non-host specific and can even attack tadpoles and salamanders. Worldwide distribution. The adult female is a rather featureless sac and is embedded into the musculature of its host with its anchor-like head region. It may even penetrate into the body cavity and embed in some viscera. The embedded parasite forms an ulcer and eventually a fibrous nodule develops around the head of the copepod. Even if fish aren't killed they lose weight and look unsightly. Reproduction does not occur below 14° so outbreaks of disease are seen only during the summer months in temperate climates. *L. cyprinaceae* and *L. polymorpha* are found in Asia, and causes severe damage to hosts, resulting in ulceration and even deformation in surviving fish. Although usually freshwater species, there are some reports of the latter species in brackishwater.

Salmonicola spp.
Another common freshwater parasitic copepod found attached to the gills, branchial cavity, skin and fins (depending on species) of salmonids.

Control of parasitic crustacea can be difficult. A number of compounds have been used as dips and baths with varying degrees of success. Organophosphates are commonly used, as is formalin. Dylox and Bromex are effective against *Argulus* spp. and larval *Lernaea*. Repeated treatments are necessary to kill the larvae of subsequent generations which will in time lead to a decrease in the adult population. Potassium permanganate will kill adult *Lernaea* but at the concentrations necessary it is dangerous to fish. It is helpful to keep adult fish which may be carriers separate from young fish.

O. ISOPODA
Many isopods are only facultatively parasitic; nevertheless, they are associated with dermal ulcerations, with the formations of pouch-like depressions in the body wall, or with pressure-atrophy type of lesions, especially in the buccal and branchial cavities. A few species are recorded from salmonids in various parts of the world, and from milkfish in the Philippines.

ANNELIDA

C. Hirudinea
Various leeches are important. *Piscicola geometra* can occur in very large numbers on fish raised in earthen ponds notably trout and cyprinids. They are approximately 2 cm long and suck the blood of the fish. Large numbers can cause anaemia or pave the way for secondary infections. They also transmit *Cryptobia*. *P. salmonisitica* transmits *Trypanoplasma* sp.

LAMPREY

Found on both fresh and marine fish and can cause very serious damage with their circle of fine sharp teeth. Recently, government support for the annual lampricide programs throughout the Great Lakes region have diminished thus raising the possibility that the lamprey may make a come back in the Great Lakes watershed.

B. MARINE

Monogeneans

Gyrodactylids are again very important e.g. *G. unicopula* in plaice. *Benedenia* spp. of the family Capsalidae (flat, oval or elliptical non-viviparous worms) affect a wide variety of both wild and cultured fish including mullet. Normally found attached to the oral mucosa but may spread to other parts of the body during heavy infections causing skin erosions and ulcerations.

Polyopisthocotylean worms (posterior adhesive organ comprising suckers or clamps with or without hooks). e.g. *Axine heterocerca*, is a problem in sea cages in Asia.

Digeneans

Again, many metacercariae from numerous genera encapsulate in the musculature, often leading to "black-spot" and a consequent drop in commercial value. All the genera of the Gasterostomata also have teleosts as their final hosts. Some have zoonotic importance.

F. Heterophyidae

Metacercariae of the *Cryptocotyle lingua* employ a wide range of teleosts as second intermediate hosts (first intermediate host is a periwinkle *Littorina littorea*) while while the final host is a wide range of piscivorous birds and mammals, including man. The metacercariae of this species have been reported to cause mortality in fry of salmonid and other species. Some genera employ invertebrates as the second intermediate hosts and fish as the final hosts.

F. Sanguilicolidae

Aporocotyle simplex is found in the blood vessels of pleuronectids.

Cestoda

O. Tetraphyllidea and Trypanorhyncha

Adults of these cestodes are found primarily in the gut of elasmobranchs and utilize a variety of fish second intermediate hosts. *Gilguinia squali* (O. Trypanorhyncha) plerocercoids (larvae) have been reported in the eyes of cultured salmon on the west coast of N. America, associated with blindness, and mortality. The lesions do not seem to correlate with the severe level of sickness observed

Nematoda

"The cod and/or seal and/or herring worm problem"

The more common genera of marine ascarids include; *Anisakis, Pseudoterranova, Terranova, Hysterothylacium, Contracaecum, not Thynnascaris. Anisakis* spp. is commonly referred to as the herring worm and *Pseudoterranova decipiens* is commonly referred to as the seal or cod worm. Sometimes these pseudonyms are exchanged. All of these genera are cosmopolitan but there are also recognized hot spots throughout the world. It presently appears that there will always be a cod or herring worm problem in terms of the aesthetic depreciation of the fish for the purposes of human consumption. However, the zoonotic dangers (gastric granulomas) can be eradicated by following some simple procedures - cooking or freezing marine fish prior to consumption.

Copepods

Various species of the family Caligidae are found on marine fish. These parasites are very mobile and can move from one host to another. They are possible vectors of blood protozoa.

The salmon lice, *Lepeophtheirius salmonis* and *Caligus elongatus* are reported to be causing big problems in sea-caged salmon culture in Norway and are starting to become a nuisance here on the east coast of Canada. *L. salmonis* females occur in large numbers, especially around the head region whereas *C. elongatus* are generally more active and are more widely distributed on the skin. The males and younger stages of *L. salmonis* are also parasitic and concentrate around the head, and can cause extensive damage in this area. Various other caligid copepods are reported to cause mortality in mullet in the Mediterranean. Nuvan and Neguvon (organophosphates) are commonly used in Europe to control sea lice, and are starting to be used here, despite the major environmental concerns which are limiting their use in Europe. Other means of control presently being investigated include Ivermectin administered in the feed, cleaner-wrasse kept in the same cages as the salmno, and pyrethrum mixed with oil and floated on the surface of the water in the cages, so that the fish become coated when they surface.

Lernaeocerid copepods e.g. *Lernaeocerca branchialis*, the adults of which are found on gadoids, can cause extensive damage, even a single parasite, owing to their habit of attaching by the head to the bulbous arteriosus of the heart, while the body is in the gill chamber.

NUTRITIONAL DISEASES

Nutritionally-related diseases are probably more common than is presently appreciated, but the weakened state of the fish renders them prone to other e.g infectious agents, and the diagnosis then becomes confused. This si especially true when alternative diets are sought for newly-domesticated species.

CARBOHYDRATES

By contrast with man and other domestic animals, CHO is not a major energy source for salmonids or catfish (and probably most other species) and although they do have the requisite enzyme systems, they don't utilize CHO efficiently. In rainbow trout, as the complexity of the CHO increases, digestibility decreases and also gastric motility increases thus decreasing the overall digestibility of the diet. This has the adverse effect of increasing fecal output which has other effects on water quality, including possibly increasing levels of fecal proteases which may have adverse effects on gill and/or skin cell surfaces, possibly predisposing to gill or skin diseases.

Salmonids reared on diets high in digestible CHO such as glucose become hyperglycemic as insulin production is not stimulated i.e. they are effectively diabetic. In addition, glycogen accumulates in the hepatocytes; this has the effect of reducing BSP clearance (sulphobromophthalein) indicating impaired hepatic function. This is reflected in practical terms by a reduced tolerance to anesthetics (increased recovery time) and increased susceptibility to water-borne pollutants eg. copper. Such an accumulation of hepatic glycogen is not seen with complex CHO.

Optimum utilization of glucose (14% in trout diets) decreases as water temperatures fall and indeed there may be no utilization at all at low temperatures, the fish relying entirely on glucose production from protein, to supply its needs for brain, erythrocytes, renal cortex and white muscle.

As already mentioned, dietary fibre is not considered to be required in fish diets. It may have the adverse effects of increasing the rate of passage through the digestive tract and decreasing the uptake

of some essential nutrients. It may obstruct enzyme activity by chelating metals acting as co-factors for the enzymes.

During starvation, glucose production from gluconeogenesis exceeds utilization, the excess being stored in the liver as glycogen. Thus starved fish may have higher levels of hepatic glycogen than fed fish. The consequences of this for the fish can only be guessed at, but it is important for the comparative pathologist to appreciate the fact.

High hepatic glycogen levels are seen in ornamental fish such as koi fed on bread.

LIPIDS

Extremely important in fish diets, especially the omega-3 polyunsaturates, levels of which increase as the temperature decreases. Such an action maintains the fluidity of cell membranes.

The essential fatty acids for freshwater fish are (1) linolenic acid (18:3 w3) or a more highly saturated fatty acid in the omega-3 series and (2) linoleic acid (w6 series). Some warmwater fish eg. *Tilapia zillii* require only omega-6 fatty acids. This lends support to the idea that omega-3 fatty acids are necessary in many coldwater species because they allow a higher degree of unsaturation, and hence fluidity, at low temperatures. The requirement of marine fish for the omega-6 series is less certain however, although they do appear to require longer chain PUFA. Thus cultured turbot are not satisfied by 18:3 w3, but are satisfied with 22:6 w3. In addition there is great variability amongst species in their abilities to elongate and desaturate fatty acids, freshwater species probably being better at it than marine species. Thus the longer chains may be more essential for marine than freshwater species, especially for larval stages or young fish.

The optimum dietary lipid concentration varies according to end requirements eg. whether a high fat level is desirable in the carcass or ease of processing (high fat diets are more difficult to pellet). In catfish, 10-12% lipid is generally considered the minimum for efficient utilization of protein although for rainbow trout, this figure is 22-24% lipid in diets containing 35% protein.

A deficiency of essential fatty acids causes dermal pigmentary change and fin erosion. In addition, cardiomyopathy and swollen pale livers are also reported. In young turbot, the gills are severely affected, with chloride cell necrosis as the first sign, and then progressive degeneration and necrosis of lamellar epithelium.

The dependency of the diets on PUFA makes them prone to oxidation, especially when they are kept in warm damp storage conditions. Various artificial anti-oxidants (eg. ethoxyquin) are incorporated to prevent this rancidity developing (no biological action) plus also vitamin E. Nevertheless, given the right circumstances of poor storage, addition of already highly oxidized fish oil to the diet, or lack of anti-oxidants, the diets may be rancid. Rancid fats probably have numerous actions on the fish:

a. consume vitamin E and probably other vitamins too (Vitamin A).
b. toxic in their own right
c. adverse effect on protein by lowering its biological value

Fish fed these rancid diets may develop lipoid liver disease; this is not uncommon in rainbow trout (brown trout kept under identical conditions and fed the same diet appear more resistant to the disease; other salmonids possibly the same). Clinical symptoms include anemia and darkening, plus often high mortality. Hepatic pallor/ bronzing is seen grossly. Histologically there is accumulation of ceroid (acid fast) within hepatocytes and fat-storing cells plus necrosis of hepatocytes and infiltration of the liver with macrophages which may congregate round the portal vessels. There may be sharply demarcated zones of degeneration, possibly a consequence of the anemia (anoxia) which may be pronounced. The

anemia is hemolytic, so there is accumulation of hemosiderin in the spleen, and also responsive, so that the peripheral blood becomes dominated by polychromatocytes (some have bizarre shapes) and there would also appear to be an inability of the erythrocytes to mature properly (use of the term microcytic, which is often applied in this case, is meaningless, as all polychromatocytes are smaller than mature erythrocytes; v.v. for mammals).

PROTEIN

A major component of fish diets (and the most expensive!) which varies between about 30% and 55%. Net utilization of dietary protein is similar to birds, but energy needs are lower, and as a result, body protein deposition is about twice that seen in chicks. Requirements decrease as the fish get older and increase with rising water temperatures. They also change with varying salinity. The essential amino acids are the same 10 as for mammals i.e. arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. Quantitative differences of these 10 vary only slightly between species (just as well, as most diets are based on those worked out for rainbow trout, catfish and carp!).

Sparing actions of one amino acid for another are noted for cystine-methionine and for tyrosine-phenylalanine i.e. the presence of dietary cystine reduces the amount of methionine needed for maximum growth. Isoleucine-leucine-valine interactions also exist in that high levels of one may compete with the other, although this varies amongst species, and is not as pronounced as in other vertebrates.

In general, a deficiency of an essential amino acid leads to depressed growth rate. Severe hypoproteinemia is occasionally seen in brood stock fish subjected to adverse feeding practices at a time of high protein demand. Lysine deficiency may lead to high mortality and fin erosion in rainbow trout (also seen with tryptophan deficiency in this species). Methionine deficiency <u>in rainbow trout only</u> leads to cataracts.

Tryptophan deficiency leads to some skeletal abnormalities in some species of salmonids eg. scoliosis and lordosis in sockeye salmon (but not chinook). These same lesions are seen in rainbow trout, as are caudal fin erosion, cataracts, shortening of opercula, nephrocalcinosis, plus elevated levels of hepatic and renal calcium, magnesium, sodium and potassium.

MINERALS

Most can probably be absorbed across gill in freshwater fish and gut in saltwater species. **Calcium** - uptake is mainly from the water through gut and gills, rather than dietary. Nevertheless, some studies in channel catfish have shown that optimal growth occurs with 1.5% calcium and a 1.5:1 Ca:P ratio. The problems arise because of differences in water hardness.
Phosphorus - it is essential for several species examined including cyprinids and salmonids. Deficiency symptoms in catfish include reduced growth and anemia, while in carp, cranial deformities and spondylolisthesis are reported. Species differ in their utilization depending on type of GI tract and pH achieved. **Magnesium** - if the Ca/Mg ratio is too high eg. 2.7% calcium versus 0.04% magnesium, rainbow trout develop nephrocalcinosis. Four percent dietary Ca, required 0.1% Mg to prevent nephrocalcinosis. Thus there is a relationship between Mg and Ca. **Manganese** - manganese deficiency of rainbow trout leads to scoliosis. **Zinc** - zinc is essential for fish, and a low dietary level (1 mg/kg dry weight) in rainbow trout leads to high mortality (50%), cataracts (50%) plus fin erosion (90%). Protein digestibility is affected. Carp have similar lesions, but no cataracts. **Iron** - iron deficiency will lead to hemoglobin deficiencies in fish as well as other vertebrates. Hypochromic microcytic anemia ("microcytic" acceptable in this situation?) seen in sea bream and common carp, and a minimum of 150

mg/kg is recommended. **Copper** - there is some evidence that small amounts may be necessary (1.5 mg/kg) but it can be absorbed from surrounding water (as is the case probably for many minerals). **Selenium** - once again, dietary levels should always take water levels into account. Maximum glutathione peroxidase levels (only known biological use for selenium) in the plasma are achieved with 0.15-0.38 mg/kg dry feed, in rainbow trout. Levels as low as 0.07 mg/kg prevents deficiency symptoms (myopathy) in presence of 400 IU vitamin E in rainbow trout. No minimum level determined however. Selenium toxicity can be seen experimentally and naturally, at 13 mg/kg diet - reduced growth plus nephrocalcinosis are seen. **Iodine** - role similar to other vertebrates, for thyroid metabolism. Minimum dietary levels for chinook salmon have been estimated at about 0.6 mg/kg, but higher levels (1.1 mg/kg) are recommended for parr, as they are thought important for smoltification. **"Mineral imbalances"** - these are suggested as the possible cause of generalized (mainly visceral) granulomatous diseases in species such as gilthead bream, rainbow trout ("visceral granuloma") and similar conditions in tropicals. Little is proved however and parasites/toxic compounds should always be suspected.

VITAMINS

Some of the B vitamins are synthesized by intestinal bacteria in species such as tilapia. A general response to a deficiency is decreased growth rate and inappetence although abnormal dermal pigmentation, hepatic lipidosis, ataxia and hypersensitivity are also commonly seen. Specific deficiency symptoms are not very common in any situation, although in general terms, as aquacultural practices tend to push fish further towards the "ragged edge", some disease entities may present as apparent deficiency type problems. This is especially the case with some of the vitamin E/selenium related problems, and differ little therefore from the situation seen in other domestic animals.

Vitamin A - essential for all species studied. The conversion of B-carotene to vitamin A is inhibited (possibly completely) at low water temperatures. Deficiency symptoms include impaired keratin formation and hence dermal and corneal atrophy with scale loss, corneal ulceration, retinal degeneration, and exophthalmia/blindness as consequences. Vitamin A toxicity (experimental) mimics deficiency to some extent, with scoliosis, epidermal and branchial lamellar atrophy (aplasia?). In the gills, the remaining epithelial cells become very attenuated and there is hyperplasia of chloride cells (due to osmotic problems?). The skeletal malformities seem to be more of a quantitative than a qualitative change in bone formation i.e. possibly differential closure of spinal growth plates.

Vitamin E - requirements for this are affected by the absolute levels and relative proportions of the polyunsaturated fats, the oxidized fats, selenium levels, and probably other vitamins such as C or A which may have sparing actions on E. Temperature is probably another important factor - low temperatures demand higher levels in salmonids. The interrelationships of all these factors are not well understood. As with other vertebrates, deficiency symptoms reported include a generalized rhabdomyopathy (heart and skeletal muscle), anemia, pancreatic atrophy and fibrosis, plus impaired immune responsiveness. The lesions seen in "lipoid liver disease" in rainbow trout, associated with rancid fat, are probably partly due to the rancid fats themselves, and partly due to the consumptive absence of vitamin E. The importance of vitamin E in Atlantic salmon diets makes itself shown in the syndrome "pancreas disease" of caged fish seen in Norway, Scotland, Eire and to a lesser extent N. America. In the U.K. and Norway, it is considered to be one of, if not the most serious production problem.

"Pancreas disease" affects caged salmon mainly in their first or second year at sea, although Norwegian broodstock are also affected. It seems to occur on dry pelleted feeds which are not rancid (low peroxide values) and which contain apparently adequate levels of vitamin E. Affected fish are dark, lethargic, inappetant, have often skin lesions (*Vibrio* spp. common isolates) and rapidly go into rigor. Pathological findings include a generalized rhabdomyopathy affecting, most seriously, cardiac muscle

(compact and spongy) as well as red and white skeletal muscle and esophageal muscle (this latter partially explains the inappetence and apparent inability of some fish to swallow pellets even in recovering animals). Anemia is not seen although erythrocytes are more fragile. Corresponding with these lesions, may be pancreatic atrophy and fibrosis with a minimal non-suppurative inflammatory cell infiltrate. The rhabdomyopathy may occur in the absence of pancreatic change, especially in older broodstock fish, in which cardiac damage is the dominant change, affecting almost exclusively spongy myocardium.

Clinically affected fish have very low plasma vitamin E levels compared to healthy fish. They also have high CK levels, corresponding to muscle damage. Of interest however, it can also be seen that apparently healthy fish are also affected at a biochemical level supporting the idea for blood sampling as a better monitor. Selenium levels also differ, but erythrocyte GPX levels are much more normal, suggesting possibly that they were responding more to changes in appetite. The mechanism whereby such low vitamin E levels are achieved is not known - need sequential clinicopathological and histo-pathological studies. Nevertheless, it is suggested that dietary vitamin E be boosted to 400 i.u./kg.

Vitamin D - probably not needed for Ca uptake from water (via gills) but needed for proper utilization and transport (in trout and catfish). A deficiency causes an increase in T_3 levels (compensatory mechanism?) hepatic and muscular lipidosis with myodegeneration restricted mainly to white fibres. Toxicity in mammals causes metastatic mineralization in a variety of tissues, but this is not seen in fish (rainbow trout). Indeed, no change was evident, even when massive quantities fed. D_3 (cholecalciferol) is much more effective than D_2 (ergocalciferol).

Vitamin C - finfish (and shellfish) require ascorbic acid. As in mammals, it is needed for collagen and cartilage maturation. Deficiency signs are similar to other animals with impaired healing, hemorrhage, scoliosis and spontaneous fractures ("broken back syndrome" of channel catfish), fin erosion and dermal depigmentation. Deficiency also impairs detoxification of various pesticides, and increased levels of vitamin C reduce vertebral damage to toxaphene. Deficiency also impairs immune responsiveness which leads to lower complement and immunoglobulin levels. Vitamin C is a reducing agent, and it works synergistically with selenium and vitamin E to prevent oxidation of phospholipids in cell membranes. It is also involved in iron metabolism. Hepatic levels <20 ug/g probably indicate deficiency.

Thiamine - a co-enzyme of many other enzymes. Important especially for central and peripheral nerve function. Deficiency symptoms in fish include hyperexcitability and darkening of the skin. Laminar degeneration of grey matter is seen in mammals, but this is not seen in fish, despite clinical evidence of nerve dysfunction. Many fish contain often high levels of thiaminase which is destroyed by cooking. Therefore mink fed fish waste can develop "Chastek's paralysis".
Riboflavin - important in respiration of poorly vascularized tissues eg. cornea. Rainbow trout deficient in riboflavin develop cataract and neovascularization of the cornea. As a result, they are often dark. Fin necrosis and scoliosis also seen in rainbow trout. Channel catfish show abnormal spinal growth leading to shortening ("dwarfs").
Pyridoxine - important as a co-enzyme in the deamination of amino acids, and therefore important to fish on a high protein diet. Despite this, requirements are roughly the same as for poultry (3-10 mg/kg diet). Deficiency symptoms appear quickly (3-8 w. in trout) and include hyperirritability, tetany, and anemia.
Pantothenic acid - a component of co-enzyme A which functions in energy release reactions. Deficiency in trout causes "nutritional gill disease" which affects the distal third of filaments (normally non-perfused -is there a connection?) causing marked fusion. Whether this is due to hyperplasia or a failure to exfoliate is unknown. Severe swimbladder distension is another symptom in rainbow trout: no lesions in the wall or duct are present however. **Biotin** - amounts required are low, but deficiency in trout causes marked gill damage - pale, mucus-covered gills with thickening of lamellae due to

hyperplasia. (These lesions not seen in catfish). Renal tubular and hepatic glycogen deposition is prominent. In chinook salmon, fragmentation of erythrocytes, and muscle atrophy are also seen.
Folic acid - a co-enzyme needed for hematopoiesis and a deficiency in trout has been shown to produce anemia with a high number of bizarre cells in circulation. Pale gills, ascites and exophthalmia may accompany this.
Cyanocobalamin (B12) - in general, needed for erythrocyte production. A deficiency causes anemia in salmonids, but not catfish nor carp, and it is possible that there is intestinal production (by bacteria) in these species. **Choline** - a quaternary ammonium compound needed for phospholipid synthesis and fat transport. Deficiency leads to fatty livers and hemorrhage. Carp do not need it for normal growth.
Niacin - deficiency symptoms in fish resemble "pellagra" of man i.e. dermal disease, which is enhanced by exposure to u.v. light.
Vitamin K - needed for normal clotting in catfish and brook trout (probably other species too).

NEOPLASIA

Description and classification of tumours in fish uses the mammalian classification, and hence a detailed description of the histological appearance is not given. With few exceptions (aflatoxin-induced hepatoma) fish tumours do not metastasize, despite often having the histological criteria for malignancy.

A. Skin

Papillomas are quite common. These are associated with overgrowth of malpighian cells obliterating other cell types such as goblet cells or club cells. They may remain flat (plaques) or become folded, with ingrowth of underlying dermal fibrous tissue. Examples include the following:
1) Papillomatosis of European eel ("cauliflower disease"). Young animals are most commonly affected in the summer in brackish water. Colder water or higher salinity leads to regression. Lesions are most common round the mouth, but are also found elsewhere on body. They may be large enough to interfere with feeding and/or respiration. The condition was originally described in the Baltic but high rates are seen elsewhere (Scotland) in N. Europe, only in European eel. Up to 30% of population may be affected in summer, 3-7% in winter. A viral etiology is suspected, but not proven.
2) Pacific flatfish papillomas. These are common in marine coastal environments of N. America, Japan and Korea. Malpighian cells proliferate but often are replaced by large rounded cells "X-cells" - which lack epidermal features of desmosomes and tonofilaments, and have ultrastructural appearance of degeneration e.g. swollen mitochondria. The identity of X-cells is unknown, as is whether they are host cells or not (protozoa?). In some areas, the incidence correlates with heavy pollution, while in others (Queen Charlotte Island in B.C.) sand sole can have an infection rate of 30% despite virtually pollutant-free water. There has been no successful experimental transmission although some virus-like particles are described in e.m.
3) Brown bullhead and white sucker papilloma. These are common in Great Lakes fish. The lip is the prime location for brown bullhead, but white sucker lesions may occur anywhere. Virus-like particles seen in both species (retrovirus in white suckers). Other species are affected with similar lesions.
4) Atlantic salmon papillomatosis. This condition is common in young fish in August, September, especially in freshwater. Hyperplastic plaques which spontaneously regress, and may become secondarily infected. Virus-like-particles are seen in e.m.
5) Oncorhynchus masou virus. This is a herpesvirus which causes papilloma in roughly 30% of fish recovering from this disease. Lesions are especially common round the mouth.

Squamous cell carcinomas are less common, but they are seen, for example in the white suckers and brown bullheads in Lake Ontario (Hamilton Harbour and elsewhere). In most cases, the lesions develop from pre-existing papillomas and are seen as downgrowths of epidermal cells through the basal lamina, into the underlying dermis with local extension and infiltration of tissues. Keratin "pearls" are not obvious

as might be expected in this non-keratinizing tissue although occasional "pearls" of epithelial cells are seen.

Pigment cell tumours. These include melanomas, erythrophoroma, xanthophoromas and guanophoromas. Melanomas are by far the commonest and may show a greatly increased incidence in hybrids (as do other tumours) e.g. swordtail/platy hybrids, in which there appears to be repression of the gene controlling melanophore production. As with mammals, some of the tumours may be amelanotic. Erythrophoromas are rare, but cells are spindle-shaped and often multinucleate, with fine granular cytoplasm. The lesions may → pressure necrosis of overlying epidermis.

Fibromas/fibrosarcomas. These are are relatively common tumours affecting a wide variety of different species. They are often relatively loosely organized (and some of these may be termed myxomas) but occasionally may be hard or extremely hard, as is the case with the specific entity "dermal fibrosarcoma of walleye" associated with retrovirus. These latter tumours may cause multifocal hard nodular growths on the body surface. They may be extremely disfiguring and may ulcerate.

Lipomas. These are occasionally encountered, but they are much less common than fibromas. As with fibromas, they may occasionally cause dermal ulceration.

Hemangiomas. These benign proliferations of small blood vessels are encountered mainly in the skin, although sometimes in the trunk musculature.

B. Gills and Pseudobranchs

In general, gill tumours are very rare, which is most interesting considering their surface area relative to the rest of the body surface and in which tumours are quite common. Chondromas arising from filamental or arch cartilage, fibromas of gill arch and one example of possible adenoma represent the mere handful of reports of spontaneous neoplasms. There is one report associated with metacerariae of digeneans. Experimental induction of branchial lamellar papillomas is reported using methylnitronitrosoguanidine in medaka.

Pseudobranchial "tumours" are not uncommon, especially in Atlantic and Pacific cod and other gadoids. They are comprised histologically of large pale cells which are virtually identical with the "X-cells" seen in the Pacific flatfish "papillomas". Again the question arises as to whether these cells are of host or parasitic-origin (Indeed a proliferation of similar cells is reported in the gills of some marine fish, just to confound the situation!). The incidence rate in Atlantic cod may be 2% round Nova Scotia and confined mainly to 1,2 or 3 yr. old fish. Incidence in Pacific fish tends to be higher.

C. Kidney

In general, renal neoplasia in fish is relatively uncommon although nephroblastomas and adenocarcinomas are both reported. In the former, there is proliferation of a pluripotential "blast" type embryonic cell to form most of the cellular elements normally encountered in the posterior excretory portion. Thus there is usually a proliferation of epithelial cells which in places try to form tubules or glomeruli. Connective tissue is present in variable amounts and either fibrous or cartilage may dominate in places, and in some tumours. We have seen nephroblastomas both in wild fish (smelt) and in farmed rainbow trout. The renal adenocarcinoma had a papillary appearance.

D. Hematopoietic tissue

Lymphoma/lymphosarcoma is well described from northern pike in Scandinavia, Eire and N. America, and in the latter, muskellunge are also affected. A retrovirus is implicated. The lesions are widely disseminated and may be leukemic. Lesions start often as small nodules in the skin which may ulcerate, and invade the underlying muscle. In late stages, spleen, liver and kidney are affected. The disease is approximately 99% fatal to muskellunge, but less so with pike and in this species, recovery may even be the norm. Disease incidence in muskellunge is highest in the spring and by summer, most fish have died. The disease has been experimentally transmitted with cell-free extracts. Many fish with lymphosarcoma have epidermal hyperplastic plaques in which it is easy (relatively) to see C-type particles.

In the Baltic, the disease was not recorded until 1950's and there is a suggestion that the incidence has increased in line with pollution, especially chlorinated hydrocarbons.

Lymphosarcoma of thymic origin (thymoma) is described, several times from salmonids. In most instances, the tumour is leukemic. Occasionally there is diffuse involvement of the kidney and such lesions must be carefully differentiated from the granulomatous nephritides, so common in salmonids and other species. Granulomas comprised of a monomorphic cell population have been mistakenly identified as lymphoma (hemogregarine infection).

E. Liver

Hepatoma and hepatocellular carcinoma are not infrequent. Rainbow trout are exquisitely sensitive to aflatoxin (along with turkeys) a product of the mould *Aspergillus flavus* which colonizes oil seeds. In the 1960's this caused large numbers of fish to die with both hepatocellular and bile duct carcinomas. This is one of the few examples (if not the only one) of a fish tumour which has been shown to metastasize - often to the kidney in this case. The disease is still seen in warm countries where feed is improperly stored.
A variety of hepatic tumours is seen in wild populations of fish, often in areas of high industrial pollution. Examination of a population of white suckers on a spawning run up Oakville Creek in Lake Ontario showed roughly 17% to have malignant hepatic tumours of both hepatocellular and bile duct origin. In some cases, as might be expected, both were found together in the same liver. Early changes are often represented by small nodules of morphologically altered cells - an increase in nuclear/cytoplasmic ratio or staining intensity, are common changes. Some of these "altered foci" may presumably progress to the hepatoma and on to (maybe directly) carincoma (or regress). Similarly bile duct epithelium frequently shows inflammatory, degenerative and metaplastic change before progressing to cholangioma or carcinoma. In general, hepatocellular carcinomas are very cystic and hemorrhagic while the bile duct carcinomas are solid and often contain retained bile, giving them a grossly distinct colour. Despite locally aggressive behaviour and cytological appearance of malignancy, none of these tumours was seen to metastasize.

Another component of the liver in some species is the melano-macrophage centre, and we have once seen a melanohistiocytoma, characterized by large aggregation of the cells tending to obliterate normal parenchyma, and by the presence of multi-nucleated syncytial cells.

F. Gastro-intestinal tract and swimbladder

Teeth tumours include ameloblastomas, invasive tumours of the odontogenic epithelium (syn, adamantinoma and enameloblastoma). Odontomas are considered to be malformations. Ameloblastomas are not uncommon, especially in salmonids, and are also reported in the Atlantic

cunner (*Tautogolabrus adspersus*), a fish which grazes barnacles and clams and which may therefore suffer mechanical damage. They are locally invasive and may ulcerate.

Other than the retrovirus-associated fibrosarcoma of Atlantic salmon which occurred in Scotland in caged fish in epizootic proportion, there are only a few other reports of swimbladder tumours in other species, notably cod.

Stomach and intestine. Tumours here are uncommon although a tumour of the cod stomach was one of the first recorded (mid 18th century). So-called adenomatous polyps are recognized in cultured yellowtails, sea bream and eels in Japan although the significance of these pinhead-sized proliferations is unknown.

A few peritoneal mesotheliomas are reported.

G. Endocrine and reproductive systems

Thyroid - differentiating physiological hyperplasia (when grossly observable = goitre) from neoplasia becomes very difficult in the thyroid and especially so when it is appreciated that in most species the thyroid is a diffuse organ, with follicles normally scattered in often remote sites such as kidney, splenic capsule, or epicardial surface. Thus the assumption of metastasis is usually mistaken. Much of the early work on iodine-related goitre was performed on fish (brook trout) in western U.S.A. and the condition in some populations of fish notably coho salmon in the Great Lakes is considered to be on the increase, possibly related to environmental pollution. Fish with grossly visible thyroids have a mass of mottled red/grey tissue (sometimes ulcerated) extending round the ventral aorta between the first and second gill arches. In severe cases, follicles extend up to the pharyngeal mucosa, invading bone and muscles and occasionally blood vessels. (In other species this apparently aggressive behaviour combined with the diffuse nature of the normal organ, so that follicles in other tissues are also hyperplastic, has erroneously led some workers to report these hyperplasias as carcinomas). The follicles generally have a high columnar epithelium and with reduced colloid, or sometimes no colloid at all. Iodine will cause involution of these goitres in roughly 16-40 days with a reduction in epithelial height and increasing quantities of colloid becoming apparent. Goitre in some species may be accompanied by increased numbers of melanomacrophage centres, which are occasionally encountered within the parenchyma of the thyroid in normal fish.

Gonads. Leiomyomas and fibroleiomyomas are quite common tumours of the testis of the yellow perch in the Great Lakes, and indeed one study showed 8% of susceptible fish to be affected. A lesser number of ovaries have similar tumours. Carp/goldfish hybrids in the Great Lakes and elsewhere also have a very high incidence of tumours. As with the tumours in the yellow perch, these may grow to a large size, causing space-occupying problems.

Sertoli cell tumours, interstitial cell tumours and seminomas are also reported as individual cases. So too are ovarian papillary adenocarcinomas.

H. Musculo-skeletal

Rhabdomyomas are usually reported as individual cases although an epizootic has been described in cultured ayu in Japan. The tumours were seen in approximately 1% of fish, affecting mainly the trunk and tail muscles and grossly distorting the fish. Chemotherapeutics were suspected, in the absence of obvious virus involvement.

Other than hyperostoses, which are not infrequently found, an example being in the mandible of pike in the Great Lakes, bone tumours are not common. They are encountered however in salmonid vertebrae, usually at the fulcrum of flexure beneath the dorsal fin, and a traumatic etiology should be suspected. Other individually reported cases occur in a variety of other species.

I. Nervous tissue

There are a few reports of central nervous system tumours (ependymomas) although by contrast, peripheral nerve tumours are quite common, especially associated with the skin. Neurofibromas (formed from endo and epineurial connective tissue) and neurilemmomas (derived from Schwann cells) are the two commonly reported, although they are difficult to differentiate from fibromas or sometimes melanomas, especially the amelanotic melanomas. While usually seen as isolated cases, they may occur in high frequency in some populations: such is the case reported for several *Lutianus* spp. (snappers) along the Florida (U.S.A.) coast, affecting the nerve branches of roughly 1% of the population. The possibility of granuloma should always be borne in mind however. Ganglioneuromas are rarely reported. An epizootic (12 of 100,000) of neuroblastomas was reported in coho salmon raised in chlorinated-dechlorinated water; they were located in the skeletal muscle adjacent to the dorsal fin. The suggestion is that the halogenation of the water may have produced carcinogens.

Retinoblastomas are also occasionally reported.

ANESTHESIA AND TREATMENT

Anesthesia and Sedation

1. Indications:
a) transport - especially tropical and valuable animals, and sometimes between hatchery and on-growing sites in farmed species.
b) surgical procedures - tumor excision, biopsy, injections, or for experimental procedures such as dorsal aorta catheterization.
c) diagnostic work - gill and skin scrapings, blood sampling.
d) treatment - topical application of concentrated drugs, I/P or I/V injections
e) euthanasia - overdose is commonly employed, especially with small fish which will be fixed "whole"
f) spawning or tagging procedures - light sedation makes the job easier and less stressful to fish, but may be some deleterious effects on eggs/milt.
g) photography - especially amongst pathologists and aquarists!

2. Rationale for use:
a) reduce the stressful impact of intended manipulations/transport, and thereby reduce self-inflicted physical or metabolic trauma (metabolic rate reduced and thus O_2 consumption reduced).
b) enable the manipulations to be carried out i.e. blood sampling a wriggling fish is quite difficult!
c) protect operator - some fish are dangerous eg. lion fish, electric eels

3. General considerations affecting response:
a) species - different species respond in different ways. In general, salmonids require lower levels than higher teleosts and within a species, large active fish succumb more readily than small sluggish ones. Always err on the side of safety with an unfamiliar species, especially if it's very valuable, and/or owner is watching! Different species have different gill area/body weight ratios (the large gill areas have more efficient uptake). Some species have accessory breathing apparati eg. anabantids (Siamese fighting

fish) and can take longer induction time when using aqueous anesthesia. Some species have a high lipid content, as may older fish or gravid females. In such animals, duration of anesthesia may be prolonged and recovery delayed when using markedly fat soluble anesthetics such as MS-222 or benzocaine.
b) water quality - temperature has a major affect, but there is no reliable pattern, and it depends entirely on the drug used. MS-222 and benzocaine require higher doses with higher temperatures. Salinity may have an affect. Most anesthetics work in seawater but barbiturates are antagonized by Ca++. pH will affect the ionization of the drug and hence its uptake.
c) general health status - sick fish are poor subjects for anesthesia, especially those with skin or gill disease. Recovery from anesthesia involves increased irrigation of gills, which in turn leads to a passive uptake of water (in freshwater situation v.v. in saltwater). Gill disease may impede gas exchange and osmoregulation and skin disease may enhance osmoregulatory problems.

4. Stages of anesthesia.

	Stage	Behaviour
I	light sedation:	movement and ventilation slower
	deep sedation:	mild analgesia; response only to vigorous stimuli
II	light anesthesia :	good analgesia; partial loss of balance; ventilation increased
	deep anesthesia:	loss of balance and muscle tone; ventilation reduced
III	surgical anesthesia:	as above. No response to stimuli. Branchial movements very slow.
IV	medullary collapse:	ventilation and cardiac movements stop. Death.

5. Practical considerations.
Use the lightest level of anesthesia necessary for the manipulation. Some marine flatfish may require no more than covering their eyes with a damp cloth even for blood sampling. Oxygenation of the water greatly improves induction and recovery - for many procedures, the fish will often be happier if the head is left in the water. Body scrapings, blood sampling, injections and topical treatments are all easily achieved by holding the body out of the water. Keep the fish out of water for as short a time as possible. Remember that its body temperature may rapidly rise to that of room temperature - a major source of stress. Epidermis may also rapidly dehydrate, especially under strong light. Never handle the fish with, or lay it on, any absorbent material, and remember to wet hands first. If the fish has to be out of water for an extended period of time as when performing eg. dorsal aorta catheterization, construction of a small operating table may help. A V-shaped trough resting on a tray with a drain at one end to collect water, is easily constructed out of plexiglass (perspex). Water containing the anesthetic solution can be delivered via a rubber tube to the table using a small submersible pump. The tube can then be divided by means of a Y-junction the two ends of the tube being then inserted under the opercula. By this means, the gills are artificially irrigated. The body surface must of course be kept moist, and the anesthetic solution is best oxygenated and may need to be maintained at body temperature. As soon as the procedure is finished, move the fish to fresh water at the same temperature. It should at least be aerated and in my experience, preferably oxygenated. Some fish such as salmonids greatly benefit from manually moving the fish backwards and forwards through the water - backward movements open the opercula. The fish may take 24 hours to fully recover. Repeated anesthesia is practised in some experimental situations with no discernible detrimental effects.

6. Types of anesthesia and anesthetic agents. Only the commonest ones are mentioned:

a. Irrigation anesthesia - drug dissolved in water. In general, this is the most commonly employed method. 1). Tricaine methanesulphonate (MS-222). A white readily soluble powder which is very commonly used, although expensive. It's acidic and is best buffered. Side effects include persistent leaching from muscle which may increase blood levels and progressively reduce branchial movements. This in turn leads to hypoxia which can cause a reduction in pH and an increase in erythrocyte size as a result. This can lead to reduced capillary perfusion, thereby compounding the hypoxia. Doses of 10-40 mg/litre are used for salmonids, but up to 100 mg/litre are required for hardier fish such as tilapia. Store at 4°C. 2) 2-Phenoxyethanol. An oily liquid which does nevertheless dissolve in water. It is also bactericidal and fungicidal and is therefore useful in this regard. It seems very good for species such as white sucker and catfish which can be induced very smoothly (MS-222 by contrast may lead to a lot of excitement in these fish, especially the former).
Doses of 0.1 - 0.5 ml/litre are quoted for salmonids. 3) Benzocaine. Insoluble in water and must therefore be initially dissolved in a little ethanol. Not acidic although similar side-effects to MS-222. Relatively cheap and effective, and considered by many to be the best all-round anesthetic. Dose rates similar to MS-222.

b. Parenteral anesthesia. May be the preferred method for lengthy surgical procedures, or possibly in fish with efficient air-breathing capabilities. The drug may be administered directly into the bloodstream via the heart (not recommended) or the caudal vein. It may be given into the peritoneum or into the skeletal muscle. The red muscle fibres running as a band down the lateral line have a greater blood supply than the white muscle, and uptake from there is more efficient as a consequence.
1). Alphaxolone (Saffan). Has been used extensively for long-term anesthesia in a variety of species, usually for experimental purposes, and can give excellent results. Some preparations may contain undesirable additives such as surfactants (Cremophor EL). Spontaneous nervous activity is usually preserved, and thus respiratory and cardiac problems do not develop. Dose rates vary from 12 mg/kg to roughly 24 mg/kg depending on species, and depth of anesthesia required. 2) Propanidid (Epontol). Dose rates of roughly 300 mg/kg given I/P are needed for effective anesthesia in rainbow trout. Recovery occurs usually uneventfully in 2-3 hours. 3) Sodium pentobarbitone (Nembutal). Effective at doses of 40-72 mg/kg given I/P. <u>But</u> anesthesia lasts a long time i.e. 6-24 hours. Most anesthetics are excreted by the gills, but not barbiturates, which may explain the lengthy anesthesia. 4)Ketamine hydrochloride (Ketalar). Respiratory problems are reported with this drug, but it is nevertheless useful as it is commonly available in many veterinary practices. A dose rate of 1 mg/200 g given I/M into rainbow trout produces deep sedation to light anesthesia, with recovery in about 10-15 minutes.

c. Other methods. 1) Carbon dioxide. Produces effective anesthesia when bubbled through water although O_2 levels may need to be maintained if recovery is desired. It can be difficult to achieve reliable recovery, and we rarely use it, except in emergency - an Alka-Seltzer tablet dropped in a beaker releases enough CO_2 to be effective. 2) Halothane. Poorly soluble in water and therefore stability can be a problem; it is not often used. It does work well however, at doses of 0.5-2.0 mg/litre, and once again, is readily available in most practices. 3) Hypothermia. Lowering water temperatures will sedate fish and thus is often used when killing farmed salmon prior to bleeding. How much cooling can be employed with other species however is less certain, and it may be dangerous with more valuable fish, possibly due to failure of osmoregulatory mechanisms (low temperature impairment of ATP-ase systems?). 4) Electric methods. AC, DC and pulsed currents all produce anesthesia, but only pulsed currents work properly in seawater. DC field strengths of 0.6 volts/cm work with rainbow trout, whereas 3.0 volts/cm may be needed for other teleosts such as tilapia. The fish recover almost immediately after the current is switched off. AC field strengths of 2-8 volts/cm produce sedation through to surgical anesthesia with exposure times of 2-16 seconds. The fish in this case do <u>not</u> recover as soon as the

current is switched off. Although poorly studied, blood chemistry changes may be just as dramatic as with chemical methods of anesthesia.

TREATMENT AND CONTROL

This is effected in several ways, external, systemic treatment with medicated feed, parenteral, and managemental manipulations.

I. External. Used mainly for treating superficial bacterial, fungal and parasitic diseases of the skin and gills. With the exception of swabbing local areas (often good for fungal infections or severe focal lesions) external treatments may be applied in one of the following ways:

a. bath - a static low concentration of chemical for 30 m - 1 hr. O_2 levels may decrease and therefore compressed air best supplied.
b. prolonged bath - very low concentrations for 24 hrs to 3-4 days.
c. dips - fish dipped in very high concentrations for 1-5 min.
d. flushes - where chemicals are added to the inflowing water to give the desired concentration. Can run into problems with improper mixing which leads to "hot spots".

Whenever treating fish in this way, several factors must be borne in mind. These include:
- O_2 levels are the most important factor - must maintain these at adequate levels. Use aerator. Low O_2 levels lead to increased respiration and increased uptake of drug.
- avoid temperature fluctuations from normal i.e. treat fish at the temperature it was at.
- don't treat in the presence of excess detritus, as these can adsorb the chemical and reduce the effectiveness of the drug.
- pH and hardness of water are major factors affecting quantity of chemical used. In general terms, soft water requires lower concentrations of chemicals.
- time of day. In outside conditions, treat when water temperature is lowest, usually at beginning and end of day and therefore O_2 levels are highest.
- starvation for 12-48 hours prior to treatment (if not off feed already) reduces O_2 demand and NH_3 production and is therefore advisable.
- check on the state of the gills as these are the most sensitive organ. If they are in a bad state, proceed with extreme caution and possibly gradually work up to therapeutic levels over a period of time.
- monitor fish at all times. If unsure of response, use a test batch of fish first.
- repeated treatments are often necessary to kill parasites developing after the first treatment.

Some drugs commonly used include the following:
1. Formalin (this is 37% formaldehyde vapour dissolved in water and is regarded as 100% formalin). Effective against most external parasites on skin, gills and fins. The therapeutic index is narrow. Increasing toxicity at high temperatures, low pH, damaged gills etc. Paraformaldehyde may be formed as a white precipitate if formalin is kept for prolonged periods of time or exposed to light. Paraformaldehyde is extremely toxic to fish and all formalin flasks with a white precipitate should be discarded. Formalin may cause allergic reactionsto human operators. Dosage: 1:4000 for 30 minutes (0.01% solution). Prophylactic: 1:6000 for 30 minutes. Use lower dosages for soft water (167 mg/litre for 1 hour). Also used to disinfect eggs and control fungal growth.
2. Malachite green. Must use zinc-free medical grade. Excellent for fungal infections of fish and eggs; also used against parasitic infections. Fallen from favour in U.S.A. owing to proven teratogenic effects, especially of cranial tissues. 1% solution as a swab, 1-2 mg/litre for 1 hour. Use lower dosage for soft water (<100 mg/litre). NB May be contraindicated for scaleless fish and neon tetras. A combination of formalin and malachite green is often used against particularly resistant parasitic infections: 5 gr. malachite green + 100 ml formalin per 400 litres water for 30 minutes.

3. Quaternary ammonium compounds such as benzalkonium chloride ("Roccal" or "Hyamine 3500"). Commonly used against bacterial gill disease in farmed salmonids, although falling from favour in Canada as mortality may be high even using therapeutic levels. The destruction of respiratory tissues is quite marked. Use 1 ppm for 1 hour if water hardness is less than 100 ppm. 2 ppm for 1 hour if water hardness is 100-200 ppm, 3 ppm for 1 hour if water hardness is 200-300 ppm.
4. Chloramine-T. Is the drug of choice for many trout farmers in Canada for treating bacterial gill disease. Use at 10 ppm for 1 hour.
5. Proflavine hemisulphate - very safe good all-round antibacterial drug. Use at 20 ppm for 30 minutes.
6. Nifurpirinol ("Furanace"). Expensive, and therefore commonly reserved for aquarium fish. Can be added to water (absorbed through gills) or to diet. Good against bacteria and parasites. 10 mg/litre for 1 hour. 0.2 mg/litre for extended treatment.
7. Freshwater dips for marine fish to treat for external parasites - for 5 minutes in aquarium, or layer freshwater onto top of the sea-cages for farmed fish.
8. Saltwater dips for freshwater fish. Good for external parasites. 10-15 gr/litre for 20 minutes.
9. Copper sulphate. Only used for marine fish for external parasites. Stock solution of 1 gr/litre. Use 1.6-2.0 ml/litre of this stock solution for a long duration bath of 3-10 days.
10. Potassium permanganate. Commonly used in Asia to treat external parasites; applied as a spray to achieve a final concentration of 10ppm for 1-2 hours. Long-term baths usually have lower levels. Coomonly used with carp. As with many of these substances, fry are more susceptible than adults; this is especially true with $KMnO_4$.
11. Organophosphates. Used largely for metazoan parasites, especially copepods and monogenes:
    a) Trichlorofon (Neguvon® Bayer, Dylox, Masoten). Used for the treatment of salmon lice (*Lepeoptheirus salmonis* and *Caligus* spp.) and fish lice (*Argulus* spp.). Powder is dissolved in a bucket of water before treatment. Dosage (according to producer): 300 ppm. Treatment time: 15 minutes at +12°C, 45 minutes at +3°C. (Many veterinarians reduce the dose to 10 0 ppm). Trichlorofon is transformed to dichlorvos (DDVP) in water (rate dependent on temp.) DDVP is far more potent (and toxic!) than Trichlorofon and the effects may be difficult to predict. TRICHLOROFON IS ABSORBED THROUGH THE INTACT SKIN (OF HUMANS) AND MAY CAUSE ACCIDENTS!!!!!
    b) Dichlorvos (Nuvan® (50% dichlorvos) is a new drug which is far safer to use than the former. As in Neguvon the dosage is temperature-dependent: If temperature is less than +5°C: 1.5 - 2.0 ppm dichlorvos 5-10°C: 1.5 ppm dichlorvos; 10-15°C: 0.5-1.0 ppm dichlorvos. If temperature is more than 15°C: 0.5 ppm dichlorvos. (1 ppm = 200 cc Nuvan per 100 cubic meters water). **There are major environmental concerns over the use of OP's in cage culture (owing to their effect on larval crustacea of all types, and alternatives are now being seriously sought. Examples include cleaner wrasse, and pyrethrums in an oil base on the surface of the water, so that fish become coated once they break the surface.**
12. Compounds derived from plants. These are quite commonly used in Asian countries. Possibly the oldest known example is the use of teaseed cake. Bundles of castor oil plants are considered effective against *Lernaea*.
13. Biological control methods, such as the use of cleaner-wrasse in salmonid cages. There is increasing pressure from environmentally-aware groups to cut down on the use of chemicals, and I believe we should and will see an increased level of research into this type of control.

II. Medicated Feed. Antibacterial and antiparasitic drugs may be mixed with the feed, encapsulated in the feed or added as a layer around the food particle (pellet). The formula should prevent any leakage of drug from the pellet to the water before it is eaten by the fish. It is most important that the medicated feed has a good taste and a soft texture as sick fish go off food rather quickly. Some of the dis-advantages of this method are: (1) all fish (i.e. healthy and sick fish) must be treated, and healthy fish may take up 2-3 times the therapeutic dosage as diseased fish have a reduced appetite, (2) medicated

feed may accumulate in the environment or may be eaten by scavenger fish. In addition to this, consider the following:
- the appetite is reduced at low (or dropping) temperatures.
- the appetite is reduced in broodfish near spawning time and during smoltification.
- the passage through the stomach and intestines is affected by the temperature (in rainbow trout kept at +3°C 99% of the feed will pass through the gut in 147 hours while the same process requires only 18 hours at +20°C. This is of great significance in drugs with a low % absorption.
- certain components of the feed (eg. Ca++) may interfere with the drug (chelates).

Excretion of drugs given in the diet is through the liver, gills and kidney. Oxytetracycline accumulates in the skin (excretion?). There is a general lack of knowledge about the exact absorption, distribution and excretion of different drugs in different species at different temperatures. Recommended withdrawal times for food fish in Europe are: 40 days if the water temperature is higher than +9°C, 90 days if the water temperature is lower than +9°C. Some of the more commonly used drugs are:
- **Oxytetracycline** (has been the "drug of choice" for many years. Probably too broad-spectrum.
- **Nitrofurazolidone**. Originally for enteritis, but has a good systemic effect in fish and is especially useful against protozoan/ bacterial diseases eg. *Hexamita*.
- **Trimethoprim/Sulfadiazine** (Tribrissen©). Very good. Expensive.
- **Oxolinic acid** (Aqualinic Powder©). Much used against furunculosis. 10 mg/kg.
- **Praziquantel** (Droncit©). Used against tapeworms (*Eubothrium* spp.) and eyeflukes. 5 mg/kg.
- **Aminonitrothiazol** (Enheptin©). Used against *Hexamita, Spironucleus* etc. 20-60 mg/kg for 3 days.
- **Nifurpirinol**. Expensive. Good for aquarium fish. Use at 2-4 mg/kg for 7 days.
- **Erythromycin**. Considered the drug of choice for BKD. 100 mg/kg/10 days.

100 mg/kg fish per day for 10 days, is a dose rate which applies to many antibiotics. For small quantities of food, melted gelatin may be used to apply the drug to the food. Oxolinic acid is often used at 30 mg/kg (this is three times the Parke-Davis recommendation) and can be applied to a 25 kg bag of feed by mixing with a 1 litre bottle of cooking oil.

Antibiotic resistance is commonly seen in aquaculture, and there is increasing concern over drug residues in food derived from farmed fish. This concern applies not only to antibiotics but to all chemotherapeutics used in fish production, including formalin, anesthetics, hormones used for genetic manipulations etc. To give some idea of the scale of use, 48 tonnes of antibiotics were used in Norway in 1987, but only 32 in 1988; thus the industry there is trying hard to get things under control. Nevertheless, from 1985-1987, consumption of antibiotics in aquaculture surpassed the combined consumption by humans and other animals during the same period. Concern is being focussed in some counties on the quinolones (eg. oxolinic acid, flumequine), which are used commonly for Gram-negative infections in fish, and which are also very useful for treating human urinary tract infections.

III Parenteral treatments.
May or may not require anesthesia. We often give I/P injections of antibiotics to valuable aquarium species which have stopped eating. Also commonly employed in a farming situation to try and prevent transfer of diseases such as furunculosis to sea-cages, or to ensure high levels of antibiotics in broodstock. Hormones are also administered by this way to induce spawning.

IV Vaccines.
Commercial vaccines are available for the major salmonid bacterial diseases of *Vibrio anguillarum, Vibrio salmonicida, Yersinia ruckeri* (enteric redmouth) and *Aeromonas salmonicida*. The efficacy of the latter is questioned by some, but in certain situations, it may prove useful. These are mostly inactivated whole cells. For other species of fish there are: *A. hydrophila, Edwardsiella ictaluri, E. tarda, Pasteurella piscicida, Pseudomonas anguilliseptica, Renibacterium salmoninarum, V. parahaemolyticus*. Protection levels afforded by these vaccines vary according to the route of administation, to the preparation used

and to the number of times it is used. Thus some are 45-50% effective while others can be 100% effective. A vaccine against *Ichthyophthirius multifiliis*, employing cilia from cultured *Tetrahymena* sp. has also been developed. Methods of administration include:

(1) oral with vaccine incorporated in the food. This is little used, but with possibly great potential, as there is virtually no stress on the fish. Formulations designed to bi-pass the stomach may be necessary. It has met with some success with *A. hyrophila, Cytophaga columnaris*, and *Vibrio*.
(2) immersion techniques, whereby batches of fish are placed for short periods of time in high concentrations of the vaccine - uptake would appear to be mainly through the gills. So-called hyperosmotic methods where fish are initially "dehydrated" in salt solutions prior to being "rehydrated" in the vaccine solution offer only a marginal increase in efficiency. Vaccines often used at a 1 in 10 dilution with exposure of 5 to 10 seconds. Longer times may not add to protection. An alternative is to use dilutions of 1 in 500, but for extended periods of time eg. 1 hour. This method can be effectively combined with transport, but water quality, especially oxygenation, needs to maintained.
(3) spray techniques - using either low or high pressure sprays. Seems to confer no advantage over immersion, and may have the disadvantage of higher stress levels, as the fish are passed on a conveyor-belt type set-up out of water.
(4) intra-peritoneal injections. Gives the best protection and is practical on a commercial scale even with small fish. 30-50 gr is considered the minimum practical size for a commercial operation, using multi-dose injectors. Up until the time the fish weigh 30-50 gr protection may be afforded by means of immersion vaccination.

Notes. Protection usually lasts at least 1 year and rate of onset after vaccination is temperature dependent - it may take 40 days at 4°C, but only 10 days at 10°C in salmon. Small fish (i.e. less than 4 gr) may lose protection more rapidly (3 months) than older fish and repeat vaccination may be necessary in these situations. There is little use of vaccines against viral diseases, although there is little doubt that this will change in the future.

V Management.
As with most farming practices, good husbandry helps prevent disease outbreaks by reducing stress to a minimum. Areas of concern include:
- use of genetically resistant stock. This is really only in its infancy, but there is good evidence that some strains of fish are indeed genetically resistant; this has been demonstated with rainbow trout against furunculosis, and correlated with high serum neutralizing antibodies (1:1256 v. 1:80) in the resistant fish. It has also been seen in protozoan diseases such as PKD; the finding of IPN virus in one Irish farm prompted the complete destruction of all rainbow trout stocks on a facility that had remained closed to new importations for 25 years. The result of this action was massive mortality due to PKD in all the newly imported "naive" stocks. There is probably substantial room for improvement in the selection of genetically resistant fish.
- stocking densities. If too high, water quality may be reduced. Compare inflow and outflow water chemistry. Of interest, it has been shown for rainbow trout that serum cortisol levels (usually taken as measure of stress) are inversely related to the stocking density; maybe this lowers the need for hierarchy. Thus the most appropriate stocking density will vary with the species of fish, independently of water quality.
- cleanliness. Check that dead fish/eggs are removed as soon as possible, as these are a ready reservoir of infection. Utensils should be regularly disinfected.
- feeding practices. Check quantity and quality of feed. Too much can frequently rapidly lead to bad water quality in an aquarium system, especially in a new tank where the filter is not properly established. Merely removing half the water and replacing with fresh may cause an almost miraculous recovery in a tank full of sick fish. Check that the size of the feed pellets are appropriate for the size of the fish - a too rapid increase in pellet sizes may be extremely stressful for Atlantic salmon in cages, and this has been

identified as a major factor predisposing to "pancreas disease". Similarly too large pellets may stick in the pharynx of young fish and predispose to mycotic infections. Merely changing the pellet sizes may effect recovery in the population. With a group of sea-cages, it may be more important than is presently recognized to ensure that the fish are fed in the same order every time (compare with pigs). With many bacterial diseases which occur at higher water temperatures, feeding levels of food to deliver antibiotics may be contraindicated (food requires more of the scant O_2 levels for metabolism). Thus merely stopping feeding altogether may be the appropriate treatment - hard to recommend sometimes. The older pracice of feeding the fish as much as they could take has largely been superceded by once or twice a day feeds in Atlantic salmon cages. This has probably resulted in more efficient uptake of nutrients, especially the fat-soluble ones, which require a longer time in the gut for proper digestion.

- record keeping. If treatment is required, a thorough knowledge of water flows, stocking densities, daily mortalities, water chemistry etc. is crucial to effective fast action. Encourage the farmer to keep such records.
- disinfection. Following outbreaks of disease, this may be necessary. Draining ponds/tanks once a year and exposing them to sunlight is most beneficial. Quicklime at 1 lb/square yard may also be useful for ponds. Hypochlorite is good for tanks.
- control of wild fish, vectors and intermediate hosts, in the case of metazoan parasites. This can sometimes be feasible, eg. the control of aquatic snails with moluscicides, scaring fish-eating birds by using wires over ponds, and the introduction of parasite-eating fish such as cleaner-wrasse, or mosquito fish.
- preventing entry of disease. Buying eggs or fish from specific pathogen free sources may be a start, but the notoriously unreliable methods currently available to detect pathogens is not a guarantee of freedom. It is wise to disinfect eggs prior to arrival at a farm using an iodophor such as Wescodyne at 50-100 ppm for 10 minutes - this will help kill surface viruses (not internal ones) but will not control some bacteria such as *Aeromonas salmonicida* which may require eg. proflavine treatment. Maintain new entries in quarantine if possible. This applies equally to aquarium fish, in which case even a routine round of treatments may be advisable, as to farmed fish entering a farm, or a country. Disinfecting effluents from stringent quarantine facilities usually employs chlorine, ultra-violet or ozone, or sometimes a combination of all three. Disinfecting the boots of visitors (if any) and the wheels of vehicles may be a sensible precaution. It is certainly a good idea for the practitioner trying to avoid the accusing finger!

Metazoan Parasites and Common Fish Diseases

Trent Bollinger, February, 1996

General Metazoan Parasitology

Many species parasitize fish but most, unless in very high numbers, appear relatively harmless. Conditions which concentrate fish and their intermediate hosts, such as fish farms, can result in parasite burdens which may be harmful to the fish. Parasites are common in wild fish and are often recognized in angled or netted fish. In this section I will review the basic life cycles of the important classes of metazoan parasites of freshwater fish and provide a few common examples.

Internal Parasites

Nematodes

Nematodes generally have a round cylindrical body and are bilaterally symmetrical. They commonly occur in both larval and adult forms in freshwater fish. Most adult nematodes occur in the stomach or intestine of fish although some occur in other tissues. Larval nematodes can occur in any tissue and their migrations can cause tissue damage. ex. *Contracaecum, Philometra*

The basic life cycle is as follows.

- adults found in fish or fish eating birds and mammals
- eggs are released into the environment, which subsequently hatch to release a free-swimming larvae; in other species free-swimming larvae are directly released into the environment.
- larvae eaten by invertebrate intermediate host where further development occurs.
- invertebrate eaten by fish where they either complete development to the adult stage or the larvae encyst in the fish, depending on species of fish.

- larvae may transfer between paratenic fish hosts before being eaten by a suitable fish or piscivorous mammal or bird, in these hosts development to the adult stage occurs.

Cestodes

Adult cestodes are common in the digestive tract of fish and are generally harmless. Larval forms occur in the abdominal cavity and/or other tissues of fish. Migrating larval forms called plerocercoids can cause extensive tissue damage which may be harmful to the fish. Plerocercoids in fillets are aesthetically undesirable. ex. *Diphyllobothrium, Ligula*

The life cycle is similar to that of nematodes and involves at least one other host. It is outlined below.

- adults are found in fish or in piscivorous birds or mammals
- adults lay eggs which are either eaten directly by an invertebrate intermediate host or the eggs hatch releasing a coracidium which is then eaten
- larval development to procercoid or plerocercoid occurs in the intermediate host.
- plerocercoids in fish or invertebrate intermediate host are eaten by the final host and develop into adult stages.
- procercoids can be eaten by a fish intermediate host where they develop into plerocercoids.

Trematodes

Monogenetic Trematodes

The majority of monogenetic trematodes of fish are external parasites of the body, fins, gills and/or oral cavity. Most species of monogeans are host and tissue specific. They have a direct life cycle. The adults produce eggs which are released into the water where they hatch releasing a ciliated free-swimming larval stage called a oncomiracidium. The larva must locate and infect a suitable host before the cycle is completed. Gyrodactylids produce larvae directly which usually remain on the same host. Transmission to other susceptible hosts is thought to be

by direct contact.

Digenetic Trematodes

Digeneans have a complex life cycle. Adults produce eggs that pass out of the gut of the final host, which may be a fish, bird or mammal. The eggs hatch into a ciliated free-swimming larva stage called a miracidium which infects a mollusc intermediate host. Cercariae develop and either directly infect a suitable definitive host to complete the life cycle or it infects another intermediate host. In the fish or invertebrate intermediate host the cercaria encysts to form a metacercaria. Ingestion of the intermediate host by a suitable definitive host completes the life cycle. Adult digeneans typically inhabit the digestive tract but some infect the swim bladder, ovary, peritoneal cavity, urinary bladder or circulatory system. Migrating cercariae can cause tissue damage. Encysted metacercariae are often found in large numbers with apparently little effect on the host. Metacercaria can render fillets unpalatable or aesthetically unpleasing. Metacercaria which are white or yellow are often referred to as yellow grub or white grub. Hypergpigmentation of adjacent tissues can make the lesions black and when this occurs it is often referred to as black spot disease.

An important trematode infection of freshwater fish is the eye fluke, *Diplostomum spathaceum*. This parasite is found in a number of species of fish. The first intermediate host is an aquatic snail. Cercaria released from the snail penetrate the skin of a second fish intermediate host where they develop to the metacercaria stage in the lens of the eye. Metacercaria can cause opacity or cataracts in the lens which, if severe, can impair vision. The life cycle is completed when the final host, primarily gulls and terns, ingest fish containing metacercaria. The adult fluke is found in the intestine of these final hosts. Other species of *Diplostomum* infect the retinal layer of the eye.

External Parasites

Many different types of organisms live on the skin or gills of fish. These often have little

effect on the host, but lesions can be severe if there is heavy infestation. Damage to the skin and gills by these parasites can result in secondary infections by opportunistic pathogens.

Leeches

Leeches are occasional parasites of freshwater fish. They are similar in appearance to flukes. They attach to the skin of the host via an anterior sucker and take blood meals. Heavy infestations with flukes can cause anemia. Leeches have a direct life cycle. Adults are hermaphroditic and produce a cocoon which they retain or attach to the substrate. Juvenile leeches hatch from the cocoon and attach to the host.

Copepods

Copepods are a type of crustacean which can be a significant pathogen in freshwater fish. Most copepods have 2 main body divisions, the cephalothorax and abdomen. Mouth parts are modified for piercing and sucking. Parasitic copepods have complex life cycle which involves several larval stages separated by moults. Eggs are laid by the female which hatch to release free-living nauplii larvae. These undergo a series of moults eventually forming a copepodid stage which may be free-swimming or parasitic on a fish intermediate host. Larval stages released from intermediate hosts or previously free-living forms develop to young adults which then mate. Females are found attached to the fish final host and can often be recognized by conspicuous egg sacs. Males are typically not parasitic.

Copepods typically are found attached to the gills and skin. Heavy copepod infections can be debilitating and can lead to secondary bacterial and fungal infections. Skin lesions are often hemorrhagic and there can be focal hyperplasia and fibrosis. ex. *Lernaea* spp. (anchor worms)

Common tumors of freshwater fish

Papillomas

Skin tumors are often referred to as papillomas. They vary in size from small flat plaques to larger protruding masses and can be found anywhere on the body. Contaminants and viruses have been associated with skin tumors. Epidermal hyperplasia in walleye and pike are multifocal hyperplastic plaques caused by viruses.

Lymphocystis

Lymphocystis is a chronic self-limiting disease characterized by pale variable sized granular masses on the skin. The disease is caused by virus which infects fibrocytes causing them to massively hypertrophy or enlarge. The enlargement of individual fibrous cells accounts for the granular appearance of the masses. Lymphocystis is rarely fatal but can be disfiguring. Transmission is thought to occur by sloughing of infective cells into the water which then infects abraded skin of another host. Virus remains viable in the water for about 1 week.

Dermal Sarcoma or Fibrosarcoma of Walleye

This tumor is a smooth , pale, firm, rounded nodule which may be focal or multifocal and occurs anywhere on the skin. The tumor is caused by a retrovirus. Walleye dermal sarcoma is more prevalent in the spring has appears to have a higher prevalence in some watersheds.

Lymphosarcoma

Lymphosarcoma (a tumor of lymphocytes) is a well recognized condition of northern pike and muskellunge caused by a retrovirus. Lesions begin as small nodules in the skin which then invade the underlying musculature. Eventually the tumor may become disseminated and involve the liver, spleen and kidney. In muskellunge the disease is usually fatal but in pike recovery may be the norm.

Miscellaneous conditions

Skeletal deformities

Skeletal deformities can involve any component of the skeletal system including: fin rays, gills, pelvic or pectoral fins, cranium, mandible, and spine. Skeletal abnormalities are common in young hatchery-reared and wild fish. Lesions can result from abnormalities in muscle, nerve, cartilage and bone. In individual cases the cause is often unknown. In farmed fish and under experimental conditions these deformities have been associated with nutritional deficiencies or excesses. Heavy metals and other contaminants have also been implicated.

Walleye Myopathy

Walleye myopathy is a syndrome characterized by degeneration, necrosis, mineralization, inflammation and fibrosis of the trunk musculature of walleye. Affected muscles have a yellow-tan appearance and are often gritty and tough. The cause of this condition is unknown but some believe it is related to deficiencies in vitamin E and/or selenium, analogous to a condition seen in domestic animals.

Fungal Infections

Saprolegniosis

Water molds are widespread in the aquatic environment. They live on dead material and are important in the decay process. Water molds can also infect fish usually in areas of skin damage caused by trauma, parasites or bacteria. Infections are characterized by pale cottony growths on the skin and gills of the host. Most are superficial infections of the skin and do not penetrate deeply into the subjacent musculature. Trapped mud or algae can alter the color of the water mold. Infection is usually via motile zoopsores in the water. Decaying plants and animals and other infected fish can be sources of infection. Most infections are thought to be associated with immunosupression of the host. Water molds are generally opportunistic pathogens; however, some can be primary pathogens. Members of the *Saprolegnia parasitica-declina* complex appear able to establish primary infections. Species of *Saprolegnia* are most commonly

isolated from infections in fish and for that reason the disease is generally called saprolegniosis. Other species of water mold can cause similar clinical signs.

NEOPLASIA AND ENVIRONMENTAL CONTAMINANTS

F.A. Leighton
February 1996

NEOPLASIA IN FISH

Neoplasms and neoplasia

Most of the lumps and bumps seen on or inside fish are neoplasms. Other terms that mean the same thing are "tumour" and "cancer", although "cancer" should be reserved for the worst kinds of neoplasms, the ones that cause great damage as they grow and often spread to many organs and tissues. Neoplasia is the process or sequence of events that result in a neoplasm.

Neoplasia often seems like a mysterious and somewhat frightening process and it is usually discussed in complicated technical language. However, we now know quite a bit about neoplasia and what is known can readily be understood, especially by biologists who have some understanding of the concept of evolution.

What is a Neoplasm ?

A neoplasm is an abnormal proliferation of cells with certain characteristics. The growing mass of cells distorts the size and shape of the normal tissue. The growth and multiplication of the neoplastic cells is not under the normal control mechanisms of the body that determine the size, shape and function of organs and tissues. These cells are beyond the control of the body and are growing independently. Stranger still, neoplastic cells continue to grow out of control even after the various agents that caused the cells to become neoplastic in the first place are no longer present - ie even after the cause has been removed.

Neoplasia

Neoplasia is a disorder of a cell's genes that causes the cell and its daughter cells to evolve the traits and characteristics we recognize as typical of neoplastic cells. To understand neoplasia, it is useful to refer to some concepts about genetics and about the evolution of species.

Concepts from Genetics:

1) Genes are strings of DNA (Deoxyribonucleic acid). These strings contain a code for a particular protein. When the gene is activated, the code is translated into the protein and the protein gives the cell certain characteristics and abilities.

2) When DNA is damaged in the cell, special repair processes are able to fix the damage so the genetic code is not changed. These repair processes involve a large number of special proteins and enzymes in the cell's nucleus.

3) Defects in DNA that are repaired incorrectly result in changes in the genetic code in a gene. Such a change in a gene is called a *genetic mutation*. A mutation may have no significant effect on the protein the gene encodes or it may alter the structure of the protein in important ways. Mutation is one way that genetic variation is created so that evolution can take place.

Concepts from Evolution:

The two key requirements for evolution of new characteristics are

1) Genetic variation - there must be new genes for new traits to arise in individuals

2) Natural selection - there must be environmental circumstances such that individuals that possess a certain trait survive and reproduce more than individuals without these traits. In this way, the new traits become established. These environmental conditions often are called "selection pressures"

Neoplasia can be viewed as the evolution of aberrant cells, created through mutations caused by agents that damage DNA and propelled through their course of evolution by other agents and conditions that give these aberrant cells a selective advantage in their survival and reproduction (division).

Four Stages in the Evolution of a Neoplasm

By studying how chemicals cause cells to become neoplastic, four stages have been identified in the sequence of events that produces neoplasms. These four stages are outlined on the attached diagram.

Initiation: This is the first key event. Something causes damage to DNA and faulty repair of the damage results in a mutation. Most mutations will not lead to neoplasia, but a very few will. These must be mutations that change the cell in some way that gives it a selective advantage in survival and reproduction under certain circumstances.

It may be that the critical mutations that lead to neoplasia occur in the genes that carry the genetic code for the molecules involved in repair of DNA. If one of these molecules is not working right, there is an increased chance of faulty repair of DNA and, thus, of more mutations. This agrees with one observed property of initiated cells: they appear to be more prone to mutation than normal cells of the same origin.

Initiation is one step in which environmental contaminants can be said to cause neoplasia. Certain chemicals can damage DNA and thus greatly increase the chances of mutations occurring.

STAGES IN THE DEVELOPMENT OF A NEOPLASM

INITIATION	→ PROMOTION	→ TRANSFORMATION	→ PROGRESSION
- GENETIC MUTATION - NEW TRAITS	- NEW ENVIRONMENT - NATURAL SELECTION - FURTHER MUTATION - FURTHER SELECTION	- INDEPENDENT GROWTH	- FURTHER MUTATION - FURTHER SELECTION - MALIGNANT BEHAVIOUR
CAUSE: AGENTS THAT DAMAGE DNA	CAUSE: AGENTS THAT CHANGE THE CELL'S ENVIRONMENT AND FAVOUR THE NEW TRAIT(S)	INDEPENDENT OF "CAUSE" (VIRUSES START HERE ?)	

Cells that have been initiated (carry mutations that might lead to neoplasia) will not develop further unless something gives them a selective advantage over other cells in the same tissue. The events that gives these cells such a selective advantage is commonly called "promotion."

Promotion: Promotion really means a change in the environment of a tissue that favours the initiated cell. The change might be exposure to another environmental contaminant. For example, suppose the mutation in an initiated cell has changed the gene for an enzyme used to detoxify certain kinds of poisons that might otherwise damage the cell, and suppose the new form of the enzyme is more efficient than the original form. Now, if the animal is exposed to a toxic chemical that its new enzyme can detoxify more efficiently than can the other cells in the tissue, the initiated cell will be able to grow better and divide more often that the other cells. In this setting, the toxic chemical is called a "promoter", but it really is just a new selection pressure that favours the growth and multiplication of the initiated cell. The end result is a small colony of cells with the new genetic mutation that first occurred in the initiated cell.

Many environmental contaminants are known to be able to act as promoting agents in the development of neoplasms. In this sense, these chemicals also are "causes" of neoplasia because without promotion - new pressures for natural selection among cells in a tissue - initiated cells do not develop into neoplasms.

It also is known that many factors other than environmental contaminants can have some influence as promoting agents. For example, chronic infection and inflammation can act as a promoting influence in the infected and inflamed tissue. Thus, promotion can be complex, in the same way that selection pressures on individual animals and plants that drive the evolution of new traits and new species can be complex. However, the basic concept is quite clear.

Multiple cycles of genetic variation (mutation) and selection (promotion): Since the initiated and now promoted cells are prone to new mutations, further cycles of mutation and selective growth of new genetically-different cells can take place. Essentially these are additional cycles of the events described as initiation and promotion, with cells developing new traits by mutation and some of those new traits conferring a growth advantage on the cells that have them.

As more and more mutations occur, eventually mutations occur in a group of genes that normally control the processes of cell growth and division. These genes have been named "oncogenes" ("onco" means neoplasm) because they appear to be so important in the development of neoplasms. When mutations occur in these genes, the cells cease to respond to the signals from the body that normally control cell growth. Instead, they begin to divide on their own schedule.

Transformation: When cells cease to respond to normal controls on growth and grow and divide on their own, they fit our definition of a neoplastic cell. These cells now can be considered "transformed" into truly neoplastic cells. A true neoplasm has developed in the animal.

Progression: The processes of genetic variation through mutation and selective growth of certain new variant cells can continue even after the cell has become truly neoplastic. Now, however, no special promoting environment is needed. The most successful cells will be those that can live and grow out of control in the normal physiological environment of the animal. Unfortunately, the traits that give an autonomously-dividing cell population a growth advantage in the body are the same traits we see in truly cancerous cells: the ability to invade and destroy tissue, and to spread to new locations. Thus, neoplasia tends to go from bad to worse as the neoplastic cells continue to evolve new and more destructive characteristics.

What Causes Cancer ?

It is evident that "cause" can be complicated when it come to neoplasia. If a neoplasm arises because of exposure to both an initiating and a promoting chemical, both chemicals are equally implicated as having caused the neoplasm. When promotion results from a complex interaction of different factors, the concept of cause is even more difficult. Some toxic substances, like cigarette smoke, contain both initiators and promoters and can cause neoplasia without assistance from other factors. Often, cause is much more complicated.

Viruses and Neoplasia: Certain viruses cause neoplasms. These viruses cause neoplasia by putting their genes right into the cell's genetic code. This alters and switches on and off those all-important genes that control cell growth and division - the oncogenes. Thus, infection with these viruses appears lead directly to cell *transformation,* bypassing the steps of initiation and promotion.

Concluding Points

1) Neoplasia develops by a process of evolution of aberrant cells

2) "Cause" often is complex in neoplasia. Thus it can be difficult to attribute a high rate of neoplasia to a particular chemical or pollutant.

3) Neoplasia involves a long series of chance events. Thus, its occurrence is predictable statistically but not in individual animals exposed to initiating and promoting agents.

ENVIRONMENTAL CONTAMINANTS AND NEOPLASIA

It is well-documented that higher rates of neoplasia, especially of the skin and the liver, occur in fish from polluted waters compared with the same species and ages of fish from un-polluted waters

Only in a very few cases has it been possible to conclusively relate neoplasia in fish to particular contaminants in particular environments. One example is flatfish living on sediments containing high concentrations of hydrocarbons. 60% of these fish had liver tumours while such tumours were very, very rare in the same species living on un-polluted sediments.

Fish in polluted environments often also have higher rates of certain infections, altered nutrition, more physical injuries and higher levels of stress than fish in un-polluted environments. All of these factors can contribute to the development of neoplasia, particularly at the stage of promotion.

The occurrence of neoplasia in fish does not necessarily imply that the fish has been exposed to environmental contaminants. However, high rates of neoplasia can be an indicator of exposure to contaminants.

Some neoplasms can occur at high rates in fish with no relationship to levels of environmental pollution. Examples are lymphosarcoma of northern pike and muskellunge and dermal fibrosarcoma of walleye (both are caused by viruses).

ENVIRONMENTAL CONTAMINANTS

There are hundreds of different chemicals that enter the environment because of human activity and can be considered environmental contaminants. A few groups of these chemicals have been selected for comment here. These groups represent some of the contaminants that are found in the aquatic environment in western Canada and may affect fish:

- Pesticides
- Metals
- Hydrocarbons
- Nitrate Fertilizers
- Radio-Active Materials

Pesticides

The majority of chemicals used in agriculture and forestry as "pesticides" are either herbicides, used to kill unwanted plants, or insecticides, used to kill insects. In general, herbicides are of relatively low direct toxicity to fish, although they can affect aquatic plants and thus lead to food shortages or low oxygen caused by rotting dead vegetation. Insecticides, on the other hand, include many extremely poisonous chemicals.

Insecticides: There are four main categories of pesticide currently in use in large quantity in North America. These are listed below together with some of their important features. A list of the various insecticides that fall into each category also is included. Note that each insecticide has an official common name, listed here, but also one or more commercial or trade names. The official common name will be found on the label in the list of ingredients.

All of the insecticides listed here are nerve poisons that kill by altering nerve transmission, particularly in the brain but also in the nerves outside of the brain.

- Organochlorines: These were the insecticides that came into wide-spread use in the 1940's. DDT is the prototype chemical of this group. In North America, use of most organochlorines has been disallowed since the early 1970's, but some, like lindane, mirex, and methoxychlor, are still used extensively. Organochlorines degrade very slowly in the environment and thus persist for decades. In addition to their action as nerve poisons, some have other toxic effects at low concentrations; some have sex hormone (estrogen) activity, others block eggshell formation in birds, etc.

NOTE: In the lists of chemicals that follow on pages 2 and 3, some fungicides and herbicides of the same chemical classes are included along with the insecticides.

Organochlorines

Aldrin
Benzene hexachloride
Chlorbenside
Chlordane
Chlordecone
Chlorfenethol
Chlorobenzilate
Chloroform
Chloroneb
Chloropicrin
Chloropropylate
DBCP
D-D
DDT
Dicamba
Dichloropropane
Dichloropropene
Dicofol
Dienochlor
Endosulfan
Endrin
Epichlorohydrin
Ethylan
Ethylene dichloride
Heptachlor
Hexachlorobenzene
Lindane
Methoxychlor
Methylene chloride
Mirex
PCNB
Pentachlorophenol
Tetrachloroethylene
Tetradifon
Toxaphene
Triclopyr

- Organophosphates and Carbamates: These are highly-toxic chemicals that replaced the organochlorines and now are widely used. They have the same mode of toxic action and so are listed together. They are much less persistent than the organochlorines but they do persist for periods of weeks to months in the environment.

Organophosphates:

Acephate-met
Akton
Azinphos-methyl
Bomyl
Bromophos
Carbophenothion
Chlorphenvinphos
Chlomephos
Chlorpyrifos
Coumaphos
Crotoxyphos
Crufomate
Cyanophenphos
Cyanophos
Cythioate
Demeton
Demeton-methyl
Dialifor
Diamidfos
Diazinon
Dicrotophos
Dimefox
Dimethoate
Dioxabenzophos
Dioxathion
Disulfoton
Ditalimphos
DMPA
Edifenphos
EPN
Ethion
Ethoprop
Etrimfos
Famfur
Fenamiphos
Fenitrothion
Fensulfothion
Fenthion
Fonofos
GC 6506
Malathion
Methidathion
Methyl parathion
Mevinphos
Monocrotophos
Naled
Omethoate
Oxydemeton-methyl
Parathion
Phorate
Phosalone
Phosmet
Phosphamidon
Phoxim
Pirimiphos-ethyl
Pirimiphos-methyl
Ronnel
Sulfo TEPP
Sulprofos
Temephos

Organophosphates (Cont'd)

Dicapthon
Dichlophenthion
Dichlorvos
Isazophos
Isofenphos
Leptophos
TEPP
Terbufos
Tetrachlorvinfos
Triaziphos
Trichlorfon
Vamidothion

Carbamates:

Aldicarb
Aminocarb
Bendiocarb
Bufencarb
Butoxycarboxim
Carbanolate
Carbaryl
Carbofuran
Dioxacarb
Diram
Ethiofencarb
Formetanate
Hydrochloride
Methiocarb
Methomyl
Mexacarbate
Oxamyl
Trimethacarb

- Pyrethroids: These are derivatives of pyrethrin, a naturally-occurring insecticide found in certain plants. They generally are less toxic to vertebrates than are the other classes of insecticide, but some are quite toxic to fish. They are not persistent in the environment like organochlorines.

Pyrethroids

Allethrin
Barthrin
Bifenthrin
Bioallethrin
Bioresmethrin
Cismethrin
Cyfluthrin
λ-cyhalothrin
d-cis,trans-allethrin
Deltamethrin
Dimethrin
Esbiothrin
Fenpropathrin
Fenvalerate
Flucythrinate
Fluvalinate
τ-fluvalinate
Kadethrin
Permethrin
Phenothrin
Resmethrin
S-bioallethrin
Synthetic pyrethrum
Synthetic pyrethrins
Tefluthrin
Tetramethrin
Tetramethrin (1R)-isomers
Tralomethrin

Metals

Certain metals can be highly toxic, for example mercury, lead, chromium, cadmium, and selenium. In general, metals tend to be general poisons that affect many tissues and organs at the same time. However, some organs may be affected more than others. For example, mercury has a particularly strong affect on the brain; selenium can accumulate in reproductive organs and cause reproductive failure as a major toxic effect.

Metals are elements and thus are not degraded and destroyed in the environment. Instead, they tend to cycle between living and non-living components of the ecosystem and to remain in the environment indefinitely.

Some metals can be incorporated into organic molecules and they become much more toxic in these organic forms than they are as pure metal ions. For example, mercury can be converted to methyl-mercury and selenium can be coupled to the amino acid methionine.

Hydrocarbons

Hydrocarbons are the main constituents of petroleum oil, coal and gas, and they are used in many chemical manufacturing processes as well as serving as fuels. Thus, they occur as environmental contaminants around many industrial sites.

There are a huge number of different hydrocarbons and related compounds. It is useful to recognize two very different type of hydrocarbon contaminants:

Polycyclic Aromatic Hydrocarbons (PAH): These are large molecules composed of multiple benzene rings (hence,polycyclic and aromatic). They persist in the environment but do not dissolve readily in water. Exposure to fish is usually by contact with contaminated sediments. Their main toxic effect is as a cause of neoplasia.

Volatile Hydrocarbons: These are small molecules that evaporate quickly but also can reach potentially-toxic concentrations dissolved in water. Benzene and hexane are examples. These hydrocarbons are able to cause acute toxicity. Their affect is similar to that of anaesthetics through alterations of nerve function. They can cause acute toxicity but also can disappear quickly from the environment.

Nitrate Fertilizers

These are included because run-off can deliver considerable quantities of fertilizer to the aquatic environment and because of a particular acute toxicity associated with nitrates. In the aquatic environment, nitrate can be converted quickly to nitrite and nitrite is acutely toxic. Nitrite reacts with hemoglobin in the blood and

changes the hemoglobin to a form called methemoglobin. Methemoglobin can not carry oxygen. The effect is the same as lowering the amount of oxygen in the water and asphyxia is the result.

Radioactive Substances

There are many different radio-active materials that can enter the environment from natural sources, from uranium mining activities and from reactors and nuclear explosions. All share the property of emitting harmful radiation. The toxicity of radioactive substances depends entirely in the dose of radiation an animal receives.

At *high doses*, radiation is immediately damaging to cells. It tends to kill cells of the immune system (bone marrow), skin and intestine. Death often is the result of overwhelming infections after the immune system has been destroyed.

At *low doses*, constant or repeated exposure to radiation primarily causes damage to genes. This toxic effect is expressed as developmental defects and as increased rates of neoplasia

Three Additional Points about Environmental Contaminants

1) Species differ in their sensitivity to various contaminants.

When contaminants kill fish, often some species are affected more severely than are others. Only at very high doses would you expect to see all species of fish killed at similar rates. Differences in sensitivity to particular poisons are due to differences in metabolism as well as to differences in behaviour, habitat preference and food habits.

2) Some contaminants accumulate along food chains.

Chemicals like mercury, selenium and many organochlorine insecticides increase in concentration at each link in the food chain. Organisms high up in the chain thus receive much higher doses. Toxic effects thus may occur selectively in fish at the top of the food chain - e.g. fish-eating fish like pike and walleye.

3) Contaminants that accumulate in fat can produce toxic effects at the time the fat is used for energy.

Some contaminants, for example, many organochlorine insecticides, dissolve readily in fat and accumulate in fat tissue. They have little toxic effect when stored in this way because the concentration in active body tissues is low. However, when the fat is used for energy, the contaminants are released from the fat stores and are re-distributed in the body to other organs and tissues where they can cause toxic

effects. Thus, the toxic effects of certain contaminants can be delayed and occur in conjunction with seasonal use of fat stores, such as during spawning, or at times of food shortage.

Field Manual
for the
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1990

Copies of this publication may be purchased from the National Technical Information Service (NTIS), 5285 Port Royal Road, Springfield, VA 22161, or from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20401, stock number 024-010-00685-4.

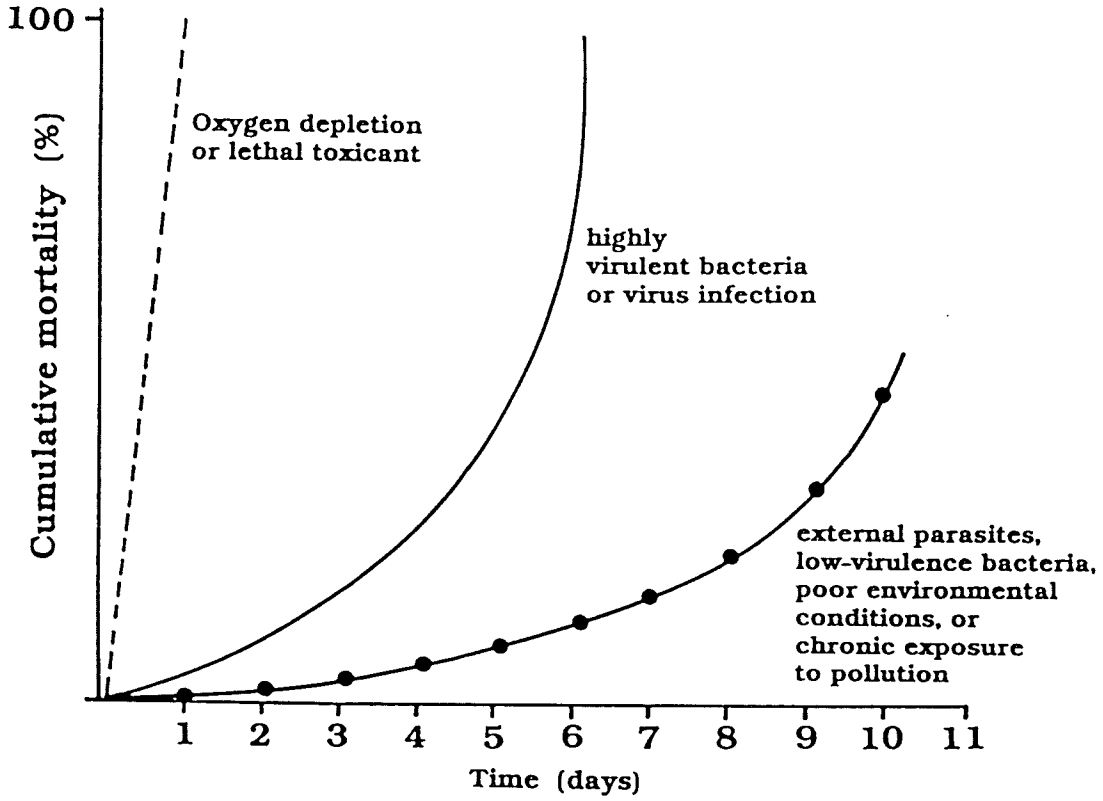


Fig. 3.1. Curves (mortality versus time) associated with three major categories of fish mortality (Wedemeyer et al. 1976).

Table 3.1. *Physical signs associated with fish mortality problems caused by oxygen depletion, toxic algae blooms, and pesticide toxicity (modified from Wedemeyer et al. 1976).*

Physical signs associated with fish mortality	Cause of mortality		
	Oxygen depletion	Toxic algal bloom	Pesticide toxicity
Fish behavior	Gasping and swimming at the surface	Convulsive, erratic swimming, lethargy	Convulsive, erratic swimming, lethargy; if organophosphate pesticide, pectoral fins extended anteriorly
Species selectivity in fish kill	None if depletion is total; common carp and bullheads may survive if depletion is partial	None, all species affected	Usually one species killed before others, depending on fish sensitivity and pesticide level encountered
Size of fish	Large fish killed first, eventually may kill all sizes and species	Small fish killed first, eventually all sizes	Small fish killed first, eventually may kill all sizes
Time of fish kill	Night and early morning hours	Only during hours of bright sunlight, about 9:00 a.m. to 5:00 p.m.	Any hour, day or night
Plankton abundance	Algae dying, little zooplankton present	Abundance of one algal species, little zooplankton present	If insecticide, no zooplankton present, but algae normal. If herbicide, algae may be absent
Dissolved oxygen	Less than 2 ppm, usually less than 1 ppm	Very high, often saturated, or supersaturated near surface	Normal range
Water pH	6.0–7.5	9.5 and above	7.5–9.0
Water color	Brown, gray, or black	Dark green, brown, or golden, sometimes with musty odor	Normal color and little or no unusual odor
Algal bloom	Many dead and dying algal cells	Abundant algae, predominately of one species	Normal bloom of mixed species unless herbicide involved; then algae absent or reduced

CHAPTER 3

Interpreting the Scene

Fred P. Meyer and Roger L. Herman

Introduction

In some instances, the cause of a fish kill is readily apparent (e.g., when an obvious toxic discharge is killing fish). The course of action then is to terminate the cause, document the situation, gather evidence, and charge the perpetrator. Because most fish kills are observed after the fact, it is usually necessary to conduct the type of investigation described in this chapter.

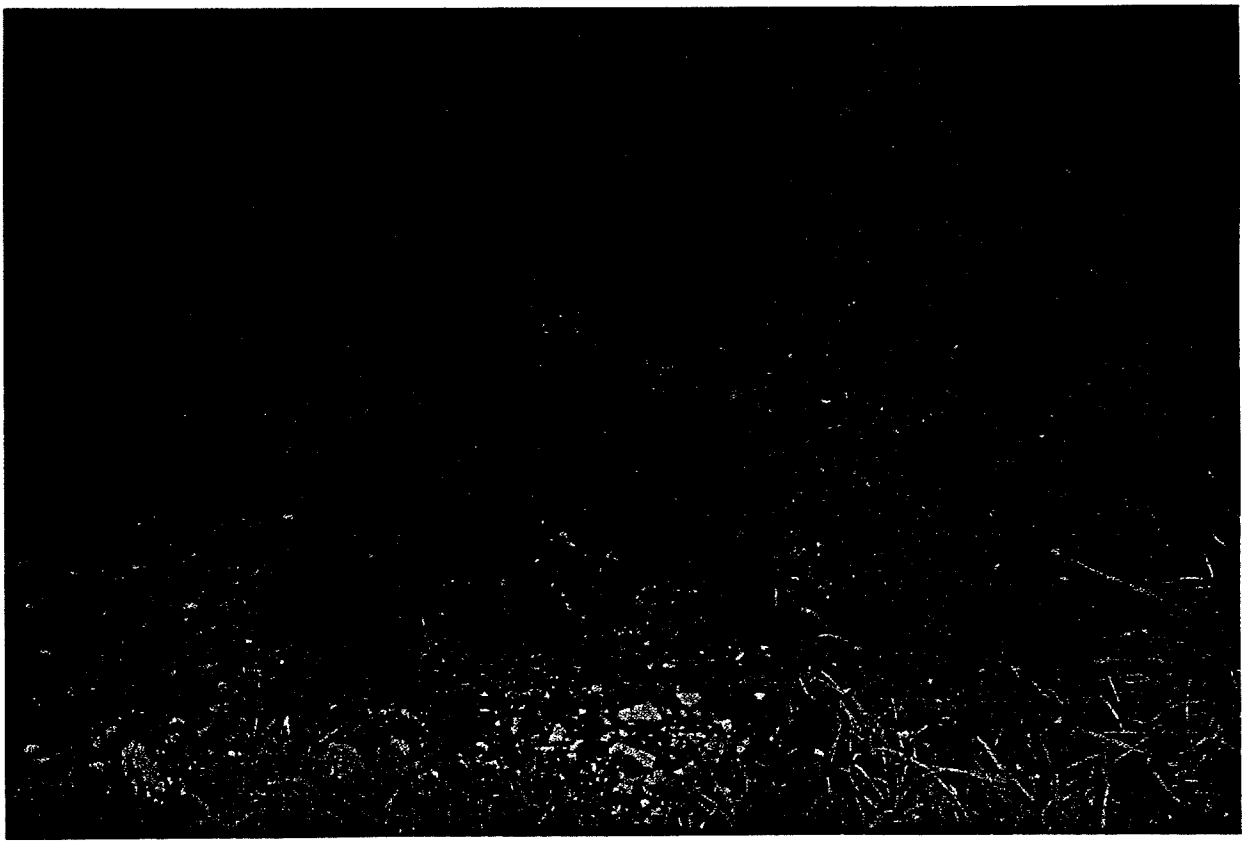
What to Look For

The first few hours after an investigator's arrival on the scene of a fish kill may be critical. It is extremely important that as much information as possible be collected as quickly as possible. Since the investigator is often working alone, it is vital that time be used effectively to gather the information and collect the samples that are likely to contribute most toward determination of the cause of the fish kill.

The presence of dead fish is often the first indication of a serious problem in the environment. (*Photo courtesy of the Missouri Department of Conservation.*)

Immediately upon arrival, the investigator should quickly survey the scene and record the following information:

1. Date and time of day.
2. Location: river, miles of river, lake and area affected, county, nearby highways, cities, or other identifying landmarks.
3. Name, address, and telephone number of person who reported or first noted the fish kill.
4. Names of persons who can provide on-scene information.
5. Time when fish kill was first reported.
6. Estimated time when kill began.
7. Water quality characteristics:
   a. Dissolved oxygen concentration
   b. pH
   c. Water temperature
   d. Conductivity
   e. Color of the water
   f. Odor of the water
   g. Salinity (if in an estuary)
8. Condition of each species of fish seen: live, moribund, dead, or decaying.
9. Condition of other organisms in the ecosystem: live, moribund, dead, or decaying.
10. Weather conditions of the day and previous day and night, such as temperature, cloud cover, recent precipitation, wind direction and speed.
11. Physical appearance of dead and moribund fish, such as gills flared, mouths agape, spinal curvature, excessive mucus, lesions, necrotic areas on gills.
12. Any unusual characteristics, behavior, or other observations of fish or other organisms, such as excessively dark color, odd position of fins, swimming at the surface, loss of equilibrium, fish or crustaceans attempting to get out of the water, excessive mucus, snails out of water on vegetation, tadpoles piping at the surface, discolored vegetation.



Fish that are affected by sublethal toxicosis, low dissolved oxygen, a heavy burden of parasites, or a bacterial epizootic may move to shallow water, vegetation, or shaded areas. They usually ignore the approach of humans.

See Chapter 7 for instructions on what additional data are needed and how the information should be documented. An analysis of this information often makes it possible to rule out several potential causes of a fish kill and may make it possible to distinguish between one or two likely or suspected causes. This reduces the number and types of samples that are required and helps reduce the personnel, equipment, and laboratory work needed when time is critical.

In recording fish kills, it is important to establish the magnitude of the mortality. The significance of a fish kill is always directly related to economic, geographical, and political factors associated with the site, as well as to the ecological effects. The losses of 100 fish in a prime trout stream or any losses due to a possible toxic discharge are always important; in other situations, the loss of thousands of gizzard shad may be of little public concern. The American Public Health Association (APHA) et al. (1985) offers the following guide for reporting fish kills:

Minor kill: less than 100 fish
Moderate kill: 100 to 1,000 fish in 16 km of stream or equivalent lentic area
Major kill: more than 1,000 fish in 1.6 km of a stream or equivalent lentic area

The rate or pattern of loss is a helpful indicator (Fig. 3.1). If all fish died abruptly or within a short time (24 hours or less), it is likely that the kill was caused by a sudden, catastrophic event that made the environment fatally toxic to fish. If the mortality began slowly and then rose sharply over the next 5 to 7 days, the most likely causes would be a slowly developing oxygen depletion or a highly virulent infectious agent. Mortality that continues at a low rate over an extended period may be due to a marginal environment, a low-virulence infective agent, or chronic exposure to sublethal concentrations of a toxic substance.

A second important piece of information is that of the sizes and species of fish affected (Table 3.1). In kills caused by toxic substances, small fish usually die before larger ones of the same species; in oxygen depletion, the reverse is true.

Establishing when a kill began and how long it continued is also often important. It is useful to know whether the kill began at night, how long it continued, and whether it was interrupted for a time and then began anew.

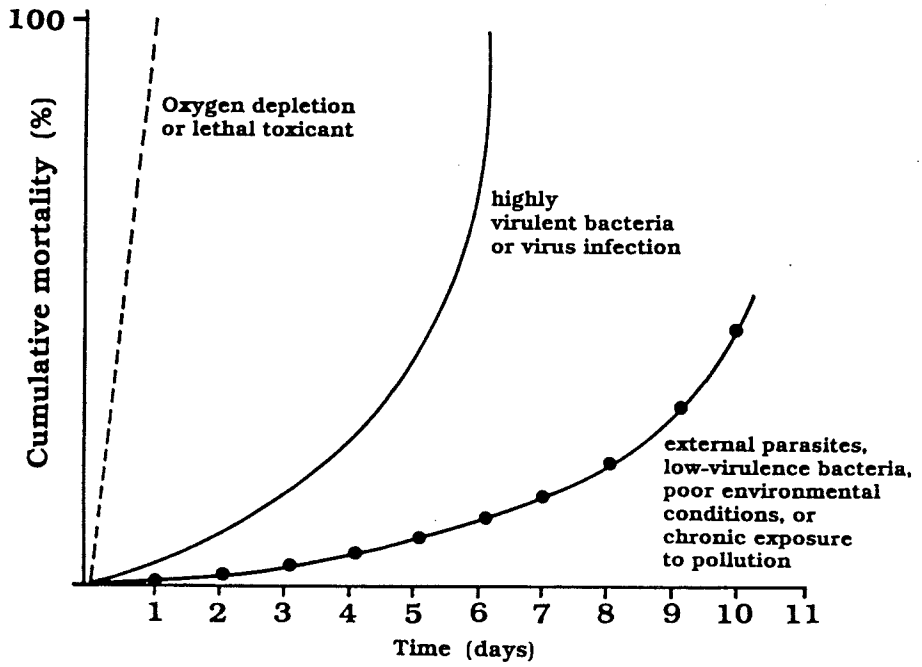


Fig. 3.1. Curves (mortality versus time) associated with three major categories of fish mortality (Wedemeyer et al. 1976).

Table 3.1. *Physical signs associated with fish mortality problems caused by oxygen depletion, toxic algae blooms, and pesticide toxicity (modified from Wedemeyer et al. 1976).*

Physical signs associated with fish mortality	Cause of mortality		
	Oxygen depletion	Toxic algal bloom	Pesticide toxicity
Fish behavior	Gasping and swimming at the surface	Convulsive, erratic swimming, lethargy	Convulsive, erratic swimming, lethargy; if organophosphate pesticide, pectoral fins extended anteriorly
Species selectivity in fish kill	None if depletion is total; common carp and bullheads may survive if depletion is partial	None, all species affected	Usually one species killed before others, depending on fish sensitivity and pesticide level encountered
Size of fish	Large fish killed first, eventually may kill all sizes and species	Small fish killed first, eventually all sizes	Small fish killed first, eventually may kill all sizes
Time of fish kill	Night and early morning hours	Only during hours of bright sunlight, about 9:00 a.m. to 5:00 p.m.	Any hour, day or night
Plankton abundance	Algae dying, little zooplankton present	Abundance of one algal species, little zooplankton present	If insecticide, no zooplankton present, but algae normal. If herbicide, algae may be absent
Dissolved oxygen	Less than 2 ppm, usually less than 1 ppm	Very high, often saturated, or supersaturated near surface	Normal range
Water pH	6.0–7.5	9.5 and above	7.5–9.0
Water color	Brown, gray, or black	Dark green, brown, or golden, sometimes with musty odor	Normal color and little or no unusual odor
Algal bloom	Many dead and dying algal cells	Abundant algae, predominately of one species	Normal bloom of mixed species unless herbicide involved; then algae absent or reduced

Kills caused by toxic substances are usually abrupt. The mortality may begin at any hour and continue until all fish have died or until the substance has been degraded, neutralized, or diluted. Small fish usually die first and affected fish often have convulsions, lose equilibrium, or show other signs of toxicosis (see Chapter 4).

A quick check of limnological or water quality characteristics will yield highly useful information (Table 3.1). If algae are alive and thriving but zooplankton and insects are dead or absent, you should suspect an insecticide as a potential cause. On the other hand, the presence of dead or dying algae, but live zooplankton, would suggest that the substance was herbicidal. If both types of plankton are dead, dying, or absent, an acid, strong alkali, heavy metal, or other highly toxic substance should be suspected.

A review of the previous information should enable the investigator to reach a judgment as to the likely cause of a fish kill and guide decisions about

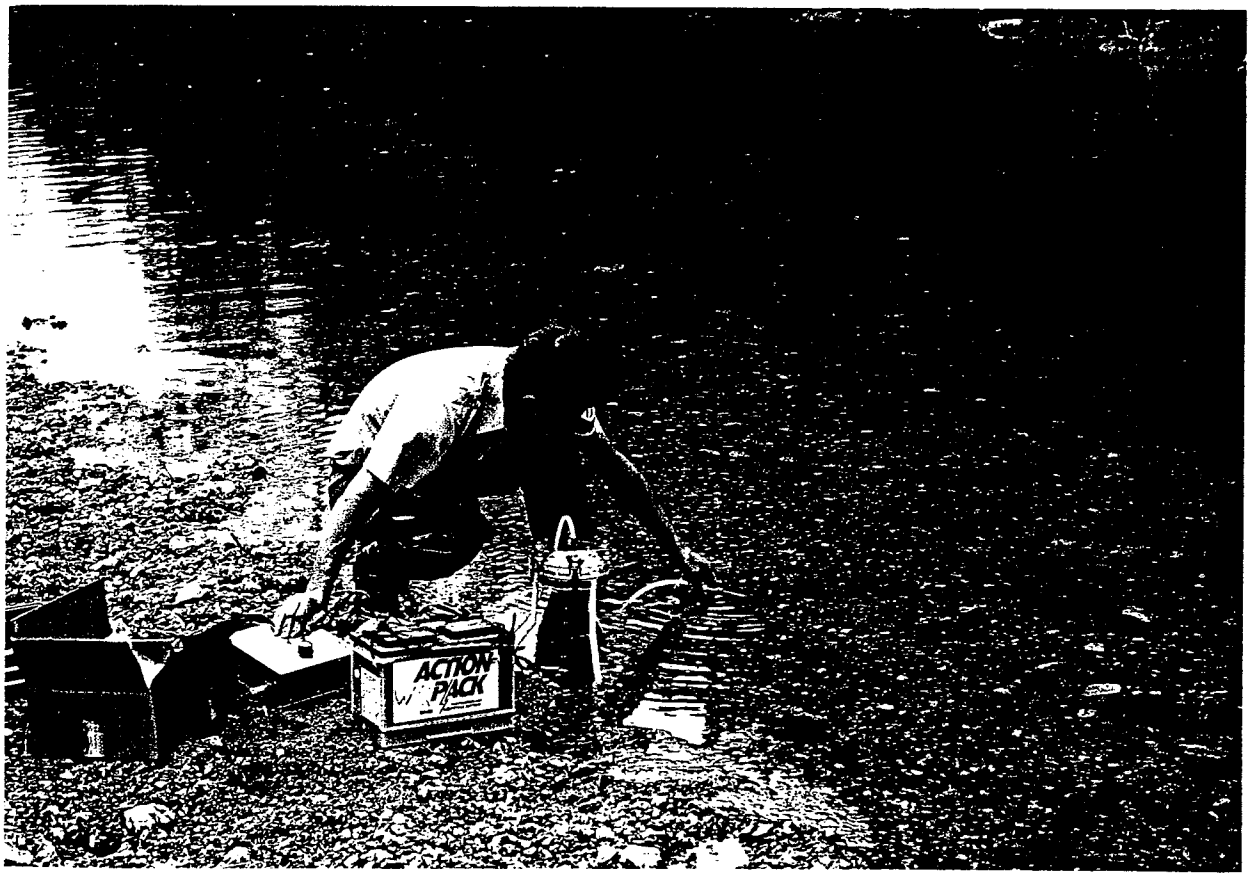
the appropriate course of action to be pursued and the types of samples to be taken. Specific details regarding procedures to follow are given in later chapters relating to each type of cause.

On-site Investigation

The investigation of a fish kill must be conducted as a forensic investigation. Data collected must be adequate to answer three basic questions: (1) What is the manner of death—natural or otherwise? (2) What is the mechanism of death—toxicosis, asphyxia, or septicemia? and (3) What is the cause of death—what started the lethal sequence of events?

Collections of fish that are affected, but not yet dead, are important to the investigation of any fish kill, but they are not always made or may not always be possible. The types of analyses to be done on the fish depend on the observed and reported circumstances of the kill. Regardless of the suspected cause, fish should be checked for the presence of infectious or parasitic diseases, preferably at the site (Chapter 6). If industrial or agricultural pollution is suspected, chemical analyses are needed, and samples must be collected and preserved accordingly (Chapters 4–6).

When an industrial or municipal discharge is suspected, water samples should be collected above, at, and below the point of discharge, as described in Chapter 4. Then plant managers or other responsible individuals should be contacted immediately to inform them of the problem, to obtain information about the possible contents of the discharge and details of plant operation (particularly just before the



Water chemistry data should be collected as soon as possible after investigators arrive at the site of a fish kill.

Highly toxic substances or high concentrations of less toxic contaminants commonly kill fish of all species and sizes.

kill), and to request permission for access to the property. This action gives plant personnel the opportunity to stop or correct the discharge if there has been an in-plant accident.

Transportation accidents should be handled in the same general way, starting with contacting the hauler, shipper, or consignee to determine what chemicals may be involved and any potential hazards associated with them. The county sheriff or highway department should then also be notified.

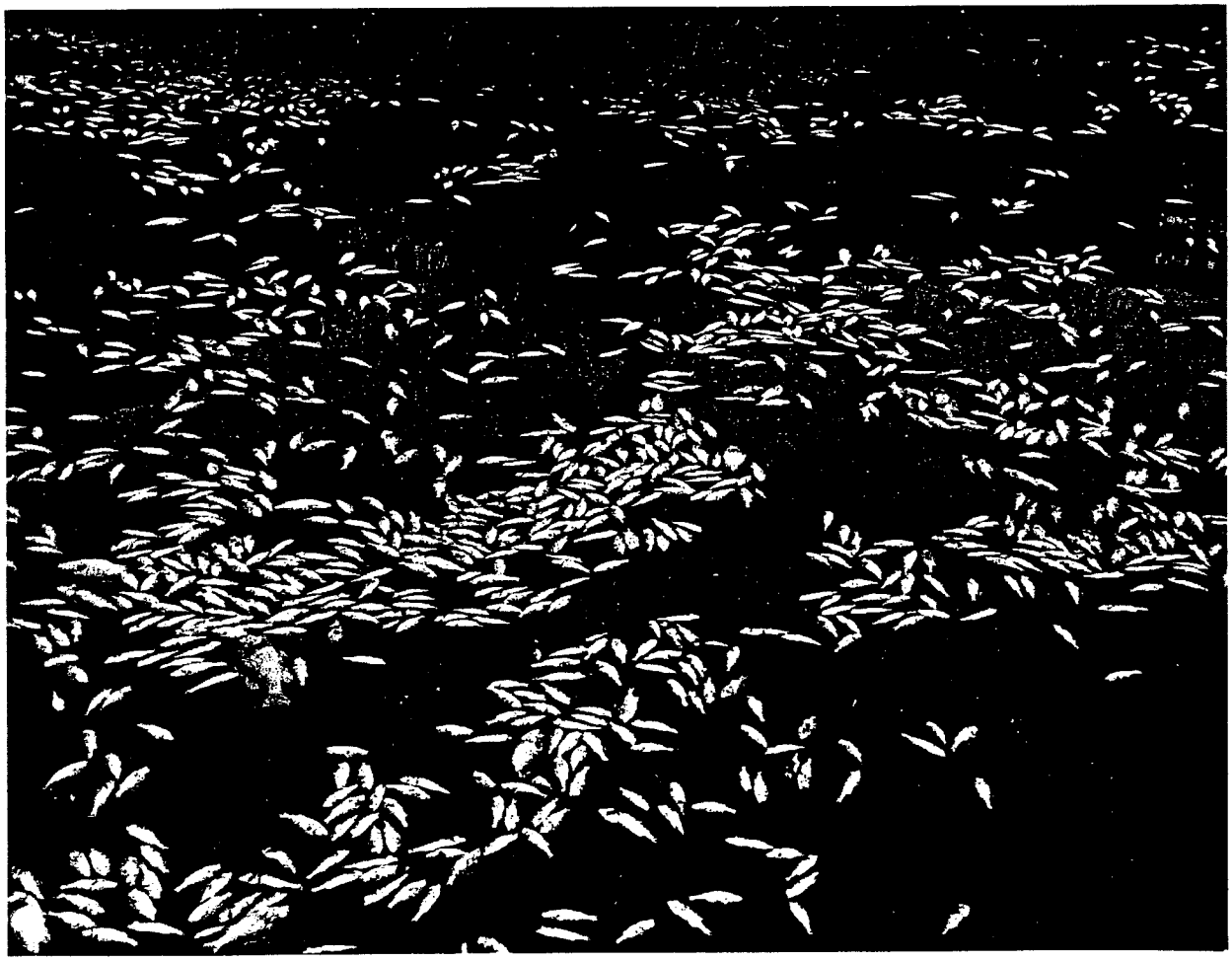
Kills due to chemicals used in agriculture or forestry are often difficult to diagnose. Runoff from fields and aerial applications of chemicals may reach bodies of water through ditches or other water conduits. This type of kill is rarely associated with obviously polluted discharges. Checks of information regarding agricultural and forestry practices in the area may suggest toxicants to be included in requested sample analyses. Water samples taken from the area must include both natural and man-made drainage systems that feed water into the area of the kill.

Observations and sampling should not be limited to fish and water. Many fish toxicants also affect other forms of life. Algae, zooplankton, benthic organisms, other aquatic vertebrates, and even rooted vegetation should be examined for signs of toxic or lethal effects.

The mechanism of death in natural kills may be easily determined but the underlying cause may not be immediately obvious. The investigation of non-pollution kills should not stop with the identification of an infectious agent or a determination of oxygen depletion. For example, low flow from a storage dam can be the cause of increased water temperatures in the stream below the dam and thus be the primary cause of a fish kill. Identifying such situations may lead to the modification of water flow management plans to prevent future losses.

Documentation must always be precise and consistent. Sample sites must be clearly identified so they can be revisited to obtain additional samples, verify any physical conditions, or conduct toxicity tests. All samples must be clearly marked so there can be no confusion as to their identity or as to when, where, how, and by whom they were collected. The chain of custody for all data and samples starts with the on-site investigator and must be continuous through any testing or other examinations that may be conducted, until the case is resolved.

Your agency may require an estimate of the number of fish lost, regardless of the cause of death, but if there is reason to believe compensation may be sought or there is a possibility of litigation, a valid estimate of the magnitude of the kill must be made. A guide recommended for this purpose is *Special Publication No.* 13 of the American Fisheries Society (1982).



Some fish kills affect only one or two species of fish; in this incident, only sunfishes were killed.

Dichotomous Key for Fish Kill Investigations

After the initial visual inspection of the scene, an investigator can sometimes make preliminary assumptions about the cause of a fish kill. By using a process of elimination based on the evidence at hand, certain types of causes may be highly unlikely. A dichotomous key is provided below as an example of how the thought process might proceed. This key is offered as a tool—not as a definitive reference—for assessing fish kills. Opportunities to use the key to help reach a presumptive conclusion concerning the cause of a fish kill are provided in Chapter 13. Seven case histories are described to help potential investigators test their skill in evaluating the information that became available during the on-site investigation. Although the thought process would be the same for ponds, lakes, streams, and estuaries, most of the examples used in preparing the key were taken from data on fish kills in ponds. In streams, where evidence at the site may be transitory because of the flow, the investigator may have to check downstream to attempt to reconstruct the scene.

1. Kill occurred in less than 24 hours 2
1. Not known when kill occurred, or kill continued for longer than 24 hours 16
2. Kill occurred between midnight and sunrise 3
2. Kill occurred at times other than between midnight and sunrise 8
3. Water dark in color, musty odor, or odor of sour cabbage 4
3. Water conditions normal in color and odor 6
4. Some fish alive 5
4. All fish dead 16
5. Large fish dead, some small fish alive 6
5. Small fish dead, some large fish alive 18
6. Dissolved oxygen less than 2 ppm 7
6. Dissolved oxygen 2 ppm or more 9
7. Algal cells absent or dead if present 8
7. Algal cells present and alive 10
8. Dead algal cells abundant .... Oxygen depletion due to enrichment
8. Algal cells absent ..... Oxygen depletion due to algicidal substance
9. Kill occurred between 9:00 a.m. and 5:00 p.m. 10
9. Kill occurred at other times as well 23
10. pH above 9.0 11
10. pH not above 9.0 14
11. Dissolved oxygen high, often saturated, or near saturation 12
11. Dissolved oxygen low or near normal for water temperature recorded 13
12. Heavy bloom of one or more species of blue-green algae ... Toxic algal bloom
12. Heavy bloom of dinoflagellate algae ..... Toxic algal bloom
13. Vegetation dead (appears burned) 14
13. Vegetation normal 15
14. Ammonia levels not high, near zero .. 15
14. Ammonia levels high .... Anhydrous ammonia spill
15. pH 6.0 to 7.0 ..... Oxygen depletion
15. pH below 6.0 ..... Possible lethal low pH or heavy metal poisoning; possible mine drainage
16. Some fish still alive 17
16. All fish dead 23
17. Kill size selective 18
17. Kill not size selective 25
18. Some small fish alive, large fish dead 6
18. Small fish dead, some large fish alive 19
19. Zooplankton and insects alive 7
19. Zooplankton and insects dead 20
20. Algal cells alive 21
20. Algal cells dead or absent .... Toxic herbicidal substance
21. Fish showing convulsive or aberrant behavior 22
21. Fish seemingly normal 24
22. Fins in normal position 23
22. Pectoral fins of fish thrust to extreme forward position .... Organophosphate pesticide
23. Kill occurred throughout day .... Pesticide poisoning
23. Kill occurred between 9:00 a.m. and 5:00 p.m. .... Toxic algal bloom (see also 11)
24. Recent temporary major change in water temperature .... Temperature kill (as from shutdown of thermal power generating plant or plant exceeding the allowed ΔT in discharge)

24. Normal seasonal change in water temperature ..... Temperature falls below or exceeds thermal tolerance—e.g., die-off of threadfin shad in cold weather; kill usually restricted to one species
25. Species selectivity evident 26
25. No species selectivity evident .... Very high level of a toxic substance
26. Lesions evident on fish 27
26. No lesions on fish .... Low toxicity or low concentration of toxic substance (see also 23)
27. Organisms in lesions visible to naked eye .. 28
27. No organisms visible 29
28. Organisms wormlike, attached to external surface of fish .... Leeches (not a cause of death)
28. Organisms resemble copepods or have jointed body parts ..... Parasitic copepods or isopods (known to kill fish)
29. Lesions not hemorrhagic 30
29. Lesions hemorrhagic .... Possible bacterial or viral cause
30. Lesions as small discrete bodies or masses in tissues 31
30. Lesions appear as gray, yellow, or white areas on body .... Bacterial or fungal cause
31. Lesion or mass filled with cellular material .. .. Cysts caused by sporozoans, protozoans (such as *Ichthyophthirius*), or helminths
31. Lesion or mass filled with gas 32
32. Bubbles of gas present in gills, fins, and behind eyes ..... Gas bubble disease, due to supersaturation with a gas
32. Odorous gas in large bubbles in necrotic lesions ..... Bacterial disease caused by *Edwardsiella tarda*

Chronic exposure to sublethal levels of contaminants may lead to tumors or other adverse effects in surviving fish. Public concern is heightened when melanomas, papillomas, and other anomalies, such as those on this black bullhead, are seen on fish.

CHAPTER 4

Toxic Substances

Joseph B. Hunn and Rosalie A. Schnick

Introduction

Fish kills caused by toxic substances fall into several categories, each with its own set of accompanying environmental evidence. Highly toxic substances act quickly and cause abrupt, extensive mortalities. Some chemicals kill both plants and animals and thus severely and dramatically affect the ecosystem. Other compounds may affect only plants, only animals, or only certain species or sizes of fish. Kills associated with these substances may be abrupt, progressive, or lingering, and may trigger a chain of adverse environmental changes. If toxic substances enter the ecosystem at sublethal levels over an extended time, the environmental effects are more subtle. Fish kills associated with such changes may appear at unexpected times of the year or long after the discharge has ended.

A fish kill is sometimes the result of long-term, chronic introduction of toxic material. The rusting 55-gallon drums shown here contained hazardous materials that were released over several years.

Biological Responses to Toxic Substances

Species of fish vary in their susceptibility to toxic substances. Unless the substance is so highly toxic or the concentration is so high that virtually all fish are killed shortly after contact, a progression of selectivity among fish species is usually evident. Because toxic substances may kill all of the biota, it is important to also check whether other organisms, such as algae, zooplankton, sandworms, snails, insects, crabs, crayfish, frogs, turtles, or snakes, are still alive. Often, some species are less sensitive than others to a toxicant, at least in the early stages of the kill.

Unless the substance is herbicidal or algicidal, the dissolved oxygen, pH, and other water chemistry characteristics may appear normal. If the substance also kills plants, the picture becomes confused by misleading indicators, such as low oxygen, low pH, high CO_2, and dying algae. The observer must be alert and consider all of the evidence to determine the true cause of the fish kill.

An array of information is needed before an investigator can determine whether a toxic substance was responsible for a fish kill. Evidence used to make such a determination must come from on-site investigations and laboratory analyses of samples taken during the investigation. Information developed from preliminary observations may include the following:

Fish
- Rate of mortality was abrupt and most fish died within 24 hours
- Small fish died first
- Some species were affected more quickly than others, although all fish eventually died
- Behavioral changes noted were indicative of toxicant poisoning (Tables 4.1 and 4.2)

Invertebrates
- Zooplankters dead, dying, or absent (suspected insecticide poisoning)
- Benthos numbers greatly reduced with a marked change in species composition
- Crabs, crayfish, and sandworms dead, dying, or absent

Other animals
- Signs of poisoning observed among other vertebrates (e.g., frogs, turtles, snakes)
- Invertebrates (e.g., snails) show signs of poisoning

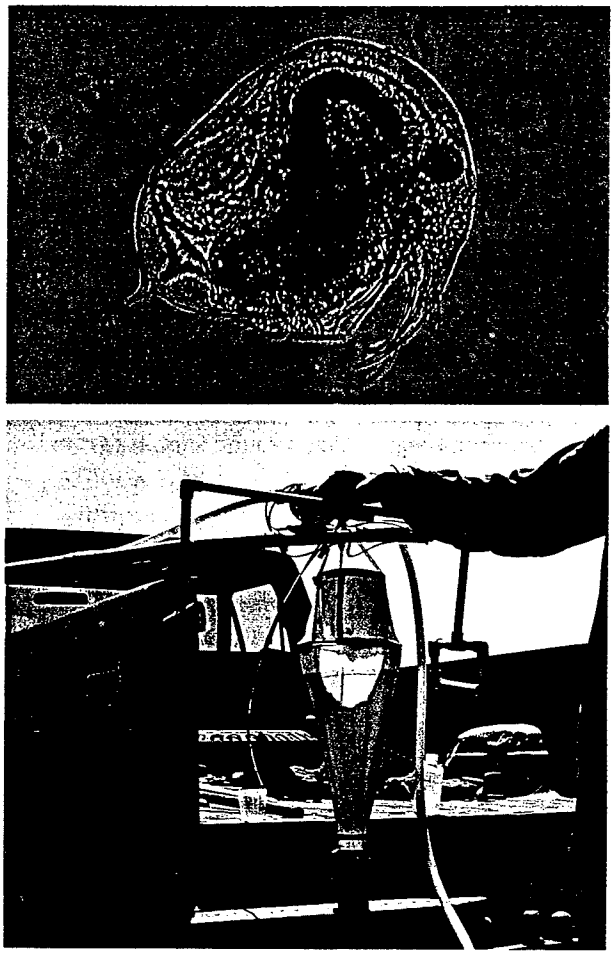
Algae
- Algae alive and normal
- Algae absent or dead (suspected herbicide poisoning)

Table 4.1. *Some observed fish behaviors and water chemistry characteristics associated with fish mortalities (modified from Davis 1986).*

Observations or water chemistry	Possible cause
Large fish coming to surface, gulping air; low dissolved oxygen. Small fish alive and normal	Oxygen depletion caused by excessive organic matter; look for a sewage treatment plant, livestock feedlot, irrigation runoff, decaying plant material, or dying algal bloom after several days of hot, calm, cloudy weather
Large fish coming to surface and gulping air in the presence of adequate dissolved oxygen	May be same as above but enough time has passed to allow for reoxygenation of water. Ammonia kills may also have these characteristics; look for possible drainage from livestock feedlot
Fish swimming erratically and moving up tributary streams to avoid pollution	Usually a heavy metal or chemical wastes discharged from a chemical complex or through a sewage treatment plant
Fish dying after a heavy rain	May be a pesticide or herbicide that has washed off adjacent agricultural fields; a spill dumped from spraying equipment; or chemicals from an aerial spraying operation
Oil sheen on water	Drilling and refinery operations; ruptured pipeline in the area; wash water discharged from oil barges; or a leaking barge
Streambanks and bottom covered with orange-colored substance; high conductivity readings in water samples	Drilling operations; look for discharge of brine water into the stream
Low pH, orange discoloration of water but good water clarity	Acid water discharge from coal mining operation
Fish hyperexcitable, rapid movements followed by death; fish may attempt to swim onto shore	High levels of ammonia or low pH
High levels of chloride, high conductivity, high salinity, and high osmolality in nonmarine waters	Possible return flow of irrigation waters that are hyperosmotic to fish
Low levels of chloride, low salinity, and low conductivity in estuarine or marine waters	Intrusion of fresh water that is hypoosmotic to fish

Table 4.2. *Fish behaviors associated with insecticide poisoning (modified from South Carolina Department of Health and Environmental Control 1979).*

Organochlorine pesticides	Organophosphorus pesticides
Central nervous system disorders	Lethargy
Increased ventilation rate	Loss of equilibrium
Rapid, jerky movements of body and fins	Dark, often reddish, discoloration; hemorrhaging in muscles and beneath dorsal fin
Erratic, uncoordinated swimming movements with spasms, convulsions, and racing	Hypersensitivity—startled fish involuntarily swim rapidly in circles
Increased sensitivity to external stimuli	Tremors, convulsions, and coughing
High excitability	Involuntary extension of pectoral fins and opercula to most forward position possible
Loss of equilibrium with successively longer periods of quiescence until respiratory movement ceases	Spinal abnormalities



Top photo. Cladocerans such as *Bosmina longirostris* are highly sensitive to toxic substances. Their presence outside the affected area but absence in the kill zone is a valuable clue to the possible cause. *Bottom photo.* Plankton nets are used for collecting zooplankton to check for toxic effects.

Chemical Changes Related to Toxic Substances

The toxicity of a substance refers to its potential for having a harmful effect on a living organism. Toxicity is a function of concentration and the duration of exposure. Acute effects occur rapidly as a result of a short-term exposure to a relatively high concentration of a toxicant. Generally, acute effects are severe and usually include mortality (Rand and Petrocelli 1985). However, fish kills may also be induced by the entry of sublethal levels of toxicants through the food chain. Such kills are usually not acute and do not occur at a particular time of year or affect a particular life stage.

Frequently, the introduction of a toxic substance causes no change in the water chemistry, but may leave residues in the water, sediment, or animal tissues. These materials should be checked because the results may yield significant information and may provide the first firm evidence that a toxic substance is involved. Preliminary analyses may provide the following information:

Water

- Water chemistry is normal for the current season and local area
- Some water constituents are abnormal and in a range known to be toxic
- A suspect toxicant has been detected in quantities known to be toxic

- Significant differences exist in the chemical composition of water between the site of the kill and the reference (control) site
- On-site toxicity tests indicate that water from the kill site is toxic, whereas that from the reference site is not

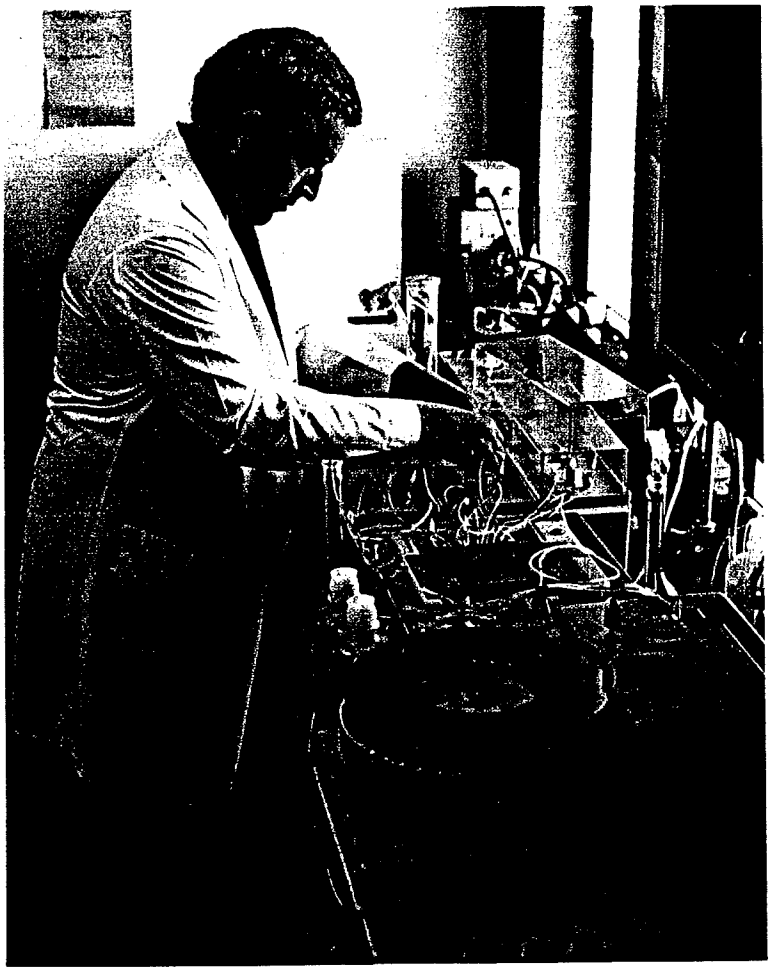
Sediment

- A suspect toxicant is present in sediments from the site of the kill
- The suspect toxicant was not found in sediments from the reference site or is present in equal or lesser quantities at the reference site
- Toxic chemical levels at the site of the kill are higher than those of background samples from the area (Kelly and Hite 1984)

Tissues

- Activity of enzymes (e.g., acetylcholinesterase in brain, ATPase in gills) is reduced in fish from the kill area
- Concentrations of toxic metals (e.g., Cd, Cu, Hg, Zn) in gill tissue are higher in fish from the kill area than in fish from the reference site (suspected metal poisoning)
- Concentrations of the suspect toxicant in tissues are greater in fish from the kill site than in those from the reference site
- Concentrations of the suspect toxicant in fish tissues are known to be toxic

Investigations of kills suspected to have originated from a toxic substance must proceed as though the cause is unknown. All factors must be checked or eliminated unless there is firm evidence that certain causes are not involved. The investigation should proceed through a process of elimination.



The use of autoanalyzers provides rapid and highly sensitive water chemistry determinations.

Diagnosis of Toxic Effects

When the initial field inspection is completed and the probable cause is believed to be a toxic substance or substances, the next step is to establish whether the suspect chemical was present in sufficient quantity to be toxic to fish. A complete water chemistry analysis should help rule out other possible causes and help identify any contributing factors (e.g., dissolved oxygen, pH) that could influence the toxicity of the suspected chemical agents. Analyses that should always be run as soon as possible are listed below (in approximate order of importance) as Priority I. Other desirable, useful analyses that should be run when possible are listed as Priority II.

Routine Water Chemistry Analyses

Priority I	Priority II
Dissolved oxygen	Biological oxygen demand
pH	Calcium
Temperature	Total organic carbon
Ammonia, nitrogen	Chlorine
Alkalinity	Chemical oxygen demand
Color	Hardness
Conductivity	Iron
Nitrite nitrogen	Magnesium
Nitrate nitrogen	Manganese
Total suspended solids	Osmolality
Salinity	Phosphate
Sulfate	
Turbidity	

Changes in pH caused by the discharge of contaminants can drastically alter the availability or activity of toxic substances. Standardized equipment, such as this digital pH meter, promptly provides accurate data.

Results of the analyses of samples taken for Priority I testing can be used to determine whether the water chemistry is within the normal range for substances that are involved in most fish kills—for example, low dissolved oxygen and high ammonia. If all characteristics are within the normal range, it may be necessary to seek further analyses such as those listed as Priority II. If the values resulting from analyses of samples from Priority I and II testing are within the normal range for the area sampled, it is a strong indication that the kill was caused by a toxic substance not usually found in the waters concerned.

Fish kills sometimes occur in situations where all environmental factors seem to be normal. Favorable water chemistry characteristics and high dissolved oxygen concentrations indicate good water conditions; the fish are normal in color and physical condition and have no lesions. The mortality rate may be slow, but continuous. Generally, predatory or omnivorous species older than 2 years are the only fish affected, and small fish and forage species may be alive and well. Such mysterious kills are most commonly seen in late fall or early winter, depending on the latitude.

These seasonal fish kills often occur in waters adjacent to areas where chemicals are used, stored, or applied. Spills, accidental spraying, or runoff can introduce sublethal pesticide levels to the environment that then become involved in the food chain by biomagnification. In kills of this type, the key indicator is that only large predatory fish are affected, whereas young-of-the-year and forage fishes seem to be thriving. Water conditions will appear to be good to excellent.

The most common cause of these unexplained fish kills is chronic exposure to sublethal levels of a pesticide. Although the daily exposure may be low, fish

The loss of large predators may indicate a fish kill caused by biomagnification of contaminants through the food chain. In such kills, young-of-the-year fish of all species may survive. (*Photo courtesy of the Missouri Department of Conservation.*)

bioaccumulate a pesticide in their energy reserve (fat) to levels that are much higher than a single acutely toxic dose. As long as the food intake meets or exceeds their daily energy requirements, the fish will continue to function normally. However, when seasonal water temperatures fall below the feeding range, the fish must rely on stored energy to survive. In fish with a high pesticide residue in their fat, mobilization of the energy reserve may release lethal levels of pesticide into the blood stream. Although signs of toxicosis are sometimes seen, the fish usually seem weak or lethargic, or unconscious. Diagnosis of such a cause requires the analysis of blood samples or brain tissue for pesticide residues. Although analysis of the fat is helpful, the results can be misleading because stored residues may be unrelated to the kill.

Under certain circumstances, selenium, a required element, enters the food chain in excessive amounts. Waterborne concentrations exceeding 3 μg/L have been detected in lentic systems—for example, in power plant cooling reservoirs and certain agricultural drainage waters. Selenium bioaccumulates in the ovaries of sensitive fish species such as centrarchids. Although selenium-laden ova can be fertilized, the young fail to survive, leading to an eventual collapse of the fish population (Lemly 1985; Baumann and Gillespie 1986).

The EPA (1986) published brief summaries of acute and chronic toxicity information for freshwater and marine species for all contaminants for which the agency has developed criteria recommendations. These criteria, which are summarized in Appendix B, are expected to be adequate to protect aquatic life. The summaries are updated to reflect recent changes in EPA's recommendations on acceptable limits for the protection of aquatic life and human health. More detailed information on

Runoff from coal mining areas can cause fish kills. Low pH in the acid mine drainage is usually the causative agent in such kills. Note the reddish iron precipitate and the conspicuous lack of life in the water.

individual water quality criteria established by EPA is provided in documents available from the National Technical Information Service (NTIS). See Appendix C.

The EPA also issued a series of documents relating to water quality criteria based on State regulations. These documents present the criteria for each State in alphabetical order. The documents are available through NTIS (Appendix D). In addition, these water quality standards are available for each State as a separate document or as part of a compilation in one document that can be purchased from NTIS.

Factors that Modify Toxicity

Laboratory and field studies have shown that many factors influence the toxicity of chemicals to fish. The origin of modifying factors may be either biotic or abiotic (Sprague 1985; Mayer and Ellersieck 1986). Biotic factors include species, life stage and size, nutritional state, general health, and parasitism. Abiotic factors include characteristics of the water (e.g., temperature, pH, hardness, alkalinity, osmolality, dissolved oxygen, salinity, dissolved organic carbon), possible binding to suspended or dissolved materials, and formulation of pesticide products.

Water hardness has little effect on the toxicity of organic compounds. However, increased water hardness (as Ca and Mg) can reduce the availability of metals such as Al, Cd, Hg, and Pb (Hunn 1985; Mance 1987). Hardness, alkalinity, and pH all influence the availability of metals, such as Cu (Sprague 1985). Hydrogen ion concentration (measured as pH) influences the toxicity of chemicals that ionize. For example, the toxicity of ammonia, cyanide, and hydrogen sulfide is influenced by the pH of the water. Un-ionized molecules usually are more lipid-soluble than ionized forms and thus penetrate membranes more readily (Hunn and Allen 1974; Spacie and Hamelink 1985). As noted by Mayer and Ellersieck (1986) in a study of 410 chemicals, pH affected the toxicity of only about 20% of the organic chemicals tested, but caused greater changes in 96-hour LC50 values than any of the other water chemistry factors examined.

Results from the analysis of water samples tested for a suspected chemical should yield positive results if that substance is present. Analytical chemistry data generated should include the concentration found, limits of detection, quality assurance, and quality control information that will help determine whether the analysis was accurate and reliable. In comparing the results from the control or reference site with those from the kill site, there should be a definite difference in concentration of the chemical. If there is not, several possibilities exist: (1) the reference site was not a true control; (2) the chemical moved downstream (in running water); (3) the compound was removed by becoming bound to sediment; (4) the substance was biotransformed, degraded, or volatilized; or (5) a combination of these possibilities.

Keup (1974) listed eight factors to consider when an investigator is attempting to interpret on-site evidence at a fish kill: (1) time of water travel (streams); (2) dilution; (3) lateral mixing; (4) season and temperature; (5) habitat characteristics; (6) delayed reactions in fish and invertebrates; (7) synergism and antagonism; and (8) suspended materials. Time of travel and dilution of the chemical can be estimated after the fact by conducting a dye study if the hydrological conditions present during the investigation are similar to those that existed at the time of the kill. For further information on how to conduct dye studies, see Slifer (1970).

Toxicity data from acute tests are usually reported as LC50's in mg/L. An LC50 is the estimated concentration of a substance in water that is lethal to 50% of the test organisms after exposure for a stated period of time (e.g., 24, 48, or 96 hours). Thus, the larger the LC50 value, the less toxic the chemical is to fish; and the smaller the value the more toxic the chemical. The relative acute toxicity of chemicals to fish (96-hour LC50) can be categorized as follows:

Toxicity rating	96-hour LC50
Practically nontoxic	100–1,000 mg/L
Slightly toxic	10–100 mg/L
Moderately toxic	1–10 mg/L
Highly toxic	0.1–1.0 mg/L
Extremely toxic	Less than 0.1 mg/L

It is important to establish some measure of the relative toxicity at the site. A valid pH measurement may be sufficient to establish whether the hydrogen ion concentration was lethal (Table 4.3). In extremely soft water, pH determinations should be made with a special electrode designed for use in waters of low ionic strength. Most substances are toxic to organisms if the concentration is high enough and

Table 4.3. *Influence of the addition of acidic or alkaline materials on the pH of receiving waters of various hardnesses.*

Total hardness (as $CaCO_3$) of receiving water	Resultant pH				
	3.0–5.0	5.0–6.0	6.0–9.0	9.0–11.0	>11.0
Extremely soft 0-9 ppm	A pH of <5.0 may be toxic, depending on species	Aluminum is most toxic to fish; other toxic metals are Cd, Cu, and Zn	At pH 8 and above, suggests algal bloom or alkali input	Indicates strong alkali input	Indicates strong alkali input
Very soft 10–39 ppm	Indicates acid input	Normal or limited acid input	Normal pH	Indicates alkali input	Indicates strong alkali input
Soft 40–159 ppm	Indicates acid input and possibility of CO_2 toxicity	Indicates acid input	Normal pH	Indicates alkali input	Indicates strong alkali input
Hard[a] 160–279 ppm	Indicates acid input and possibility of CO_2 toxicity	Indicates acid input	Normal pH	Indicates alkali input	Indicates strong alkali input
Very hard[a] 280–399 ppm	Indicates strong acid input	Indicates acid input	Normal pH	Indicates alkali input	Indicates strong alkali input
Extremely hard[a] >400 ppm	Indicates strong acid input	Indicates acid input	Normal pH	Normal in alkaline waters	Indicates strong alkali input

[a]As hardness increases, the toxicity of metals decreases.

Fish kills due to insecticides may destroy all fish and invertebrates but have no effect on plants (as shown here by the thriving duckweed among the dead fish).

the length of exposure is long enough. Although data obtained from 24-hour exposures are most appropriate for use in evaluating an acute kill situation, data from 24-, 48-, and 96-hour tests can also be used to estimate the toxicity of a substance suspected of causing the kill. The 95% confidence interval establishes a range for the LC50 and is helpful in determining whether the concentration of chemical found in the field was high enough to cause acute toxicity (Mayer and Ellersieck 1986).

Sources of Toxicity Information

One of the best sources of information on toxicity developed since 1970 is the data base AQUIRE. It includes information on acute and chronic toxicity, bioaccumulation, sublethal effects, chemical substance information, details on test organisms, study protocols, experimental design details, and results. Bibliographic references to the original sources are included. AQUIRE is one of the Chemical Information System components sponsored by the Office of Toxic Substances of EPA. The data base focuses on the toxic effects of chemical substances on freshwater and saltwater organisms, other than aquatic mammals, birds, and bacteria. As of July 1988, about 68,000 records were available on more than 4,000 chemicals.

The following references are sources for toxicity information: McKee and Wolf (1963); EPA (1973, 1977, 1980–1989, 1983–1989, 1986); Thurston et al. (1979); Alabaster and Lloyd (1982); Rand and Petrocelli (1985); U.S. Department of the Interior (1985–1989); Mayer and Ellersiek (1986); Mance (1987); Mayer (1987); and Weed Science Society of America (1989).

Clinical Signs of Toxicosis

Few of the signs related to fish poisoning are unique to a particular compound or group of compounds. For example, if adequate oxygen is available in the water at the time of exposure, cyanide poisoning results in bright red gills and blood because the available oxygen cannot be used at the tissue level. This condition might lead an investigator to assume that water conditions were normal; however, there will be hemorrhages and blood clots in the liver and viscera.

Acetylcholinesterase-inhibiting compounds (e.g., organophosphates or carbamates) reduce brain levels of cholinesterase activity, induce a forward positioning of the pectoral fins in moribund scaled fishes, and may induce spinal abnormalities.

High concentrations of nitrite can induce methemoglobinemia, a condition that is characterized by brown blood. However, hydrogen sulfide can also bind to hemoglobin to produce sulfhemoglobin, which also results in dark, chocolate-colored blood. Exposure to sulfide reduces the level of cytochrome oxidase in fish tissues and increases the levels of thiosulfate in the blood, kidney, and spleen.

The clinical signs listed must be observed in freshly dead or moribund fish because they disappear soon after the fish die. Other signs that have been observed in relation to toxicant-caused fish kills are listed in Table 4.4. It should be noted that the listed signs and behavioral responses (Tables 4.1 and 4.2) are not strictly diagnostic as to the cause of death, but they provide useful information in developing evidence.

Table 4.4. *Clinical signs associated with toxicosis in fish (modified from U.S. Department of the Interior 1970).*

Sign	Possible causative agent
White film on gills, skin, and mouth	Acids, heavy metals, trinitrophenols
Sloughing of gill epithelium	Copper, zinc, lead, ammonia, detergents, quinoline
Clogged gills	Turbidity, ferric hydroxide
Bright red gills	Cyanide
Dark gills	Phenol naphthalene, nitrite, hydrogen sulfide low oxygen
Hemorrhagic gills	Detergents
Distended opercles	Phenol, cresols, ammonia, cyanide
Blue stomach	Molybdenum
Pectoral fins moved to extreme forward position	Organophosphates, carbamates
Gas bubbles (fins, eyes, skin, etc.)	Supersaturation of gases

The gills of fish are delicate, highly sensitive tissues. Injury or other damage caused by corrosive or toxic chemicals is readily evident to a trained observer. Parasites, bacteria, or fungi may also cause gill damage.

Sample Collection for Suspected Toxic Substances

"It is an old axiom that the result of any test can be no better than the sample on which it is performed" (APHA et al. 1985). When a toxic substance is suspected as the possible cause of a fish kill, it is critical that the investigators collect samples properly, use appropriate containers, follow preservation and storage methods that are consistent with accepted methodology, and ship samples properly and promptly. The following sections discuss proper procedures for the collection, handling, storage, and shipment of samples for fish, water, sediments, invertebrates, and plants.

An essential element is a field log in which there is an entry for each sample collected for analysis, its identification number, the site where collected, the date, and the name or initials of the collector. These entries provide backup identification if sample labels are damaged, are lost, or if confusion develops over when and where certain samples were taken.

Fish Samples

A representative size series of moribund or recently dead fish of each species affected should be collected. If possible, healthy fish of the same species and sizes from the unaffected area should also be collected to provide background data. Methods that are used to preserve the various samples should always be noted on the label. For samples to be analyzed for pesticides or other toxic organic substances, the whole fish should be rinsed with clean water, wrapped in aluminum foil (with the dull side toward the specimen), and frozen as quickly as possible. Samples to be analyzed for metals or other elements should be collected separately, placed in polyethylene bags, and frozen. Subsamples of tissues such as brain, gills, or blood that are needed for special analyses should be taken immediately after sampling and frozen in separate clean glass containers. Special analyses may include measurements of enzymatic activity (e.g., acetylcholinesterase in brain or Na, K-ATPase in gill tissue) or metals in gill tissue (e.g., Cd, Hg, Zn, or Cu).

Tissues for histological examination should be taken from moribund fish—never from dead fish (in which postmortem changes are likely to have occurred). Fish that have been dead longer than 10–15 minutes are not suitable specimens. Tissue samples for histological examination should not be frozen. It is imperative that tissue specimens be placed into a suitable fixative as soon as possible, preferably at a ratio of 1 part tissue to 10 parts fixative. A 10% solution of buffered neutral formalin is readily available and is an acceptable fixative. Check with the histopathologist who will do the tissue analyses for his or her choice of fixative and for other instructions on fixation techniques. Fish tissues that were preserved in a fixative for histological examination should be transferred to 70% ethanol for storage. They can then be held for a year or more if the solution is renewed periodically. For further information, see Morrison and Smith (1981) or Yasutake (1987).

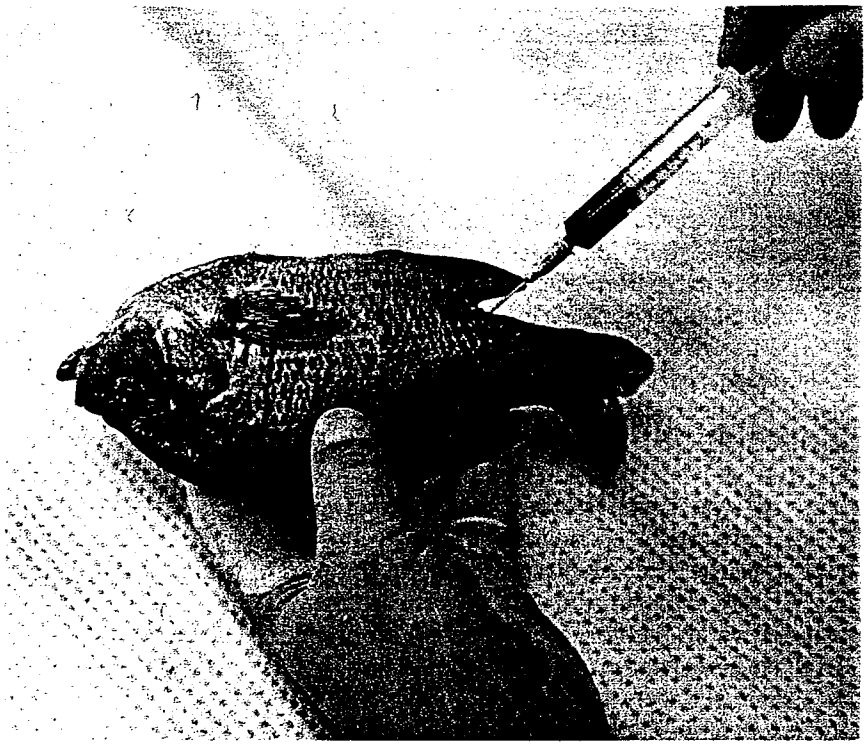
For analytical purposes, it is better to collect several small fish than one large fish from each species that is affected. The numbers collected, amount of tissue needed, and preservation techniques depend on the types of analyses to be performed. The following general guidelines apply:

Inorganic analyses
: Per sample: at least three fish or as many as needed to provide 100 g of whole body tissue as the minimum total sample; collect three samples for each species from each site

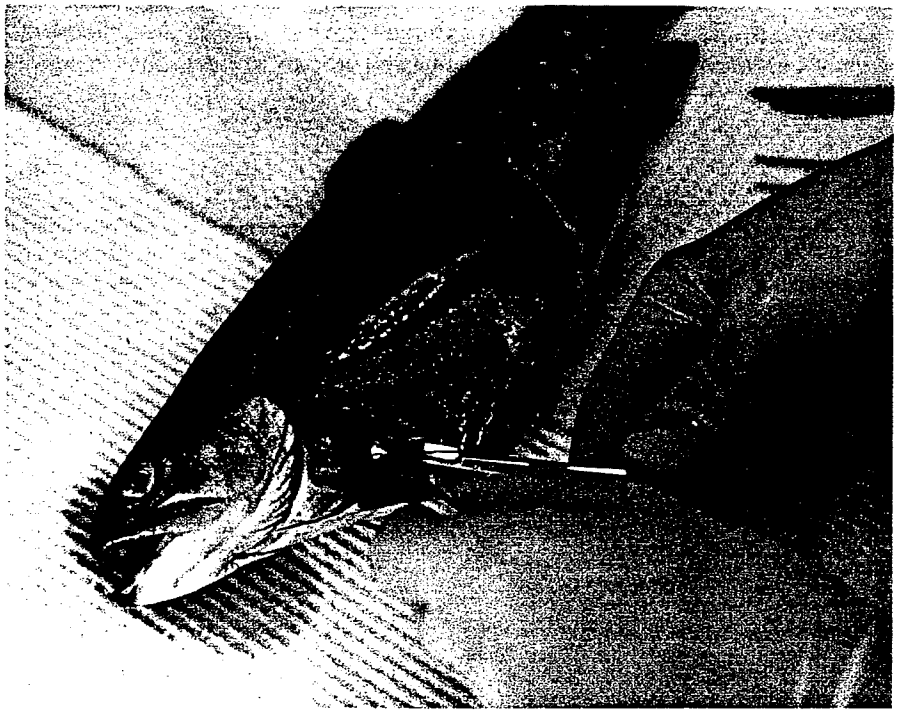
Organic analyses
: Per sample: at least three fish or as many as needed to provide 250 g of whole body tissue as the minimum total sample; collect three samples for each species from each site

If it is suspected that the causative agent is a volatile substance, about 100 g of tissue should be placed in containers that can be sealed airtight and frozen.

The composite samples of three or more fish should be separately wrapped in foil and placed in a single bag, properly labeled, and frozen. Samples of all types should be frozen as quickly as possible and kept frozen at $-20°$ C or lower until analyzed. For a large kill with many species, the investigator must select the species to be collected. Samples should include representatives from all trophic levels that are affected—for example, herbivores, omnivores, forage fish, and predators. It is critical that the same species of fish (and preferably of the same sizes) be sampled from the control or reference area as from the kill area. The numbers and types of samples collected will depend on the extent of the



Blood samples taken from surviving fish often provide insight into the nature and identity of a toxic substance associated with a fish kill.



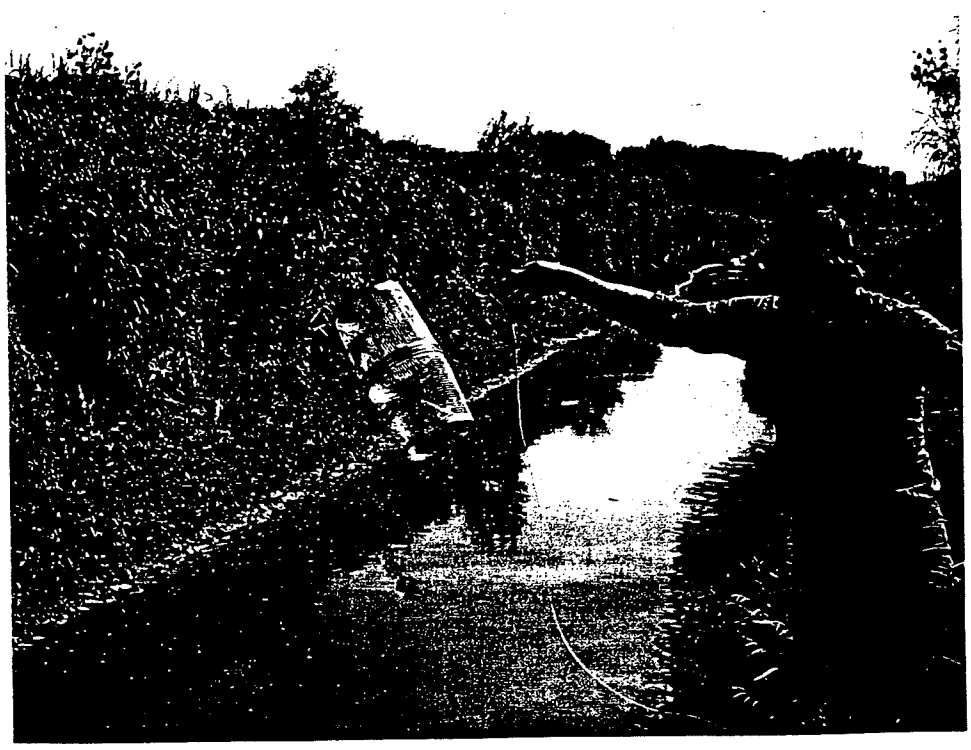
The liver is a major site for detoxification or biotransformation of toxic substances in fish. Consequently, it is often analyzed for residues of suspected contaminants or their metabolites.

kill, the number of species involved, agency protocol, instructions from the analytical facility, and the estimated costs of analyses.

Water Samples

After tests of water quality characteristics, such as dissolved oxygen, pH, conductivity, salinity, and temperature, have been completed, grab samples of water should be taken. (For information on the type of container needed, sample size, and methods of preservation, see Table 4.5.) At a minimum, samples should be collected above, within, and below the kill area (Figs. 4.1 and 4.2). The specific types of sampling and analyses to be run must be determined on a case-by-case basis by the on-site investigator. Before the sample bottles are filled, each bottle should be rinsed two or three times with the water that is being sampled (unless the bottle contains a preservative or dechlorinating agent). Water samples must be refrigerated at 4° C in amber bottles and stored in darkness (Table 4.5). The number of samples to be taken and sampling methods should be determined by consultation with the agency that is to perform the analyses (Keith et al. 1983). If no guidance is available, as many samples as convenient should be taken over the area. Although it may not be necessary to have all samples analyzed, there may not be another opportunity to collect useful samples. Sampling protocols should be in place, if possible, before investigative sampling is begun. For further information, see Hill (1983), Keith et al. (1983), and APHA et al. (1985).

The minimum volume needed for water samples varies with the type of analysis to be performed on the sample. In general, a 1-liter sample is sufficient. It is important that properly cleaned, prepared containers be used to collect and store the samples. In general, samples to be analyzed for inorganic compounds can be taken with plastic (polyethylene or equivalent) bottles that have been acid washed and rinsed with distilled water. For preservation, samples taken for metals analysis should be acidified to pH 2 with redistilled nitric acid. Samples taken for suspected pesticides or other toxic organics will require glass bottles with Teflon-lined caps. The glass bottles should have been rinsed with hexane and dried before use. If volatile organics are suspected, the sample bottles should be filled to overflowing and capped, leaving no air space. Recommended methods of preservation and storage times are given in Table 4.5. Properly cleaned and stored sample bottles and preservatives should be part of the fish kill investigation kit (see Chapter 12); such containers are commercially available. Ampules that contain premeasured amounts of acid for preserva-



Small, baited traps can be used to collect surviving forage fish from the site of a fish kill or from a control area.

Table 4.5. *Summary of special sampling or handling requirements for water samples*[a] *(modified from and permission to use granted by American Public Health Association et al. 1985).*

Determination	Container	Minimum sample size (mL)	Preservation	Maximum storage (d = days, h = hours, m = months)	
				Recommended	Regulatory[b]
Acidity	P, G(B)	100	Refrigerate	24 h	14 d
Alkalinity	P, G	200	Refrigerate	24 h	14 d
Biological oxygen demand	P, G	1,000	Refrigerate	6 h	48 h
Boron	P	100	None required	28 d	28 d
Bromide	P, G	—	None required	28 d	28 d
Carbon, organic, total	G	100	Analyze immediately; or refrigerate and add H_2SO_4 to pH <2	7 d	28 d
Carbon dioxide	P, G	100	Analyze immediately	—	—
Chemical oxygen demand	P, G	100	Analyze as soon as possible, or add H_2SO_4 to pH <2	7 d	28 d
Chlorine, residual	P, G	500	Analyze immediately	0.5 h	2 h
Chlorine dioxide	P, G	500	Analyze immediately	0.5 h	2 h
Chlorophyll	P, G	500	30 days in dark; freeze	30 d	—
Color	P, G	500	Refrigerate	48 h	48 h
Conductivity	P, G	500	Refrigerate	28 d	28 d
Cyanide					
Total	P, G	500	Add NaOH to pH>12, refrigerate in dark	24 h	14 d
Amenable to chlorination	P,G	500	Add 100 mg $Na_2S_2O_3$/L	—	—
Fluoride	P	300	None required	28 d	28 d
Grease and oil	G, widemouthed calibrated	1,000	Add H_2SO_4 to pH <2, refrigerate	28 d	28 d
Hardness	P, G	100	Add HNO_3 to pH <2	6 m	6 m
Iodine	P, G	500	Analyze immediately	0.5 h	—
Metals, general	P(A), G(A)	—	For dissolved metals, filter immediately, add HNO_3 to pH <2	6 m	6 m
Chromium VI	P(A), G(A)	300	Refrigerate	24 h	48 h
Copper by colorimetry[b]					
Mercury	P(A), G(A)	500	Add HNO_3 to pH <2, 4° C	28 d	28 d
Nitrogen					
Ammonia	P, G	500	Analyze as soon as possible or add H_2SO_4 to pH <2, refrigerate	7 d	28 d
Nitrate	P, G	100	Add H_2SO_4 to pH <2, refrigerate	48 h	48 h
Nitrate + nitrite	P, G	200	Analyze as soon as possible or refrigerate; or freeze at −20° C	0	28 d

Table 4.5. *Continued.*

Determination	Container	Minimum sample size (mL)	Preservation	Maximum storage (d = days, h = hours, m = months)	
				Recommended	Regulatory[b]
Nitrite	P, G	100	Analyze as soon as possible or refrigerate; or freeze at −20° C	0	48 h
Organic, Kjeldahl	P, G	500	Refrigerate; add H_2SO_4 to pH <2	7 d	28 d
Odor	G	500	Analyze as soon as possible; refrigerate	6 h	—
Organic compounds					
Pesticides	G(S), TFE-lined cap	—	Refrigerate; add 100 mg $Na_2S_2O_3$/L if residual chlorine present	7 d	7 d
Phenols	P, G	500	Refrigerate, add H_2SO_4 to pH <2	[a]	28 d
Purgeables by purge and trap	G, TFE-lined cap	50	Refrigerate; add 100 mg $Na_2S_2O_3$/L if residual chlorine present	7 d	14 d
Oxygen, dissolved	G, BOD bottle	300			
Electrode			Analyze immediately	0.5 h	1 h
Winkler			Titration may be delayed after acidification	8 h	8 h
Ozone	G	1,000	Analyze immediately	0.5 h	—
pH	P, G	—	Analyze immediately	2 h	2 h
Phosphate	G(A)	100	For dissolved phosphate, filter immediately; refrigerate; freeze at −10° C	48 h	48 h
Salinity	G, wax seal	240	Analyze immediately or use wax seal	6 m	—
Silica	P	—	Refrigerate, do not freeze	28 d	28 d
Sludge digester gas	G, gas bottle	—	—	—	
Solids	P, G	—	Refrigerate	7 d	7–14 d
Sulfate	P, G	—	Refrigerate	28 d	28 d
Sulfide	P, G	100	Refrigerate; add 4 drops 2N zinc acetate/100 mL	28 d	28 d
Taste	G	500	Analyze as soon as possible; refrigerate	24 h	—
Temperature	P, G	—	Analyze immediately	—	—
Turbidity	P, G	—	Analyze same day; store in dark up to 24 hours	24 h	48 h

[a] See text for details. For determinations not listed, use glass or plastic containers; preferably refrigerate during storage and analyze as soon as possible. Refrigerate = storage at 4° C, in the dark. P = plastic (polyethylene or equivalent); G = glass; G(A) or P(A) = rinsed with 1 + 1 HNO_3; G(B) = glass, borosilicate; G(S) = glass, rinsed with organic solvents; TFE = Teflon.

[b] U.S. Environmental Protection Agency, Proposed Rules, *Federal Register* 44; No. 244, 18 December 1979.

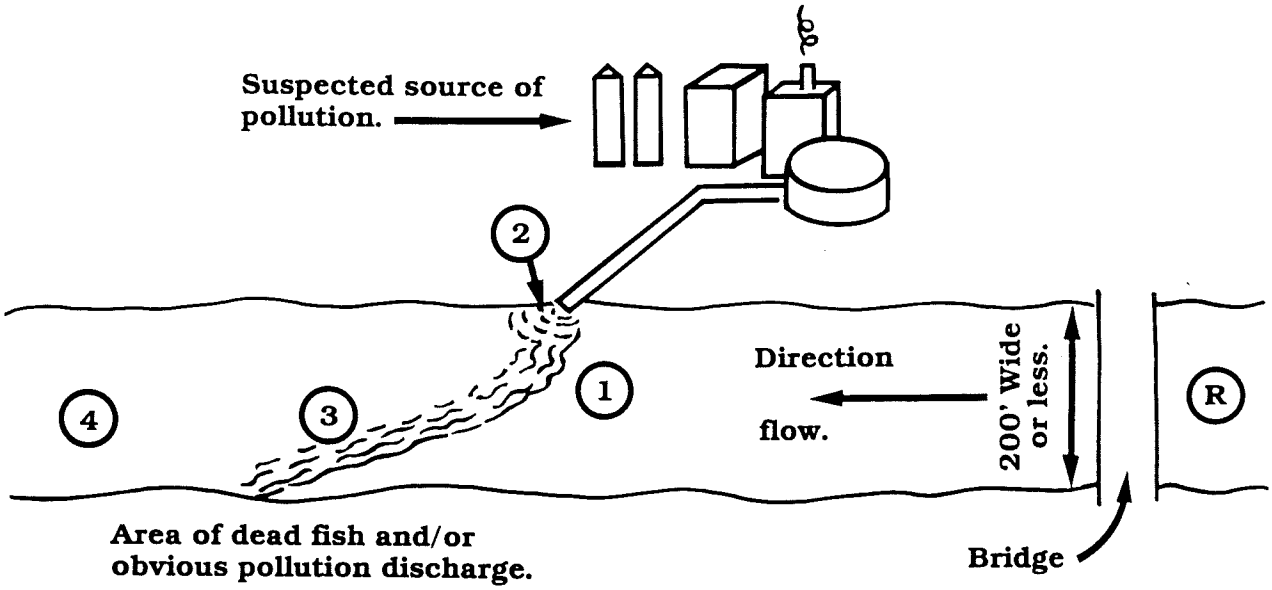


Fig. 4.1. Suggested sites for collecting samples related to a fish kill in which only one source is suspected. The *circled numbers* indicate where samples should be taken to look for the toxic substances. Site *R* is a reference site above the affected area (modified from South Carolina Department of Health and Environmental Control 1979).

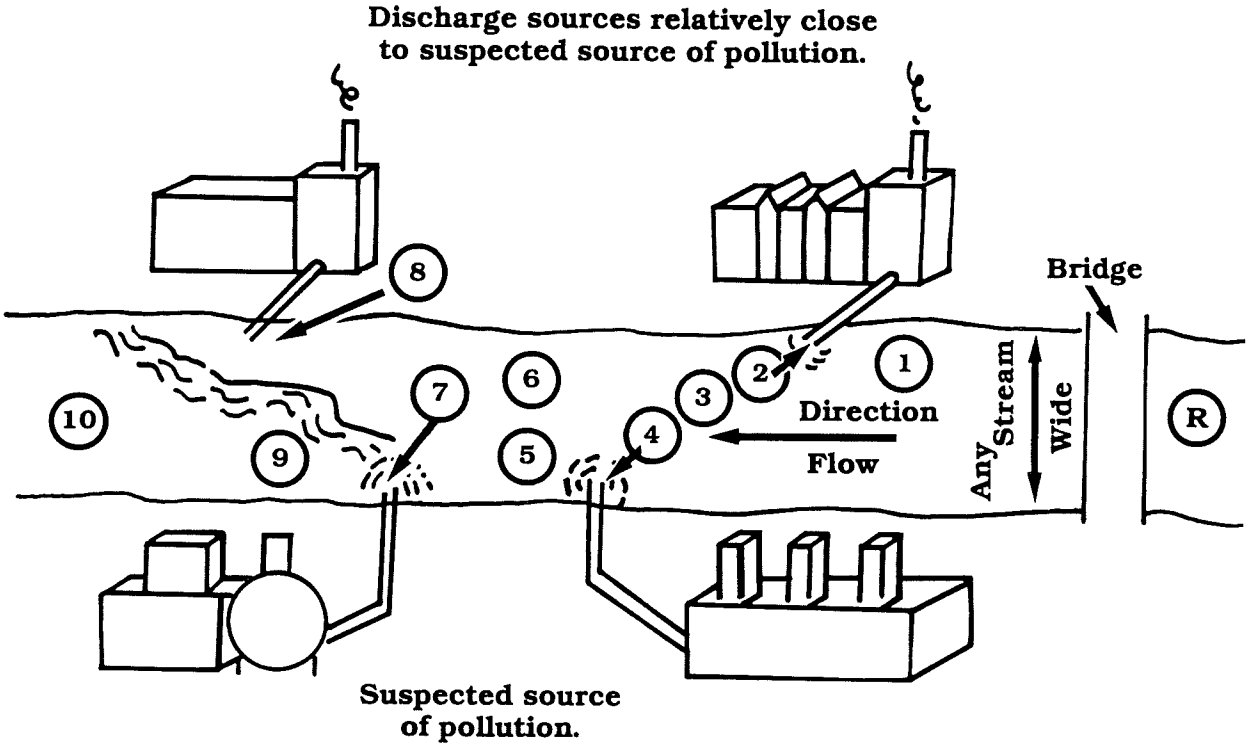
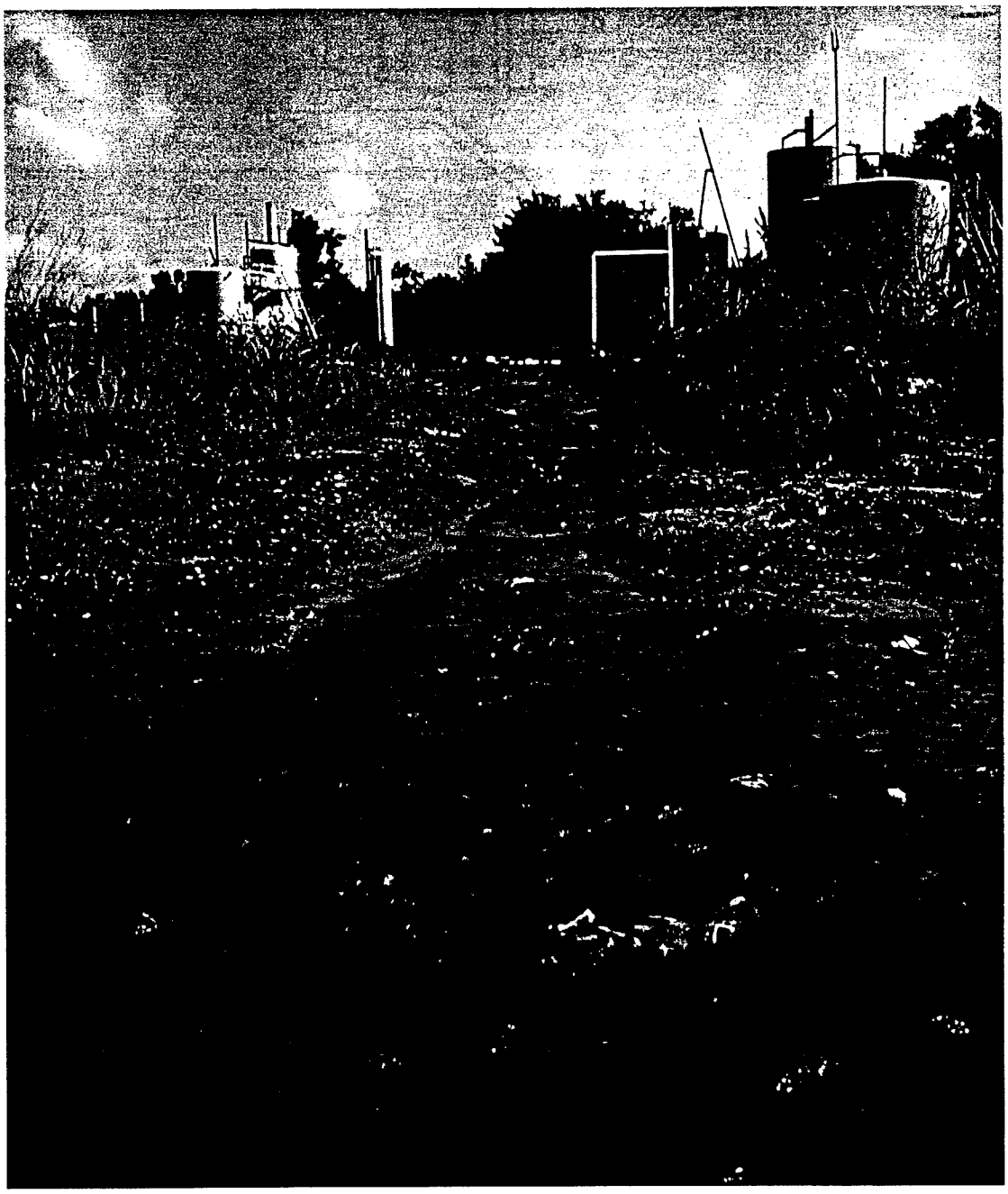


Fig. 4.2. Suggested sites for collecting samples related to a fish kill in which multiple sources might be involved. The *circled numbers* indicate sites where samples should be taken to look for possible toxic substances. Site *R* is a reference site above the affected area (modified from South Carolina Department of Health and Environmental Control 1979).



During an investigation of a fish kill, it is important to check all point-source discharges in the area. Although the flow shown in this photo is relatively low, the contaminants being released are having an obvious adverse effect on the receiving stream.

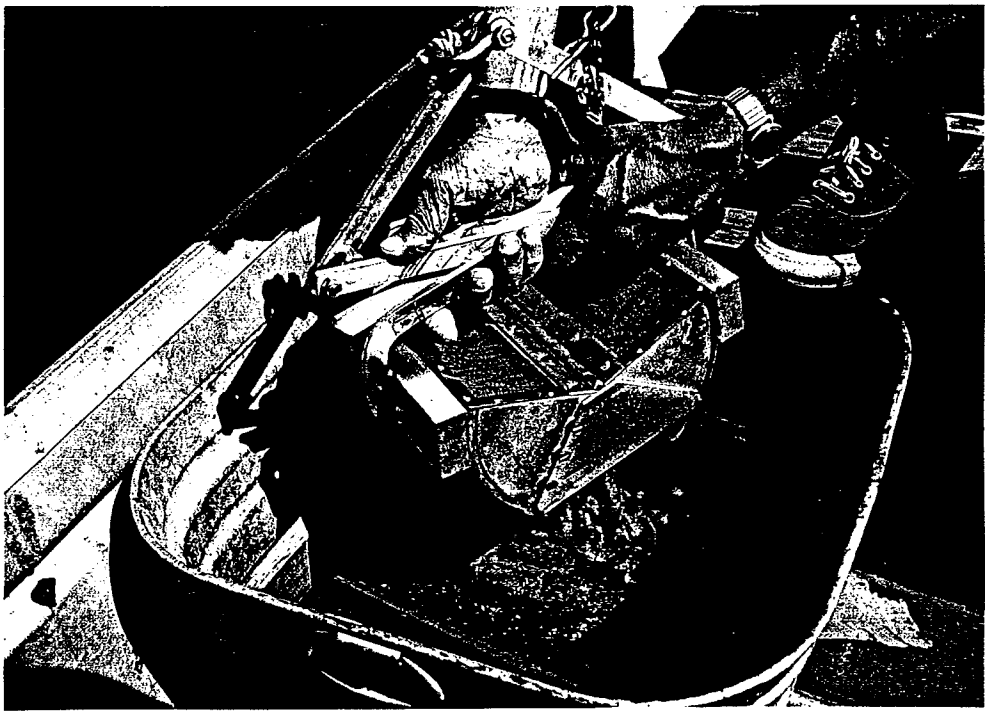
tion of water samples are also commercially available. Use of these ampules reduces acid leaks in sampling kits.

The on-site circumstances should indicate where and how many samples should be taken. As a minimum, samples should always be taken outside and inside the kill area. The control or reference site (outside the kill area) should always be free from the influence of the suspected toxic water. In a stream, one sample should always be taken above the kill area or above any point source potentially associated with the kill. If involvement of an effluent discharge is suspected, a sample of the effluent should be collected, as well as water samples collected downstream from the outfall (Figs. 4.1 and 4.2), to delineate the contaminated zone. For streams more than about 60 m wide, samples should be taken at two or more points along a transect across the stream. In large streams, it may also be necessary to take samples at various depths. Sampling devices that can be used to take water samples are outlined in Chapter 12; others are given by EPA (1982), Hill (1983), and APHA et al. (1985).

Sediment Samples

It may not be necessary to collect sediment in all fish kill investigations. However, samples should be consistently taken from the same sites where water samples were taken (above, within, and below the kill area). Special sampling sites below point source inputs may be desirable and should always be carefully documented. The method of handling the samples after collection and before analysis is determined by the type of test to be run. Samples should always be kept cool (4° C) or frozen and stored at −20° C or lower (EPA 1982; Palmer 1984; Tetra Tech 1986). If samples are to be used in toxicity tests, they should always be kept cool (4° C), but never frozen (M. K. Nelson, National Fisheries Contaminant Research Center, Columbia, Missouri, personal communication).

Sediments are usually taken with a corer or mechanical grab dredge (EPA 1982; Palmer 1984; Tetra Tech 1986). The needed sample size is usually not less than 50 g (Table 4.6). One-quart wide-mouthed glass jars with screw cap lids are accept-



To determine if benthic organisms have been killed, samples of the bottom materials must be collected with equipment such as this Ponar dredge.

Table 4.6. *Recommended quantities, containers, preservation techniques, and holding times for sediment samples to be analyzed for selected variables (modified from Tetra Tech 1986).*

Variable	Minimum sample size (g)[a]	Container[b]	Preservation	Maximum holding time (d = day; m = month)
Particle size	100–150[c]	P,G	Cool, 4° C	6 m[d]
Total solids	50	P,G	Freeze	6 m[d]
Total volatile solids	50	P,G	Freeze	6 m[d]
Total organic carbon	25	P,G	Freeze	6 m[d]
Oil and grease	100	G only	Cool, 4° C, HCl; Freeze	28 d[d] 6 m[d]
Total sulfides	50	P,G	Cool, 4° C, 1N zinc acetate	7 d[d]
Total nitrogen	25	P,G	Freeze	6 m[d]
Biochemical oxygen demand	50	P,G	Cool, 4° C	7 d
Chemical oxygen demand	50	P,G	Cool, 4° C	7 d

[a] Recommended field sample sizes for one laboratory analysis. If additional laboratory analyses are required (e.g., replicates), the field sample size should be adjusted accordingly.
[b] P = polyethylene, G = glass.
[c] Larger samples are required for sandy sediments than for muddy ones.
[d] This is a suggested holding time. No U.S. Environmental Protection Agency criteria exist for the preservation of samples or quantities needed for determination of this variable.

able containers. The caps should be lined with Teflon sheeting (metal analysis) or aluminum foil (organic analysis). All jars, lids, sheeting, or foil should first be washed with a nonphosphate, laboratory-grade detergent, and triple rinsed with tap water. They should then be rinsed with reagent grade nitric acid (1:1) and tap water, followed by a rinse with 1:1 hydrochloric acid (reagent grade), and a triple rinse with distilled water. The containers and materials should then be rinsed with acetone, followed by pesticide grade hexane, and dried in a contaminant-free area. Commercially prepared containers are available. Clean jars should be stored in the sample kit with lined caps screwed on the jars.

When widemouthed glass jars are used, the jars should be filled almost to the top with sediment, topped off with water from the site, and sealed with a Teflon-lined cap or aluminum foil beneath the lid. After appropriate labeling, the samples should be stored at 4° C. If samples are to be held for long-term storage, jars should be only two-thirds full, including the cover water. Samples should then be immediately frozen and stored on dry ice for transport. For short-term storage (less than 7 days), they should be refrigerated at 4° C; for long-term storage, they should be frozen and kept frozen until analyzed.

Invertebrate Samples

Samples of benthic invertebrates can be used to determine the extent of the kill and to document recovery after the kill. Samples should be taken in the same areas in which water and sediment samples were taken. If sufficient invertebrates, especially unionid mussels, are available, tissue can be used for residue analyses. Tissue samples should be frozen in a suitable clean container and properly tagged and labeled.

In most investigations, benthic invertebrate samples are not needed for toxicant residue analyses. If information on residues in the benthos is desired, a sample of at least 100 g is required for analyses. Generally, large invertebrates such as crayfish or unionid mussels suffice as samples for analytical purposes. Samples should be frozen in the same type of prepared containers as those used for sediments, and stored at −20° C until they are analyzed.

It is usually difficult to collect enough zooplankton for residue analysis. Generally, a record of its

The survival or death of invertebrates, such as crayfish, is a valuable clue to the cause of a fish kill.

Fish kills sometimes affect large areas. Many millions of fish were killed along several hundred miles of the Mississippi River and over large areas in its delta at the Gulf of Mexico by the dumping of a large quantity of a pesticide.

presence or absence and whether living or dead is sufficient.

Zooplankton samples can also be used to document the nature of the cause and the extent of the kill. The presence or absence of live animals can be useful information in determining the cause of the kill (see Chapter 3). The choice of sampling gear used to collect the zooplankton depends on the types of organisms present and the body of water to be investigated. To collect zooplankton, 30 liters of water are filtered through an 80-micron mesh plankton net. For a discussion of sampling techniques, see APHA et al. (1985) or Weber (1973). To preserve zooplankton, use 70% isopropyl alcohol or 5% buffered formalin. Do not store the sample in formalin longer than 48 hours before transferring it to 70% isopropyl alcohol.

Plant Samples

Phytoplankton and macrophyte samples are not normally used for residue analyses. However, in certain situations, for example, petroleum hydrocarbon contamination, residues can be rinsed from the plant surfaces and used to document the presence of particular hydrocarbons.

Phytoplankton

Samples of phytoplankton should be examined for the presence and abundance of live algae. Closing samplers, pumps and filters, or fine-mesh plankton nets can be used to collect samples. For quantitative determinations, the volume of water filtered must be recorded. If live samples are wanted for analysis, the samples should be refrigerated after collection or kept chilled at 4° C. For fixing and preserving samples, Lugol's solution is recommended (Weber 1973; Vollenweider 1974; APHA et al. 1985). (See Appendix E for the formula for Lugol's solution.)

Macrophytes

The distribution, abundance, and general physical condition of macrophytes should be noted if it is suspected that the plants are causing a decrease in the dissolved oxygen concentration, especially in early morning hours.

Environmental Factors, Stress and Fish Diseases

Trent Bollinger, February, 1996

The Environment

Epidemiology is the study of disease under natural conditions with the purpose of trying to understand the factors which determine health or disease in populations. Disease in its broadest context means any impairment of the normal structure or function of an organism. Disease can be further divided into infectious, noninfectious and genetic diseases, to name a few. A concept basic to the study of epidemiology is that disease is determined by the interplay of the host, the disease causing agent or agents and the environment. This is sometimes referred to as the epidemiological triangle (Figure1). In basic terms it means that for disease to occur it is simply not a matter of bringing a disease agent, such as a bacteria or virus, into close proximity to a host; instead, environmental factors through their effect on the host and disease agent (and vice versa) are equally important in determining whether disease will or will not occur.

Another way to present this concept is that the agent is part of the environment (Figure 2). Within this framework an agent is just one of many environmental factors which determine health status of a host. In some cases the agent is weather, in others its an infectious agent, in others its a toxic chemical, in mosts cases its combination of several agents.

For this discussion on the effect of pollutants on fish I am going to pull pollutants out as the primary agent of concern realizing that it is in fact a component of the environment (Figure 3). The fate of chemicals in the environment is determined by their chemical composition. Some remain relatively unchanged but more commonly pollutants are modified by the environment. Their chemical composition can be altered by biotic and abiotic factors making them more or less toxic to fish. They may be bound to substrates in the environment essentially preventing exposure to fish. In other cases they accumulate in the tissues of food species and are

found at increasing levels in organisms as one moves up the food chain. The bioaccumulation of persistent fat-soluble compounds such as organochlorines is probably the best example. Toxic effects on other species can alter the aquatic environment affecting food supply, predation, vegetation, spawning habitat, etc. Indirect effects such as these can have significant impacts on the health of fish populations. Direct and indirect effects of pollutants, in conjunction with other environmental factors, act to stress the host.

Stress

Stress in organisms has been defined by a number of individuals. The definition I will use is that of Wedemeyer and McLeay (1981), which states: stress is " the sum of all the physiological responses by which an organism tries to maintain or reestablish normal metabolism in the face of physical or chemical changes". In this and most other definitions stress is the response of the host and factors eliciting these responses are referred to as stressors. Some other definitions include the concept that the stress response some how impairs the survival of the host. Acute or severe stress, such as occurs when fish are exposed to high concentrations of a toxic agent or to an extremely virulent infectious agent (bacteria, virus, parasite), are obvious and often result in rapid death of the organism. Low level persistent or repeated stress, referred to as chronic stress, is usually less obvious.

Selye studied the stress response in mammals, including humans, and his observations can be generally applied to all vertebrates. He observed that the stress response could be divided into 3 phases. The first is the alarm phase in which the host invokes physiological processes to oppose the new stressor. If the stressor persists and does not kill the organism, it moves into the adaptation or resistence phase. In this phase the physiological processes of the host stabilize at a new level of activity in response to the stressor. The third or exhaustion phase occurs when the stressor is of sufficient duration and intensity that it exhausts the hosts physiological response and the organism dies. These phases have been referred to as the general adaptation syndrome.

Biomarkers/Bio-indicators

In study the effect of pollution, researchers have been trying to develop tests or biological indicators of chronic stress which can be applied to populations of fish and other aquatic organisms. The basic concept behind the use of biomarkers or bioindicators is that effects of sublethal stress are first seen at the lower levels of biological organization, in molecules and biochemical processes. Alterations at this level than manifest themselves as alterations in the form and function of individual tissues which ultimately lead to alterations in the overall health of the organism. Effects on the individual, such as reduced survival or reproduction, are then seen as effects at the population or community level. The expression of stress at one level of biological organization has its explanation in the levels below. To be useful, biomarkers should detect environmental stress prior to significant changes at the population level. Numerous biomarkers have been proposed, but all have their limitations and many are unproven. A major critisism of many of the biochemical markers is their unproven biological signifigance. For example, polycyclic aromatic hydrocarbons (PAH) typically induce increased production of a certain class of detoxification enzymes, called mixed function oxygenases, in the liver but at our current level of understanding it is difficult to relate these changes to significant ecological effects. Metallothionein is another example. Metallothionein is a metal binding protein found in a wide variety of species which is important in normal physiological regulation of metals in tissues. Exposure of fish and other organisms to heavy metals such as cadmium, copper, zinc and mercury induces tissues, such as the liver, to synthesize metallothionein. The effects of metallothionein induction on higher levels of biological organization is still not understood. It is unlikely that a single biomarker will be found that predicts ecological effects and instead it is recommended that several levels of organization be measured when trying to assess effects of pollutants.

Another nonspecific biomarker which has been proposed, involves measuring the induction of the generalized stress response, as discussed above. Acute stress in vertebrates elicits a well described nonspecific response culminating in the production of catecholamines (adrenalin, etc.) and corticosteroids (cortisol, etc.). These hormones and neuropeptides have a

number of secondary effects which may be detrimental to the host. In fish, this response begins with sensors in the body detecting stresses and relaying this information to the brain. A portion of the brain called the hypothalamus processes this information and responds by inducing a glandular structure at the base of the brain, called the pituitary gland, to secrete a chemical (ACTH) into the blood stream. This chemical travels to the kidney where it induces interrenal cells to produce and release cortisol into the blood. This system is sometimes referred to as the hypothalamic-pituitary-interrenal (HPI) axis. In mammals corticosteroids are produced by the adrenals. The hypothalamus also induces cells to produce catecholamines. Corticosteroids and catecholamines have a number of physiological effects often referred to as secondary stress effects. These include increased breakdown of protein and energy stores in the body and depression or impairment of the immune response. Examples of stressors which can elicit this response are: capture and handling, high stocking density, poor water quality, infection, and pollutants.

Immunity

Suppression of the immune response is thought to be one of the reasons why occurrence of infectious disease in fish is strongly associated with stress. Corticosteroids have been shown to depress host defenses at various levels. Skin, gill epithelium and gastro-intestinal mucosa provide the first defense against pathogenic organisms. These structures produce mucous which traps and sloughs microorganisms. Mucous contains proteins and cells which can lyse bacteria. If an organism penetrates the mucosal layer the thick epidermis provides a physical barrier to further advancement. The effects of cortisol on the stucture and function of epithelial barriers and mucous has been poorly studied in fish. Direct toxic effects of chemicals on epithelial cells and alterations in composition of the mucous layer can allow water borne pathogens to enter the host. If a microorganism penetrates these initial defenses, it then confronts nonspecific defenses within the host. These are generally components of the inflammatory response and include proteins in the blood and tissues, such as, lysozyme, compliment and interferon. Phagocytic cells (cells that engulph and destroy microorganism), primarily macrophages, are also important

in this defense, as are nonspecific cytotoxic cells. Macrophages are a critical component of the nonspecific and specific immune reponse. Cortisol significantly impairs macrophage function.

The specific immune sytem responds to specific microorganisms and allows for the induction of "memory". Immune memory is the ability to mount a more effective response with subsequent exposures. The specific response consists of an afferent or induction pathway and an efferent or effector pathway. The afferent pathway involves the engulfment and digestion of microorganisms by macrophages and the presentation of components of the digested microorganism on the cell surface of the macrophage. Presentation of specific components of the invading microorganisms stimulates cells called lymphocytes to divide and produce antibody. Antibody and certain populations of lymphocytes can assist in destruction of other similar microorganisms. Cortisol has been shown to inhibit most stages of this process.

Infectious Diseases of Fish

Many of the bacteria which infect fish are those which are common in the aquatic environment. Therefore, for disease to occur something must upset the balance between the agent and the host. For example, environmental factors can favor survival and reproduction of the microorganism allowing it to build to numbers which overwhelm the host. Alternatively, environmental factors can depress the physiological responses of the host, such as immunity, making it more susceptible to infection. In virtually all cases it is a combination of factors which result in disease. Disease surveillance, therefore, can be used as an indicator of environmental degradation. In some cases infectious disease can be attributed to natural stressors such as seasonal changes in water temperature and water quality, or natural physiological stressors sush as spawning. Well planned studies are required in order to differentiate these effects from the effects of pollutants.

Summary

Pollutants are modified by the environment and modify the environment, both of which may be detrimental to fish. Pollutants can be considered part of the environment and are one of

several factors which can stress or alter the physiological state of fish. Biomarkers or bio-indicators are tests which can be used to identify chronic stress in fish prior to development of significant effects at the population or community level. A single biomarker will likely never be found which will predict ecological effects and therefore tests at several levels of biological organization are recommended. Environmental changes which stress fish or favor development of infectious organism can result in outbreaks of infectious disease. Disease surveillance in fish can be used to identify potential environmental problems.

FISH HEALTH RESOURCE LABORATORIES AND PERSONNEL

IN WESTERN CANADA

General Information:

Canadian Cooperative Wildlife Health Centre
Western & Northern Regional Centre
Department of Veterinary Pathology
University of Saskatchewan
52 Campus Drive
Saskatoon, SK S7N 5B4

Tel: 1-800-567-2033 (Canada)
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Email: ccwhc@sask.usask.ca

Alberta:

Animal Health Laboratory Branch
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Tel: 403-422-4830
Contacts:
- Bev Larson (Fish & Wildlife)
- Jim Hanson (Agriculture)

- General diangosis of fish diseases and advice of field investigations. Bev Larson is the fish disease specialist with Alberta Fish and Wildlife and she works in close association with Jim Hanson and the staff of the Animal Health Laboratory.

British Columbia:

BC Environment
Fisheries Branch, Fish Culture Section
Fish Health Unit
c/o Science and Technology Dept.
Malastina University/College
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- Sally Goldes
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Contacts:
- Dorothee Kieser
  Tel: 604-756-7069
- Carl Westby
  Tel: 604-756-7057
  - FAX: 604-756-7053

- Responsible especially for federal fish health regulations and for anadramous salmonids; available for general consultation and assistance.

Animal Health Centre
Ministry of Agriculture, Fisheries & Food
1767 Angus Campbell Rd
Abbotsford, BC V3G 2M3

Tel: 1-800-661-9903 (BC)

Contacts:
- Ron Lewis
  Tel: 604-556-3003
  - FAX: 604-556-3010

- This laboratory does not specialize in fish diseases but can provide general diagnostic services for infectious and non-infectious diseases of fish.

Manitoba

The Freshwater Institute
Department of Fisheries and Oceans
501 University Crescent
Winnipeg, MB R3T 2N6

Contacts:
- Brian Souter
  Tel: 204-983-5125
- Andy Dwilow
  Tel 204-983-5128
  FAX: 204-984-2403

- Responsible especially for federal inspection and certification of fish hatcheries. Available for general consultation and assistance with infectious diseases of fish.

Animal Health Centre
545 University Crescent
Winnipeg, MB R3T 5S6

Contact:
- Mark Swendrowski
  Tel: 204-945-8838
  - FAX: 204-945-8062

- This laboratory does not specialize in fish diseases but can provide general diagnostic services for infectious and non-infectious diseases of fish.

Northwest Territories

Renewable Resources
Wildlife Management Division
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Yellowknife NW X1A 2L9

Contact:
- Brett Elkin
Tel: (403) 873-7761
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- Responsible for wildlife diseases in general. Can provide consultations, advice and referrals.

Saskatchewan

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Western & Northern Regional Centre
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University of Saskatchewan
52 Campus Drive
Saskatoon, SK S7N 5B4

Tel: 1-800-567-2033 (Canada)
or: 306-966-5099

Contact:
- Trent Bollinger
- FAX: 306-966-7439

- General diagnostic services, consultation, and assistance with field investigations of infectious and non-infectious diseases.

Yukon

Fish and Wildlife Branch
Box 2703
Whitehorse YT Y1A 2C6

Contact:
- Phil Merchant
- Tel: 403 667 5285

- Responsible for wildlife diseases in general. Can provide consultations, advice and referrals.

Helpful References:

Introduction to Fish Health Management, 2nd Edition. 1995.
Edited by Becky A. Lasee. U.S. Fish and Wildlife Service, LaCrosse Fish Health Center, 555 Lester Avenue, Onalaska, Wisconsin,54650 USA
(Available from this address)

- An excellent general reference to diseases of freshwater fish

Field Manual for the Investigation of Fish Kills. 1990.
(U.S. Department of the Interior, Fish and Wildlife Service, Resource Publication 177)
Edited by Fred P. Meyer and Lee A. Barclay
Available from: National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161 USA

- An excellent practical guide to the investigation of disease outbreaks in wild fish