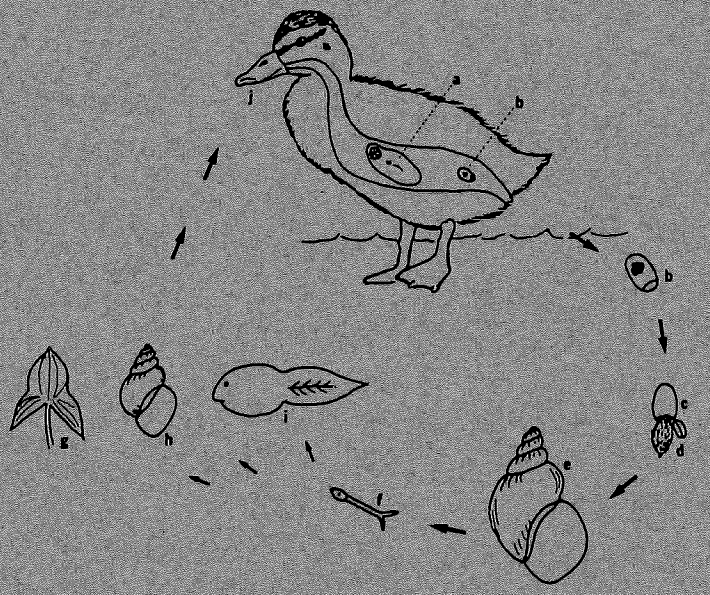
WATERFOWL DISEASE and WETLAND MANAGEMENT



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WHAT IS DISEASE?

Gary Wobeser

Although we use the term daily, and we each have a mental picture of DISEASE, it is surprisingly difficult to produce a definition that includes everything we want included and that still excludes items that are not disease. We use the word disease differently in different situations, e.g., we will be talking about waterfowl disease, and in this context people normally equate disease with dead birds, usually in large piles. In contrast, a group of veterinarians might discuss "*production diseases*", by which they mean that the cows don't give as much milk as desired or the pigs take a few extra days to reach market weight, but few animals die. We are even more liberal in what we class as disease when we talk about humans. It is usually not necessary to die to take a day off work because of disease, and people talk about obesity as a disease.

The definition that I like is that **disease is any impairment that interferes with normal functions of an individual**. Note that this definition doesn't say anything about the cause, duration, or severity of the disease. Certainly if a duck is dead, its normal functions are impaired and we can apply the term disease but we can also use the term to describe a condition in which reproductive function is impaired, so that it lays less eggs; or a condition in which a bird's normal behaviour is disrupted, so it is less attentive to its nest. The latter might occur after sublethal exposure to a pesticide. Just because a disease is not spectacular we should not discount its potential impact on a population. A disease that reduces reproductive success might have a greater impact on the population than a disease that killed a few thousand ducks, even though the latter event is much more spectacular.

The general term disease can be sub-divided in many different ways. The most simple classification is to divide diseases into **infectious** and **non-infectious** groups.

Infectious diseases are caused by living organisms that live on or in the bird "*host*". We usually refer to these organisms as "*disease agents*". We can divide disease agents in various ways. One way of separating agents is on their life-style, and by whether or not they are dependent on the host for growth and reproduction. Using this method, we can recognize two groups of infectious agents:

- **obligate disease agents** include those which are totally dependent on the bird at some stage of their life. (Often this is the stage in which they reproduce). Examples of obligate disease agents in waterfowl include the herpesvirus that causes duck plague and all other viruses, the bacterium *Pasteurella multocida* that causes avian cholera, and the various protozoa and worms that we usually lump as "*parasites*". These agents are only able to reproduce in or on a suitable host.

- **opportunistic disease agents** include those that will grow in or on a host if given the opportunity, but which are fully capable of living and reproducing away from the bird.

An example of an opportunistic disease agent in waterfowl is the fungus Aspergillus fumigatus that causes the disease Aspergillosis. This fungus is everywhere in the environment, living happily in decaying organic matter and producing spores to reproduce itself. Birds (and humans) inhale spores everyday but, in a normal individual, the spores are quickly dealt with by the body's defence system. However, if a bird is in an environment where there are massive numbers of spores (for example if the bird is feeding on mouldy grain), or if the bird's immune system is compromised in any way, some of the spores may germinate in the lungs or airsacs, cause tissue injury and death of the bird. There are also agents that usually live as harmless commensals in the body without causing disease, but which can overgrow and cause injury, if the bird's defences are injured or impaired in some way. We believe that this is the case in the disease necrotic enteritis of geese, in which the bacterium *Clostridium perfringens*, which is present in the gut of normal birds proliferates and produces toxins that injure the intestine.

Another way to sub-divide infectious agents is into so-called **microparasites** and **macro-parasites**. As the names suggest, agents in the first group are generally smaller than those in the second. There are several differences between the two groups but the most important one is in the type of disease they produce and how the host responds.

In general, **microparasites** cause rather short-lived disease. Animals that survive the infection have good resistance to the agent, so they are resistant to re-infection, and the agent is not found in healthy animals. Measles in children is a good example of a microparasite; it causes a short sharp disease but, once a child recovers the virus is gone and he or she is resistant for years if not for life. Most viral and bacterial diseases of birds probably act in a similar manner; one important exception is the virus that causes duck plague. Birds infected with this virus probably remain infected for life.

In contrast, **macroparasites** generally result in very prolonged infections; many healthy animals can be infected with a small number of the agents; and there is poor resistance to reinfection. Thus, we expect to find a few worms in most ducks. Clinical disease caused by these agents is usually the result of an abnormally large number of parasites.

Finally, infectious agents can be **contagious** or **non-contagious**. A contagious agent is one that spreads from one bird to another. An example is *Pasteurella multocida* that causes avian cholera; this bacterium is transmitted from bird to bird. An example of a non-contagious agent is the fungus *Aspergillus fumigatus* which is acquired from the external environment and does not spread from a diseased bird to others

Non-infectious diseases are caused by a great variety of "*causative factors*", including toxins and poisons (e.g., botulism, lead, blue-green algae, selenium), physical features (e.g., cold, heat), chemical features (e.g., salinity), deficiencies (e.g., vitamin A deficiency, starvation), and genetic and developmental defects.

Cause and Effect

We often assume that disease can be explained by a simple equation such as :

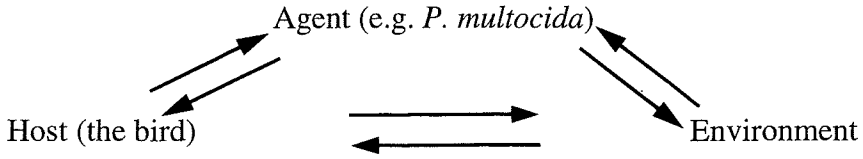
bird + *Pasteurella multocida* = avian cholera

or

ducks + *Clostridium botulinum* type C toxin = botulism outbreak

However, while this simplistic approach may work in the laboratory when you inject *P. multocida* into a bird, or force ducks to ingest botulinum toxin, it can very seldom explain what actually happens in nature.

Another concept has been developed to explain the relationship, and in this equation there are three interacting components, rather than two:



The basic feature of this concept is the notion that other factors in the environment can alter the relationship between a disease agent and its potential victim. Although none of us may refer back to this triangle again directly during the remainder of the course, this concept is central to everything that we will be discussing, both in trying to explain how and why disease occurs, and in speculating how we might manage to reduce disease.

I want to use a simple hypothetical example to illustrate how the concept works. Let us assume that we have an infectious disease agent "A" and some birds. There are several steps that have to occur before A will produce disease. The first is that a bird must contact A and we call this step **exposure**. The amount and type of exposure is likely to be influenced by many environmental factors, as well as by the density and distribution of agent A, and of the birds. If a large number of birds are crowded together in an area where A is plentiful, the risk of exposure of at least one bird is great. The second step is that A has to enter into the body of the bird and become established. We call this step **infection**, and it is influenced by the degree of exposure (how many of A entered the body) and by the **resistance** of the bird to infection. The bird's resistance is influenced by many factors including its species, age, sex, nutritional state, prior experience with the agent (particularly if A is a microparasite), presence of other disease agents in its body, and the state of its immune system. Obviously, many of these components can be altered by environmental factors. For example, a bird that is malnourished, has many intestinal parasites, and high levels of corticosteroid because of stress from being crowded on poor quality water during a cold spell, may have poor resistance to infection. If A is able to become established in the bird, it may or may not produce *an impairment that interferes with normal functions*, i.e. disease. Some individual birds will be less effected by A than other birds, and resistance to the effects of A is also influenced by a great variety of environmental factors. If the bird does become diseased, this entire process of exposure and infection must be repeated before

the disease is transmitted to another bird.

We will be stressing the importance of **exposure** and **resistance** throughout this course, and trying to show how habitat changes and management activities can inadvertently or intentionally alter these factors.

During the course we will also use a few terms to describe the way that diseases occur within waterfowl populations. Certain patterns of disease have been recognized and categorization of disease in this way is often helpful in thinking about the significance of disease. In human medicine, the terms *endemic* and *epidemic* are used; when referring to disease in animals, the equivalent terms *enzootic* and *epizootic* are often used. (There are no obvious reasons for having different terms for animals and humans, other than past practice). These terms depend upon having a history of how the disease has occurred in the past in an area or population.

An **enzootic** (endemic) disease is one that occurs in a population (or area) at a regular, predictable or expected rate. For example, we have found a low level of mortality due to avian cholera among Snow Geese migrating north through Saskatchewan every spring since 1977, so the disease at that location and time period is enzootic. Similarly ,we expect to find that about 10% of adult Northern Shovelers will have *Sarcocystis rileyi* parasites in their breast muscles so infection with that parasite is enzootic.

An **epizootic** (epidemic) occurs when a disease occurs at a time or place where it is not expected, or at a rate substantially greater than expected for the time. An outbreak of avian cholera that occurred among moulting Redheads in 1988 on Manito Lake in western Saskatchewan was considered to be an epizootic, because it had never been detected on the site before (or since). A single case of duck plague recognized in a wild Mallard near Saskatoon in 1984 was also, by definition, an epizootic.

A single disease may occur in both patterns on a single site; e.g. botulism occurs at a low (predictable?) level in most years at Eyebrow Lake, but at irregular intervals many birds die. The pattern of botulism on this wetland is enzootic with periodic epizootics.

The terms *die-off* and *outbreak* are less precise in meaning, and are usually used to describe situations where many birds die over a short period of time. An outbreak or die-off is not necessarily synonymous with an epizootic.

HOW IS DISEASE DESCRIBED AND MEASURED?

Trent Bollinger

Disease is described or understood at several levels. Disease can be described at the level of the individual by describing the typical clinical signs such as diarrhea, leg paralysis, lethargy, difficulty in breathing, etc. The etiology or cause (ie. *Pasteurella multocida*, organophosphate poisoning, botulism) and the pathogenesis or development of the disease is described. Determination of the pathogenesis involves understanding alterations in physiological processes at the system, organ, cellular and biochemical level. Finally, we can describe the response of the individual to treatment and vaccination. However, not all individuals respond in a similar fashion to disease-causing agents, due to differences in genetic makeup, environmental factors, level of exposure to the agent, and so on. To account for this variability we observe effects of disease at the level of the population, describing the frequency of disease occurrence in groups of individuals. Here the emphasis is on quantifying the effect of disease and is accomplished by grouping individuals into categories based on common characteristics such as species, age, sex, and so on.

Individuals are categorized by disease status, diseased or non-diseased, using a case definition. The case definition is a list of characteristics or clinical signs which are used to categorize diseased individuals. The case definition can broad and include several causes. For example, all cases of feedlot pneumonia in cattle, as defined by individuals with elevated respiratory rates, coughing, elevated temperature, and lethargy which will include individuals infected with bacteria and/or viruses. The case definition may be more refined or specific and include only those cases with specific pathological lesions or from which a specific agent, such as a bacteria, is isolated.

In wildlife die-offs our case definition is often very broad. For example, in a botulism die-off the diagnosis is typically based on the results of tests on approximately 6 individuals and once the diagnosis of botulism is made all carcasses collected from that marsh are categorized as having died of botulism. In these cases botulism is likely the cause of death in the majority of birds but because the case definition is so broad, basically anything dead, other diseases are missed. A certain unknown percentage of birds will have died of other diseases such as starvation, blue-green algae toxicity, trauma, predation, etc. The case definition must be sufficiently specific to meet the needs of the study. In outbreaks of long duration, carcasses and sick individuals should be necropsied at regular intervals to ensure that the same disease is responsible for the mortality.

The third level at which we can describe disease is at the biological or ecological level. At this level we describe the interaction between the host, the agent and the environment sometimes referred to as the epidemiological triangle. These relationships are complex and often poorly understood. As an example, for botulism to occur spores of *Clostridium botulinum* must be present, the bacteria must be infected with a virus or bacteriophage which causes it to produce

type C toxin, environmental conditions must be conducive for germination of the spores and finally the toxin must be ingested by a susceptible host species. The interaction of *C. botulinum* with other bacteria in the water and sediments, the role of invertebrates and vegetation, and so on, are all part of the ecology of botulism. Throughout this course diseases of waterfowl will be described at these three levels. You will see that for the majority of diseases understanding is best at the level of the individual and we are just beginning to make inroads in understanding disease at higher levels.

When trying to understand disease at the population level it is important to identify the larger target population or the group of individuals whose characteristics we'd like to understand. Examples of a target population might be moulting dabbling ducks on Pakowki Lake, Alberta or the entire continental population of mallards. The choice of the target population is dependant on the questions asked. Waterfowl biologists or managers often ask the question, what is the effect of disease on the population. In this situation the target population is the continental, flyway, or perhaps regional population of waterfowl. Since each individual in the target population is rarely counted there is typically a sampled population which must be representative of the target population. For example, a common source of information on disease occurrences are the records from veterinary diagnostic laboratories. This information is usually not representative of disease occurrence in the wild due to the biased nature of the sample. The records can be used to detect or identify diseases of concern, geographic range, etc. but by themselves cannot be used to make estimates on the prevalence of disease in wild populations. In the same sense using mortality rates from a few large die-offs or from the surveillance of a few large marshes is not an appropriate sampled population in order to extrapolate to continental populations. Undetected enzootic and chronic disease on other marshes may be more significant at the continental population level than the mortality which occurs during epidemics or outbreaks which tend to receive the most attention.

There are 3 characteristics which need to be measured in order to describe disease in a population: 1) number affected, 2) number at risk and 3) time. All three parameters are required to address questions of population effects of disease. However, in most waterfowl disease studies or outbreak investigations this information is lacking. Typically what is known are the number of carcasses collected which is a minimum estimate of mortality. These counts cannot be meaningfully used to compare differences in disease occurrence over time or from location to location.

There are many reasons why more complete population data on waterfowl disease is not collected but the main reason is that wild birds are elusive and mobile which makes measurement of these 3 parameters difficult. In contrast when dealing with disease in a pen of feedlot cattle we can count the number at risk, we can recognize all disease individuals and we know or we can follow these individually identified animals over time. This is not the case for wildlife and in particular for waterfowl.

Let's look at each of the 3 population parameters individually. The number of individuals affected by disease is the most basic parameter to measure but there are several factors which increase the uncertainty of estimates. Sick birds often seek seclusion in heavy vegetation and many waterfowl species are cryptically coloured to avoid detection. Researchers studying the effectiveness of carcass searches experimentally have found that only a relatively small percentage of carcasses are found. For example, Stutzenbaker *et al* (1986) placed 100 banded duck carcasses in a 100 acre shallow marsh in Texas. Thirty minutes after the carcasses were placed, 8 individuals searched the marsh and collected any carcasses they found. Of the fifty carcasses placed in cover, none were found and of the fifty placed on top of vegetation in exposed positions, 6 carcasses or 12% were found. In another study Cliplef and Wobeser (1993) used marked carcasses during routine carcass clean-up operations of a botulism die-off to estimate the proportion of carcasses found. Under these conditions approximately 1/3 of the marked carcasses were collected and there were distinct differences amongst species in the proportion of carcasses picked up; approximately 53% of marked mallard carcasses were recovered as compared to only 25% of carcasses of smaller species such as American coots and blue-winged teal.

Removal of carcasses by scavengers is another factor to consider when enumerating the number of diseased individuals. The rate of disappearance of carcasses is dependent on number and type of scavengers in the area, species of bird involved and density of carcasses. Large die-offs appear to overwhelm the ability of scavengers to remove carcasses. The rate of disappearance of carcasses is therefore very specific to the situation. In some circumstances 50% or more of duck to goose-sized carcasses have been reported to disappear within 4 days while in the case of a botulism die-off only 1 in 42 duck carcasses was disturbed during the 4 days immediately following death.

In some situations, such as when the marsh is too large for complete carcass clean-up or in situations where carcass clean-up is not instituted, it may be decided to estimate the mortality using a sampling strategy such as line transects, etc. Application of wildlife population survey techniques, taking into account visibility, rate of carcass loss and rate of development of new cases need to be applied in these situations. Statistical sound sampling for disease individuals has rarely been applied to disease studies or investigation in waterfowl.

Estimation of the number of animals at risk in a population is often even more difficult. As with enumerating diseased individuals, visibility of birds is a problem. In addition waterfowl are highly mobile and counts on a marsh can vary depending on time of day and from day to day. During migration populations on a marsh can be highly variable. Surveys of large marshes usually require aircraft and if mortality occurs over an extended period of time, surveys must be repeated to account for bird movements. The cost of these surveys can be high; however, to obtain meaningful data on disease at the population level, estimates of number at risk must be made.

Finally this monitoring must be repeated over time in order to provide estimates of the probability of disease occurrence. A single survey can provide an estimate of the prevalence of disease in a population but this is only a snapshot of disease occurrence at that point in time. To be meaningful the rate of new disease occurrence over time, referred to as the incidence, must be determined.

Now that we have identified the parameters needed to measure disease in population and inherent difficulties in measuring these quantities, lets look at some of the methods which have been reported or proposed to allow measurement of these quantities. It must be remembered that there will not be a single best way to measure these parameters but instead the techniques employed will be determined by circumstances, budgets and information needs.

Some examples of solutions to the problem of counting diseased birds are as follows. The use of marked carcasses has been discussed previously and has been used during botulism clean-up operations to determine the percentage of carcasses actually retrieved during clean-up operations. With this information a correction factor can be applied to the number of carcasses collected to estimate total mortality. Cliplef and Wobeser used a correction factor of 3 to determine the mortality at Eyebrow Marsh. This correction factor has been used for botulism outbreaks at other locations and in other outbreaks different correction factors have been used, the value chosen often based on intuition. Unless this value is measured during each die-off the correction factor is at best a guess. Similarly, rates of carcass removal by scavengers and decomposition are very much dependent on the circumstances and should be assessed for each disease investigation.

I've already discussed rates of carcass scavenging but will also add that Wobeser, when studying the occurrence of avian cholera in spring migrant geese in Saskatchewan, left marked carcasses in the field to determine their rate of disappearance and found that they were completely removed by 1 wk. Based on this, they knew they could repeat their aerial counts of carcasses weekly without recounting diseased individuals. In this study they also determined a visibility factor for aerial counts of dead birds by repeating the counts on the ground in selected areas. This ground-truthing is a well-established aerial survey technique used in spring waterfowl breeding pair counts, etc. We have done some preliminary work on estimating mortality on Old Wives' Lake using randomly located line transects plotted on a map of the lake and by using hand-held GPS units and an air-boat to count carcasses on these transects. With this technique we were able to estimate the mortality on the entire marsh. Although this technique has been reported to be a relatively inefficient way of collecting or counting carcasses based on carcasses collected per person hour, it does allow statistical estimates of mortality on the entire marsh. Other methods of carcass counting do not. This technique can be improved stratifying the sampling based on vegetation types and/or concentrations of carcasses. This should reduce confidence intervals and make the sampling more efficient. Random sampling of a marsh during carcass clean-up operations could also be used to assess the effectiveness of carcass clean-up operations and to estimate total mortality.

Estimates of number of waterfowl at risk during a disease investigation are costly but are needed to describe disease in populations. Aerial and ground surveys of bird populations were done at Pakowki Lake in 1996 and these gave a preliminary estimate of waterfowl use of the lake. Counts need to be repeated over the summer during prolonged outbreaks, such as those occurring at Pakowki Lake, Whitewater Lake and Old Wives' Lake, to account for movement of migrant birds. Indices of abundance such as counts on study plots, catch per unit effort, mark and recapture, etc. have been used to compare relative abundance of animals over time and could be used in conjunction with surveys to better estimate numbers at risk.

There are several methods in which the number at risk has been accurately determined. Radio telemetry has been used to monitor birds and animals over time allowing accurate assessment of number diseased, number at risk, and time. These have the disadvantage of cost and number of individuals which can be monitored at one time. In other studies birds have been banded, treated and released and band returns have been used to estimate probability of mortality. Bellrose (1959) trapped and banded several thousand mallards and prior to release gave them 0, 1, 2 or 4 lead shot orally. The outcome of the experiment was monitored via band returns and from these results he was able to extrapolate to larger populations. More recently geese have been captured, banded and either vaccinated for avian cholera or left unprotected and susceptible to the disease. Band returns are again being used to assess the outcome.

Rocke and Brand (1994) have used captive-reared mallards kept in enclosures on botulism prone marshes to determine the daily mortality rates during botulism epidemics. The ability to collect all diseased individuals and monitor the captive ducks (the population at risk), over time, allowed for the calculation of site-specific mortality rates.

Only with better information on population parameters can we answer the questions of effectiveness of management strategies, factors involved in the development of disease, and extrapolation of information from outbreaks and studies to the larger target population. Samuel (1992) used the Mallard Annual Cycle Model to assess the influence of 3 diseases (lead poisoning, botulism and avian cholera) on population dynamics of mid-continental mallards using existing records and reports on the occurrence of these 3 diseases. He found that the model was very sensitive to estimates of daily probability of mortality and the proportion of birds that are at risk to these diseases and we need better estimates to assess these population effects. Estimates on numbers affected, population at risk and duration of risk are needed.

In summary, the number of diseased individuals and the number of individuals at risk over time must be measured or estimated in order to describe disease in populations. Without all 3 parameters we cannot adequately address hypothesis on causes of disease or effectiveness of control measures, or extrapolate to effects on continental populations. This aspect of disease investigation and research warrants more emphasis than it has received in the past.

AVIAN CHOLERA

Gary Wobeser

Avian cholera is an infectious and contagious disease of birds caused by the bacterium *Pasteurella multocida*. Infection with the same species of bacterium in domestic poultry is called *"fowl cholera"*. It is likely that all or most species of birds are susceptible to infection with *P. multocida*; however, birds vary in their resistance to the agent and different strains of the bacterium occur in different species. (The strains causing disease in wild waterfowl are different from those causing disease in chickens and turkeys; and strains in birds in general are different than strains that occur in mammals).

Avian cholera has a long and interesting history in North American waterfowl. It was first recognized among wintering waterfowl on a single refuge in California and another in Texas in 1944. The disease recurred every following winter on these two sites but was not recognized elsewhere until the mid-1960's, when a few outbreaks were recognized on other wintering areas, and among nesting Common Eiders on the Atlantic coast of the USA and in the Gulf of St. Lawrence. Since the early 1970's, the disease has been recognized very widely throughout North America, with very high mortality (>100,000 birds) in some sites. Major sites with recurring large outbreaks among wintering and staging birds in the USA are the Central Valley of California; northern California (Tulare Lake, Klamath Basin), the Texas Panhandle, and the Rainwater Basin of Nebraska. Avian cholera was first recognized in waterfowl in western Canada in 1977 among Snow and Ross' geese migrating north through western Saskatchewan. A low level of mortality has occurred in these geese each spring since 1977. The first recognized outbreak among nesting waterfowl (other than Common Eiders) was on the west coast of Hudson Bay among Lesser Snow Geese in 1979; since then periodic outbreaks have been recognized among nesting Snow and Ross' Geese in other colonies in the central and western Arctic. Avian cholera has been recognized among southward migrating geese in Manitoba and Saskatchewan on several occasions. Usually, at least some of the birds involved were from colonies in the arctic that had experienced an outbreak during the summer. Mortality among nesting Common Eiders has occurred sporadically. An outbreak occurred among nesting Double-Crested Cormorants at Lac La Biche, Alberta and an outbreak occurred among moulting Redheads on Manito Lake in western Saskatchewan. Lesser Snow Geese seem to be involved in the early stages of many outbreaks, and movement of the disease has coincided with movement of this species on several occasions.

Knowledge of the ecology of Avian Cholera is still imperfect. The most perplexing problem is where the bacterium hides when it is not causing disease. In domestic poultry, it has been known for many years that apparently healthy individual birds can carry the organism in their nasal cavity and shed the bacterium in secretions, and that these carrier birds can infect other birds. The same is thought to occur in wild waterfowl, but it has been very difficult to find carriers; probably because the prevalence of carriers in a population is very low, so that very large samples have to be examined to have a reasonable chance of finding a carrier. *Pasteurella*

multocida can spread from bird to bird via close direct contact and this may be method of transmission during periods when only very low level mortality is occurring, e.g. among northward migrating geese passing through Saskatchewan each spring. The bacterium can also survive for days to weeks in surface water. If carrier birds are shedding the bacterium regularly into water, the number of bacteria could build up over time, until transmission occurs through the water. This may explain why sudden large outbreaks involving many species occur on crowded wintering and staging refuges. Nasal discharges from birds that die of avian cholera contain many *P. multocida*, so that even an occasional death from the disease (which could go unrecognized) would lead to water contamination.

Very little is known about the infection stage of avian cholera. If birds are exposed experimentally to *P. multocida*, most will die within 24 hr. Many wild waterfowl also die very acutely, but is not known what proportion of birds that are exposed are resistant to infection, what factors effect resistance to infection, or how carrier birds are able to have the bacterium in their body without becoming diseased.

Most wild waterfowl with avian cholera are found dead and sick birds are very rarely observed. Dead birds are generally in excellent body condition and pathology is limited to small hemorrhages on the heart and internal membranes, tiny white spots within the liver (these are foci of acute necrosis), and excess mucus within the intestine. Many birds have no lesions and it is common to find recently ingested food in the esophagus. (Scavenging birds, such as crows and gulls, may become infected by feeding on waterfowl carcasses. Because these birds seem to be more resistant to the effects of *P. multocida,* so they survive longer after infection and may have more advanced lesions with large amounts of fibrinous exudate in their body cavity).

Avian cholera should be suspected in any situation in which waterfowl in good body condition are found dead with minor or no lesions. If the bird is fresh when found, a smear of heart blood can be examined for the presence of small cocco-bacilli bacteria, but a confirmed diagnosis requires isolation and identification of the bacterium in the laboratory. Whenever possible, intact birds kept chilled should be submitted for examination. We have found that *P. multocida* can be isolated from bone marrow in the wing bones for an extended period after the death of a bird. Collection and submission of wings is useful in circumstances where only scavenged carcasses are available or where it is difficult to recover and submit entire birds.

There are currently no general management strategies to reduce the occurrence of avian cholera. In some instances, where exposure occurs through water in a limited area, it may be possible to reduce exposure by altering the distribution of birds, or by habitat changes. Collection of carcasses during outbreaks may lower the amount of environmental contamination with *P. multocida.* Reducing the effects of other stressors, such as food availability might increase the birds' resistance, but the basic problem on wintering areas is compression of large numbers of birds on small water areas for extended periods of time.

DUCK PLAGUE

Ted Leighton

Duck Plague is a disease of ducks, geese and swans caused by a virus from the family of viruses known as herpes viruses (cold sores and chicken pox are diseases of people caused by herpes viruses). This disease first appeared in North America in the late 1960's. In the winter of 1973, a massive outbreak that killed some 40,000 Mallards occurred at the Lake Andes National Wildlife Refuge in South Dakota and terrified waterfowl management agencies across the continent. It caused the US Fish and Wildlife Service to establish a wildlife disease unit, now the National Wildlife Health Center in Madison, Wisconsin, which has since provided international leadership in the incorporation of disease management into wildlife management. Fortunately, Duck Plague never became the devastating disease that many had feared. While it remains an issue and a controversial disease in terms of management policies, and while important information about the disease remains to be gathered, it has been only a minor source of waterfowl mortality in North American.

Duck Plague was first described in the Netherlands in the 1920's in domestic ducks. The virus was characterized and the disease named Duck Plague in 1949. Other names that have been used for this disease include "duck virus enteritis" and "anatid herpes virus infection". Duck Plague is known to occur in Europe, Asia and North America. It first was recognized in North America on commercial duck farms on Long Island, New York, in 1967. That same year, it was diagnosed in a feral Mute Swan and then in several hundred wild ducks found dead in the same general geographic area as the affected commercial farms. Only two other outbreaks have been recognized in free-flying waterfowl in North America: Lake Andes in 1973, where several hundred Canada Geese died in addition to the Mallards (mortality rates were approximately 40% in Mallards and 3% in Canada Geese), and an outbreak in 1994 in the Finger Lakes region of New York State that killed approximately 1150 waterfowl out of the 50,000 at risk in the area (2%). There have been numerous other small outbreaks in the United States and a few in Canada. These have all centred on small flocks of captive or feral ducks, often including Muscovy Ducks, and domestic ducks or domestic-wild hybrids in habitat potentially shared with free-flying wild waterfowl.

Duck Plague most often appears as a cause of death without a recognized clinical course of disease. Thus, it must be considered as a possible cause of death for any duck, goose or swan found dead. At necropsy, lesions highly suggestive of Duck Plague may be evident. However, in the most susceptible species, death occurs so rapidly that there may be no observable abnormalities in the dead birds. When present, the lesions typical of Duck Plague include areas of necrosis on the surface of the lower esophagus and of the cloaca, small pale areas of necrosis in the liver and spleen, hemorrhage and necrosis of patches of tissue along the intestinal tract (lymphoid tissue) and hemorrhage more or less anywhere, including bleeding from the mouth or anal vent. None of these lesions is completely diagnostic. Microscopic examination of tissues often reveals lesions highly suggestive and sufficient for a preliminary diagnosis. A definitive

diagnosis requires identification of the causative virus. Only a few laboratories are equipped to isolate and identify this virus. Thus, it is particularly important to secure and freeze (dry ice, liquid nitrogen or a -70C freezer) liver or spleen from suspect birds as early as possible in the investigation, and to forward these to an appropriate laboratory.

The virus that causes Duck Plague is known variously as the Duck Plague Virus, Avian Herpes Virus 2, and Anatid Herpes Virus. It appears to be one virus but to exist as multiple strains that vary considerably in their ability to cause disease (virulence). The virus infects only members of the family Anatidae - ducks, geese and swans. The virus survives outside the body of its host birds for sufficiently long periods to be transmitted through water, contaminated food material, etc. Virus could be isolated from water from Lake Andes held at 4C for up to 60 days. This "horizontal" bird-to-bird transmission, either direct or via contaminated water or other material in the environment, is considered to be the usual route of transmission. There also can be "vertical" transmission in which the virus is passed from mother to offspring through the egg. There also is evidence that birds that survive infection carry the virus for long periods and shed virus periodically, possibly for the rest of their lives. This behaviour is typical of herpes viruses in general. For example, the human cold sore virus (herpes simplex virus) persists in the nerves that innervate the face. When infected people are stressed, the virus is activated, moves from the nerve to the skin, and causes the small areas of necrosis we recognize as cold sores. Studies to date suggest that Duck Plague Virus is similarly latent in at least some birds that survive infection and that the virus becomes active and is shed from such carrier birds from time to time. Experimentally, such carrier birds have transmitted infection to ducks held in the same cages or pens and also to their own ducklings vertically through the egg. Carrier birds may or may not have antibodies to the virus in their blood. Thus, it is not possible to use surveys of antibodies to assess the proportion of carrier birds in a population.

Waterfowl species differ in their susceptibility to virulent strains of Duck Plague Virus. Based on experimental infections with the virus isolated during the Lake Andes outbreak, Blue-winged Teal are the most susceptible and Pintails are the least susceptible. Muscovy, Wood Duck, Canada Goose, Redhead, Gadwall and Mallard are moderately susceptible. Muscovy ducks are repeatedly associated with small outbreaks of the disease in North America and the United Kingdom.

The appropriate response of regulatory agencies to outbreaks of Duck Plague has become highly controversial, particularly in the United States. The controversy centres around a scientific issue that is unresolved: is the Duck Plague virus widely present in carrier birds in wild waterfowl populations or does it persist only in the small captive flocks in which most outbreaks have occurred ? Currently, most agency policies call for eradication of all affected flocks. Drainage and disinfection of ponds has also been carried out. This policy is based on the presumptions that these diseased flocks represent important potential sources of infection for wild waterfowl and that the disease is a significant threat to wild waterfowl. The policy is logical within the philosophical paradigm of many wildlife agencies in which wild waterfowl are held to have great value while small feral or captive flocks of domestics, hybrids and Muscovys are held

to have relatively little value. However, this view is increasingly at odds with the views of the people among which the small flocks reside and who place a high value in retaining these birds within their communities. For them, eradication is not a logical regulatory act necessary for the greater good but an unjustified act of brutality that deprives them of a valued aesthetic component of their environment. Given our collective knowledge about Duck Plague in particular, and herpes viruses in general, it is hard to justify the presumption that wild waterfowl are significantly threatened by Duck Plague or that small flocks are the only likely source of virus that might infect wild birds. It is at least as likely that the virus is widespread in carrier birds in wild populations throughout the continent and that outbreaks in small flocks pose no significant threat to wild waterfowl.

SALINITY AND SELENIUM

Gary Wobeser

Excess salinity and selenium are problems that result from the leaching of minerals from the soil, with the subsequent accumulation of these compounds in wetlands. Both are associated with semi-arid areas in which evaporation exceeds precipitation, and they are of particular concern on terminal wetlands with no little or no outflow.

Excess Salinity

Saline wetlands are common on the prairies, and the normal process of salinisation may be exacerbated by the same agricultural practices, e.g., summer fallowing, that lead to soil salinisation. The chemical constituents of individual saline wetlands are highly variable. Sodium, calcium and magnesium are the most common cations; bicarbonate and sulphate are the common anions. In general, calcium-bicarbonate water is common in dilute wetlands, whereas sodium and/or magnesium-sulphate water predominates in more highly saline wetlands. Many saline wetlands are used heavily by waterfowl, although birds may have to move to freshwater sites to drink on more highly saline wetlands.

Three specific disease problems have been identified on saline wetlands:

1) toxicity for ducklings
2) salt encrustation in association with dropping water temperatures in autumn
3) direct salt toxicity in adult birds.

Each of these is associated with a circumstance in which birds are unable to move to freshwater.

Duckling toxicity

It has been observed for many years that ducklings are scarce or absent on highly saline wetlands and, when they are present, they are usually closely associated with freshwater inflows. The degree of salinity that causes deleterious effects to ducklings depends upon the ions present in the water, and the species and age of the ducklings. Conductivity can be used as an approximate guideline to the likely toxicity of individual wetlands. Growth depression and sublethal effects may occur if ducklings are confined on water with conductivity $\geq$ 4000 μS/cm; increasingly severe growth retardation with some deaths is likely on water with conductivity from 13,000-15,000 μS/cm, and newly hatched ducklings are unlikely to survive for more than 1 day on water with conductivity > 20,000 μS/cm. There are no specific lesions in ducklings that die after exposure to saline water. Some wetlands that are otherwise highly suitable for duckling production may be of limited use because of salinity. Small freshwater impoundments at inlet streams, at which ducklings could drink, have been suggested as a management.

Salt Encrustation

Salt encrustation appears to be a condition that occurs on certain highly saline wetlands when falling temperature during autumn result in super-saturation of the salt in the water. Crystallization of the salt can then occur on the bird's feathers and individual birds can become coated with kilograms of salt. This condition has been recognized on two lakes in Saskatchewan and one in Alberta, and likely also occurs in North Dakota. Birds may die from a combination of dehydration, salt poisoning, exhaustion, and muscle injury as a result of struggling to fly. Many individual birds that have been rescued, washed in freshwater to remove the salt, and moved to freshwater have recovered; a few died of muscle injury.

Salt poisoning of adult waterfowl

This condition has not been reported in the Canadian prairies, but has occurred in North Dakota and on playa lakes in Texas, in circumstances where birds are forced to use highly saline wetlands because of lack of other water. (In northern locations this was because all freshwater marshes were frozen; in Texas it was simply a lack of water). Some of the lakes in Texas contained run-off from potash deposits, others contained NaCl from mining activity. Some of the birds in these circumstances were also salt encrusted. Gross lesions were minimal, but there was microscopic damage to the eyes, and to the mucus membranes of the alimentary tract. The brain of poisoned birds contained >2000ppm sodium and this seems to be a suitable level to use for diagnosing this disease. A related condition was recognized once in Saskatchewan when Tundra Swans landed on hyper-saline brine ponds around a potash minevery late in the autumn. It appeared that the birds suffered frostbite to the face, eyes, feet, bill and esophagus from water that was colder than -20C. No other open water was available.

Selenium

Selenium is a required micronutrient but if ingested in excessive amounts it is highly toxic. Soils in large areas of western North America were derived from ancient seabeds and are rich in selenium. Poisoning of waterbirds has been reported in California, Utah and Wyoming; and is suspected to occur in Colorado, New Mexico, Nevada and Montana. These situations are associated with run-off water from agricultural irrigation that contains high levels of selenium. The chemical form of selenium that is likely most important in biological systems is L-selenomethionine.

Gross lesions described in poisoned waterfowl include weight loss and emaciation, loss of feathers on the top of the head and dorsal midline of the neck, degeneration of the "nail" on the bill, and degeneration and loss of the toenails. Tissue concentrations of selenium are useful for indicating exposure to high levels of selenium but there is a poor correlation between tissue concentrations and lesions in individual birds. In addition to direct mortality, excessive intake of selenium is associated with severely impaired reproduction, and the production of abnormal embryos. Concentrations of about 3 ppm selenium (wet weight) in eggs are considered the

threshold for reproductive impairment. Exposure to excessive selenium has also been linked to impairment of the immune system in birds.

Ducks appear to recover quickly after they leave areas with high selenium concentrations and it is thought that they should not suffer reproductive impairment after migration to areas with no contamination. Irrigation drainwater in some areas also contains high concentrations of arsenic and boron.

Disposal of irrigation runoff water has become a serious problem in some areas, because of its toxicity. The initial method proposed was to use evaporation ponds but these were highly attractive to waterbirds, especially in arid areas such as California. The contaminated ponds have now been drained and filled, but the problem of what to do with water that is considered a "*hazardous substance*" remains.

LEAD POISONING

Trent Bollinger

Lead poisoning is caused by the ingestion or inhalation and absorption of toxic levels of lead. In waterfowl lead exposure is usually the result of ingesting lead shot in the environment. Lead fishing sinkers are also a source of lead, primarily in fish-eating birds such as loons. Less commonly, exposure can occur as a result of environmental contamination from mine and mill operations. Birds of prey, and in particular eagles, can be exposed by eating the flesh of hunter killed or crippled birds containing lead shot.

Lead in rural and urban areas is ubiquitous in soil, air and water at levels far above natural background concentrations. This increase is due to the widespread human use of lead-based products. Most animals are exposed to lead in the environment and have accumulated lead in their tissues to varying degrees. Lead has no known biological function in the body and even low levels result in some physiological change; however, exposure to current background levels of lead does not appear to impair the health of wildlife populations. Lead poisoning in waterfowl due to the consumption of lead shot used for hunting was a significant problem in the United States and in parts of Canada. Prior to the ban on lead shot in the United States 2400-3000 tonnes of lead were deposited annually by waterfowl hunters and it was estimated that it affected 1 to 2 million waterfowl a year.

Lead poisoning is typically a chronic disease with ongoing sporadic mortality which frequently goes undetected. Lead poisoned birds are most common late in the hunting season or in the period following the hunting season. Lead shot deposited in marshes typically becomes less available to waterfowl as time progresses and in most cases the lead is unavailable after 1 year. Lead shot is available to waterfowl for longer periods in water bodies with hard bottom sediments and little vegetation.

Lead impairs cell processes throughout the body but most clinical signs are related to impaired function of the nervous and digestive system. Clinical signs are dependant on the amount of lead absorbed and the time since exposure. Early in the disease birds are reluctant to fly and often remain on water bodies after other birds have left. These birds are often mistaken for hunting cripples. Birds appear weak and uncoordinated. Lead-poisoned Canada geese may have an altered tone of their call and fly with a bent neck. As the disease progresses and the birds become flightless, wings may be carried in an unusual position over their back or the wings may droop. Severely affected birds generally seek isolated locations under vegetation. They often pass green bile-stained liquid feces and there may be a fluid discharge from the bill. The presence of bile-stained feces along shorelines and other loafing areas is suggestive of lead poisoning and further searching in the area for sick and dead birds may be warranted. Occasionally a bird is exposed to high levels of lead in a short period of time and clinical disease rapidly results in death. Absorption of lead into the body and clinical disease is dependant on a number factors including: age, sex, physiological condition and nutrition.

Lead-poisoned birds are frequently emaciated. Fat stores are reduced or absent from under the skin and from around internal organs. Muscle wasting is common and is most pronounced in the pectoral muscles resulting in a prominent sternum or keel. The feathers around the cloaca or vent are often stained with bright green feces. The carcasses are usually pale due to anemia and in some cases the head may be swollen due to fluid accumulation or edema. Impaction of the esophagus or proventriculus occurs in about 1/4 of affected birds. The gallbladder is usually distended with green bile and the normal yellow gizzard lining is green bile stained. Lead pellets which are eroded, black and flattened may be present in gizzard contents. Birds that die acutely may have few if any lesions.

The lesions described above are suggestive of lead poisoning but are not pathognomonic. Starved birds can show many of the same lesions. Tissue lead levels are the best measure of lead exposure and poisoning. Liver is the tissue of choice in dead birds. Liver lead concentrations equal or greater than 6 ppm wet weight are suggestive of lead poisoning. Lead in blood and soft tissues usually remains elevated for weeks to months following exposure and will eventually decline if the exposure is not prolonged. Bone, on the other hand, accumulates lead over the lifetime of the bird. Lead is also not as rapidly deposited in bone and may not be significantly elevated in acutely poisoned birds. Lead poisoning can be diagnosed in live birds by demonstrating depressed aminolevulinic acid dehydratase activity or elevated lead concentrations in the blood.

Banning the use of lead shot for waterfowl hunting is an obvious method of reducing mortality caused by lead poisoning. The use of nontoxic shot has been required for all waterfowl hunting in the United States since 1991-92 and will be required in Canada by fall, 1997. Habitat manipulation in heavily contaminated areas, such as altering water levels and tillage of basins to reduce the amount of lead shot available to water fowl may be required on water bodies were problems of lead poisoning persist.

NECROTIC ENTERITIS

Gary Wobeser

Necrotic enteritis is a newly recognized disease of wild geese, that has occurred on a number of wetlands in southern Saskatchewan and Manitoba since 1983. Individual outbreaks have usually involved >100 dead geese, some have involved >1000. Almost all of the outbreaks have been among geese using saline wetlands during autumn migration; all of the goose species present on these wetlands have been involved but ducks, coots and shorebirds on the same wetlands have apparently not been effected. Most of the geese have been found dead, so clinical signs of the disease are unknown. Many of the dead birds have grain in their esophagus and gizzard, suggesting that the disease has a very abrupt onset and short course.

The birds are in excellent body condition. Lesions are restricted to the small intestine, in which there is inflammation (enteritis). The content of the small intestine may vary from thin fluid contains flecks of fibrin and necrotic tissue, to hemorrhage, to a fibrin "*cast*" that completely fills the lumen and covers the mucosa.

The cause and pathogenesis of necrotic enteritis are still unclear. The only potential disease agent that is found consistently in the intestine of these geese is the bacterium *Clostridium perfringens*. This bacterium is found in the intestine of normal birds, but it is an opportunistic pathogen than can overgrow within the intestine and produce potent toxins that destroy the epithelial cells lining the intestine. A very similar necrotic enteritis, caused by *C. perfringens*, occurs in domestic chickens, and in capercaillies. In those species, the disease is associated with various stressors, particularly abrupt changes in diet; especially if the diet is changed to one higher in carbohydrates or protein than that to which the birds are accustomed. In chickens there is a higher prevalence of necrotic enteritis, and greater mortality from the disease, among chickens on a small-grain diet (wheat, barley, rye or oats) than in chickens on a corn-based diet.

It is suspected that necrotic enteritis in geese is associated with the abrupt dietary change that occurs when arctic-nesting geese reach the prairies in autumn and begin eating a diet that consists almost entirely of grain. This, and perhaps a change to saline water, may cause disruption of the normal intestinal bacterial flora, allowing *C. perfringens* to overgrow, produce toxins, and damage the intestine.

The extent of mortality that occurs from this disease is unknown. The first outbreak in Saskatchewan was initially overlooked because it was thought that the few dead birds visible on a cursory examination of the lake represented crippling loss from hunting. The sudden death of birds in very good body condition, with few or no gross lesions, is strongly suggestive of avian cholera and laboratory examination is needed to differentiate among the diseases.

It is also unclear whether this is truly a "new" disease, or if it went unrecognized prior to 1983. Because the disease is associated with a certain type of wetland, and outbreaks have occurred in more than one year on some wetlands, these wetlands should receive special surveillance. The disease could likely be "managed" by dispersing birds from these areas, if the losses are considered to be significant.

PREFLEDGING MORTALITY

Gary Wobeser

A high mortality rate among the very young is typical of most populations, and waterfowl are no exception. It is known that large numbers of young birds disappear, but the causes of this disappearance are very poorly understood. By extrapolation from domestic animals and humans, we also know that the very young are the most susceptible group in the population to a variety of infectious diseases. This susceptibility may be because the young have undeveloped defence mechanisms, i.e. that their resistance to the disease agents is inadequate or impaired, or it may be that young birds are exposed in a different manner than adults. It is likely that infectious diseases play a significant role in post-hatching mortality, but the study of birds during this time period is extremely difficult. Nestlings tend to be camouflaged and secretive, so they are difficult to detect while alive; sick hatchlings are prey to many species and their bodies are very small so they are removed rapidly by scavengers after dead. This means that ducklings or goslings seldom reach a diagnostic laboratory for examination. Use of radio-transmitters on either hatchlings or adult females will be very helpful in understanding mortality among these birds.

Examples will be used to illustrate some potential effects of infectious disease on young birds. It has to be stressed that these give only a key-hole peek at what may be occurring.

Renal Coccidia:

Coccidia are single-celled protozoan parasites that live and multiply in intestinal cells of their host. Most coccidia inhabit the intestinal tract but some have adapted to live in epithelial cells of the tubules within the kidney. Coccidia have a direct life-cycle, i.e., only one animal host is required. Resistant stages (oocysts) that are passed in the bird's urine persist in the environment, until they are consumed by a suitable host. After being consumed, the renal coccidia pass to the kidney and enter epithelial cells. Within the epithelial cells, the parasite divides into a number of offspring, which then break from the cell and each offspring enters a new host cell. There are several generations of multiplication of this type before oocysts are produced; an important feature is that every time the parasites leave a host cell, that cell dies. Thus, the number of organisms present is very important in coccidial infections. If only a few organisms are present, the bird can replace its cells rapidly enough and the parasite has little effect. However, if many coccidia are present, the bird can not replace damaged cells rapidly enough and kidney function is impaired (i.e., disease occurs). Coccidial infections are an example of a macroparasite. Birds tend to remain infected for a long time and resistance to re-infection is dependent on the presence of a few parasites in the body.

Renal coccidial infection is common in Lesser Snow Geese, some adult birds have a long-lived mild infection that probably has no significant ill-effect. However, these birds serve to introduce the parasite to breeding colonies, where they deposit oocysts on the ground in their urine. Because of dense populations of geese and over-grazing on many Snow Goose colonies,

goslings receive a very high level of exposure to oocysts (high contamination, grazing close to ground). Because they have had no previous experience with coccidia they have little resistance, and their resistance may also be impaired by malnutrition. Heavy kidney infections result. The actual effects of renal coccidiosis have never been quantified, but the infection undoubtly has an energetic cost to the bird:

-cost of replacing the epithelial cells destroyed by the parasite
- cost of nutrients lost in urine because of damaged kidney tubules
- cost of defences mustered to "fight" the parasite.

Domestic goslings with kidney lesions equivalent to those seen in Snow Goose goslings die of the infection. Undoubtly, some snow Goose goslings also die of coccidiosis, but more important may be the increased energetic costs for birds in an environment with reduced nutritional availability. (How do you separate nutritional costs of parasitism from reduced nutritional availability?)

Myiasis in Ducklings

The second example illustrates the peculiar susceptibility of young birds to some disease agents and the fact that mortality of the very young can be found if one looks intensively. Flies of the genus *Wohlfahrtia* are "*fleshflies*" that require a living vertebrate host for reproduction. These flies deposit either eggs or larvae on the skin of vertebrates, and the larvae then penetrate the skin and develop in the subcutaneous tissues until they are ready to pupate, when the leave the animal and drop to the ground as pupae. The critical factor is that the larvae can only penetrate very thin delicate skin, so that this type of infection does not occur in adult animals. However, the larvae can penetrate the skin of very young animals. This parasite doesn't differentiate among species, although rodent "pups" are probably most commonly used. Infections occasionally occur in human babies. There is only one report of this type of myiasis in waterfowl, and that was of three cases in one year among ducklings on a study site near Saskatoon where the fate of duck broods was being followed intensively. In each of these cases the ducklings died from the infection. The ducklings that were known to have died of this parasite represented a significant proportion of the total known production on the area. *Wohlfahrtia* spp. are ubiquitous, suggesting that mortality may be more common than realized.

Leeches

Leeches of the genus *Theromyzon* are called the duck leeches, because they occur commonly on waterfowl. It has been known for many years that waterfowl that are sick for other causes, particularly birds with botulism, may acquire large numbers of leeches in their eyes and nasal cavities. Similarly, birds that are captured in traps often have heavy leech infestations. Probably leech infestation occurs in both of the above situations because normal preening behaviour does not occur, and leeches are able to reach a site where preening is ineffective. Leeches can withdraw a significant amount of blood and likely contribute to the debility in birds with botulism and other diseases.

Leeches have been reported to be an important cause of mortality among Trumpeter Swan cygnets on some wetlands, but why this species is particularly susceptible is unclear. (A disquieting thought is that swan cygnets are not more vulnerable than other species, but instead are larger and more easily found that ducklings, and are being monitored closely as a threatened species?). Mortality of adult waterfowl from leech infestation has been reported in South Africa and reproduced in the laboratory. The birds rapidly developed a *"paralysed state"* resembling botulism, and the investigators stated that *"chicks, of course, stand no chance of surviving"* on some of the effected wetlands.

The purpose of this discussion is to point out that very little is known about the role of disease during the stage of the life history of waterfowl that has the highest incidence of mortality. Infectious disease agents may kill some nestlings directly, but it is more likely that most infections result in an energetic cost to the bird that impairs growth, increases susceptibility to predation, or increases vulnerability to factors such as inclement weather.

PETROLEUM OILS

The usual association between oil and birds is large-scale mortalities of marine birds in association with tanker accidents or other oil spills into the ocean. Waterfowl have been victims in many such spills into oceans, lakes, rivers and estuaries. Less is known about the extent to which oil poses real or potential problems to waterfowl on inland wetlands. However, mineral extraction takes precedence over many other uses of public and private land in Canada, and wetland habitat is no exception. Thus, petroleum oil is a potential contaminant of some wetland habitats.

Petroleum oil is not one substance but many. Crude petroleum is composed of a huge variety of chemicals and the chemical composition of crude oils can be very different. The vast majority of chemicals in oils are hydrocarbons. These come in two major classes from the point of view of their potential toxicity: hydrocarbons composed of chains of carbon atoms (aliphatic hydrocarbons) and those composed of one or more benzene rings (aromatic hydrocarbons). In addition, oils contain chemical with oxygen, sulphur or nitrogen attached in various ways, and some oils contain considerable quantities of metals such as vanadium and mercury. Major petroleum products such as fuel oils of various kinds also differ in chemical composition, depending the make-up of the oil from which each was distilled. Very little is known about the potential toxicity of most of the chemicals in petroleum oils. A few are well studied, but most have not been studied at all. In general, where this has been carefully examined, the aromatic hydrocarbons with two or more benzene rings have been found to be the class of compounds among which the toxicity of oils to birds was located.

Petroleum oils have deleterious effects on birds in two different ways. The most common and important effect is the external oiling of feathers. The properties of feathers that cause them to be waterproof do not resist penetration by oils, and oiled plumage becomes matted. This destroys the critical function of insolation, waterproofing and buoyancy. Oiled birds lose vast amounts of heat through oiled plumage, the more so if they must remain on water, especially cold water. They also can become waterlogged and drown. They usually are prevented from feeding normally. Together, all these factors result in rapid depletion of energy stores and death from cold (hypothermia). This effect of oil on feathers is its most significant effect in causing bird mortality.

Oil also can be a poisonous substance with toxic effects produced by at least some of its constituent chemicals when they are absorbed by birds. Possible routes of absorption include inhaling the volatile components (particularly in the immediate area of a fresh spill), absorption through the skin, absorption through the digestive system and absorption through the egg shell. There is evidence that the latter two routes, at least, can be important.

Birds ingest oil when trying to clean their plumage. A lot of studies have been done on the toxic effects of ingested oils. It is hard to sort out all this information because so many different species of birds, doses of oils and different oils have been used. However, a few

common themes emerge, and most of the data for these have been generated in experiments with ducks. Ingestion of oil appears to be highly stressful to birds. When combined with other stress-causing stimuli, such as salt water to drink and cold temperatures, ingestion of oil has resulted in significantly increased mortality over time. This approach to the study of oil toxicity is highly relevant to what occurs in nature, especially in winter when waterfowl may be both cold and in a marine or brackish environment. It also is relevant to spring and fall on saline prairie wetlands. Ingestion of oil also has reduced reproductive success, however such success has been measured: hatching success, fertility, egg structure, hormone cycles, incubation behaviour. The mechanisms here are not clear nor necessarily always the same, but the results recur in experiment after experiment. Ingestion of at least some oils also have been shown to cause toxic destruction of red blood cells and, thus, anemia.

Avian embryos are extremely sensitive to the toxic effects of oil that contaminates the egg shell. Micro litre quantities (millionths of a litre) of oil are sufficient to kill most embryos during the first half of incubation and to prevent hatching of those that survive the acute exposure. The quantities of oil delivered to eggs by a lightly-oiled incubating bird are sufficient to have this effect. Thus, a minor oil spill at the right time and place could lead to a major reduction in reproductive success in the affected area.

There have been no estimates of the potential threat to prairie wetlands posed by extraction, processing or use of petroleum. A casual inspection of oil-producing areas of western Saskatchewan in the spring quickly revealed oil wells in low-lying basins filled with both water and waterfowl. How much extraction activity occurs within permanent wetlands important to waterfowl appears not to have been assessed. The oil industry strives for spill-free, clean operations and generally achieves this. Where oil extraction or related activities occur on wetlands, oil should be considered a contaminant of potential importance. Involvement of the responsible corporation in the development of management plans for the wetland may be the best approach to minimizing this hazard and encouraging dialogue and cooperation.

BLUE-GREEN ALGAE

Blue-green algae are abundant in prairie wetland waters. When conditions are favourable, they can reproduce quickly, resulting in algal blooms in which waters become opaque from the high density of algal cells and large surface mats of algae may form and raft onto shore according to prevailing winds. Many of these algal species can and do produce powerful toxins. If mammals or birds (or fish) drink or feed in these waters, they can become poisoned and die within a few minutes to a few hours. Blooms can disappear as quickly as they form, and toxins are dispersed or degraded. Thus, algal poisoning can defy detection, all signs other than the dead animals having disappeared before the event has even been recognized.

Over 40 species of blue-green algae are known to produce toxins. Species of the genera *Anabaena, Aphanizomenon, Microcystis* ("Anny, Fanny and Mike"), *Nodularia* and *Oscillatoria* dominate most toxic algal blooms. Blooms are favoured by warmth, sunlight and abundant nutrients. Blooms are toxic when toxin-producing species are in abundance; 40-70% of blooms were toxic in various surveys. Run-off or other water sources containing fertilizer, manure, sewage and other nutrient sources will favour development of blooms. The toxins produced are, in general, either hepatotoxins (toxic to the liver) or neurotoxins (toxic to the nervous system). There are multiple forms of both kinds of toxins. One well-studied hepatotoxin is microcystin which has a potency roughly ten times that of strychnine. Neurotoxins produced by these algae include toxins that act like organophosphate or carbamate insecticides by inhibiting the enzyme acetycholinesterase and blocking nerve transmission, and toxins like saxitoxin which also is the principle agent responsible for paralytic shellfish poisoning in marine environments. Lethal doses of neurotoxins usually produce death within 30 minutes to 1 hour while hepatotoxins require 2-4 hours.

Algal poisoning should be suspected when waterfowl mortality occurs in warm summer weather, particularly in association with dense populations of algae. Investigative field observations should include the location of dead animals relative to wind direction in the recent past, occurrence of blooms, presence of beached mats of algae, and the number and range of species affected, including domestic animals. In addition to dead birds or other animals collected for diagnosis, samples of algae in the area of mortality should be collected for identification of algal species and for toxicity testing. Specimens for identification should be preserved (equal parts 10% formalin and water containing the algae is recommended by one author). Specimens for toxicity testing should consist of concentrated algal cells kept cool and presented to the laboratory as quickly as possible where they can be frozen or lyophilized pending testing. The presence of toxins can be demonstrated by mouse inoculation, but the identification of the precise toxin(s) involved requires special analysis.

Diagnosis of death due to algal poisoning requires demonstration of the algal toxins in the birds. This is best done through analysis of intestinal content. Such analyses are available only from a few specialized laboratories. It also is important to rule out other possible causes of

disease, particularly botulism, which is likely to occur in the same species and habitats at the same time of year.

There has been virtually no recent research into the occurrence or importance of blue-green algal poisoning in waterfowl. New tools to detect and identify the many different toxins produced by these algae now are available, and new studies are sorely needed. A particular focus for attention should be the relationships among botulism and algal poisoning. As noted elsewhere in this course, outbreaks of botulism may be initiated by any cause of mortality of vertebrates in the affected wetland. Thus, algal poisoning is one potential cause of such mortality. The mid-summer initiation of many botulism outbreaks is coincident with the peak occurrence of algal blooms. Many of the clinical signs of birds affected with the two diseases are similar, and, thus, some mortality attributed to botulism may be due to algal toxins.

INSECTICIDES

Ted Leighton

There are many different kinds of chemicals used in agriculture, forestry, industry and urban living that might have some toxicological impact on wetland birds. Herbicides, for example, can alter plant communities and habitat. Insecticides are highlighted here because among the insecticides in current use are some very poisonous substances that readily kill birds. Killing birds is not the intended use of these chemicals, and both package labels and manufacturer advertising and information programs emphasize procedures to avoid negative impacts on non-target areas and species. Wetlands on the Canadian prairies generally exist as islands in a sea of cultivated lands on which monocultures of fragile but productive plants are maintained by chemical-based, high-input farming practices. Thus, exposure of wetlands to some of the most toxic of these chemical inputs may be inevitable from time to time, despite precautions.

Four classes of insecticides are currently in use on relatively large scales, or have been so used in the recent past: organochlorines, organophosphates, carbamates and synthetic pyrethroids. A list of insecticides, together with a few herbicides of the same chemical classes, is appended as a reference to help place the various insecticides available into the appropriate chemical group.

Organochlorines: Few of these chemicals currently are in use. These were the first insecticides to become widely used in agriculture. DDT is the prototype for this class and the use of this and related insecticides revolutionized agriculture in the decade after World War II. However, significant negative effects of this use were widely recognized by about 1960. Rachel Carson's book *The Silent Spring* (1962) catalysed a societal reconsideration of the use such chemicals and DDT itself was banned in North America in the early 1970's. While organochlorines can kill birds directly if ingested in relatively high doses, the more significant problem with these chemicals is their persistence in the environment for years and years and their accumulation and bioconcentration in animal tissues. Thus, concentrations in the environment increased with repeated applications and animals high in the food chain accumulated toxic doses. In birds, the most important effect of DDT was reduced reproductive success. This is due principally to the unfortunate facts that birds lay fragile, hard-shelled eggs which they then sit on, and that DDT and related compounds inhibit shell production, resulting in thin-shelled eggs readily broken during incubation. Some organochlorines also have caused increased mortality of embryos and decreased survival in hatchlings in the absence of thin egg shells.

Organophosphates and Carbamates These are very different kinds of chemicals to a chemist but they have nearly identical toxicological effects. These two classes of insecticide kill both target and non-target species alike by blocking the action of an enzyme, acetylcholinesterase, which is

essential in transmission of nerve impulses. Compared to the organochlorines, these chemicals are extremely poisonous, but they persist in the environment for weeks or months rather than for years and decades. Thus, they do not accumulate with time. Also, they are quickly broken down by animal metabolism so they do not persist or bioaccumulate in tissues. The principle affect recognized in waterfowl has been acute mortality associated with feeding on treated seed, grass, trees or other food material. Sub-lethal exposure of birds to these chemicals has produced altered reproductive behaviour in adults and abnormal responses to environmental stimuli in ducklings. It is not clear at what magnitude such affects may occur in the field.

Synthetic Pyrethroids These chemicals are synthetic versions of the natural plant product pyrethrin. While extremely toxin to invertebrates, the toxicity to vertebrates is very, very low. Thus, direct toxicity to birds is not an issue with respect to the pyrethroids. The major concern relative to wetland management has been the possible reduction in invertebrate food resources should significant quantities of these chemicals be applied, intentionally or unintentionally, to wetlands. This same concern exists for other insecticides, particularly where aerial applications occur on landscapes that are mosaics of target cultivation and non-target wetlands. Under these circumstances, significant drift onto non-target land is difficult to avoid.

Diagnosis of death due to insecticide poisoning is difficult for organochlorines and relatively straight-forward for organophosphates and carbamates. The mechanism of toxic action of organochlorines is not completely known and leaves no diagnostic changes or lesions in its wake. Diagnosis is based on finding significant concentrations of organochlorines in brain tissue. However, it is not certain what concentration of the various chemicals or of combinations of these chemicals will cause death. Thus, there can be considerable uncertainty in the diagnosis. Death due to poisoning with organophosphates and carbamates is associated with a marked reduction in the activity of acetylcholinesterase in the brain, which can be measured. This does not reveal which of the many chemicals of these two classes was involved, however. This latter information can be had only by chemical analysis or from knowledge of what chemical was used in the area where the birds were found.

It is difficult to assess how great a threat insecticides pose to wetland birds. While some modern insecticides are extremely toxic, they are, in general, used appropriately and with due caution. Exposure of wetland birds is most likely to occur through ingestion of treated food materials on adjacent agricultural land, via runoff if wetlands receive agricultural drainage waters from areas of intensive insecticide use, or through mistaken direct application. New biotechnologies are rapidly changing many aspects of agriculture. Genetic manipulations may lead to crops resistant to many of the insect pests currently controlled by insecticides. In addition, low-input agriculture is growing in popularity, particularly with consumers. Thus, insecticide use on major crops may be reduced in the future.

Names of Insecticide Chemicals:

There are many insecticides on the market that are members of these categories of chemicals. Organophosphates and carbamates are among the most widely used insecticides in North America, but the pyrethroids are becoming widely used as well. Organochlorines are generally used in smaller quantities, but their use continues. Each insecticide has an official common name and is sold in one or more commercial products or formulations, each of which also will have a commercial or trade name. The official common name will appear on the label in the list of ingredients. (Some fungicides and herbicides of the same chemical classes are included in the lists that follow.)

Organophosphates:

Acephate-met
Akton
Azinphos-methyl
Bomyl
Bromophos
Carbophenothion
Chlorphenvinphos
Chlomephos
Chlorpyrifos
Coumaphos
Crotoxyphos
Crufomate
Cyanophenphos
Cyanophos
Cythioate
Demeton
Demeton-methyl
Dialifor
Diamidfos
Diazinon
Dicapthon
Dichlophenthion
Dichlorvos
Dicrotophos
Dimefox
Dimethoate
Dioxabenzophos
Dioxathion
Disulfoton
Ditalimphos
DMPA
Edifenphos
EPN
Ethion
Ethoprop
Etrimfos
Famfur
Fenamiphos
Fenitrothion
Fensulfothion
Fenthion
Fonofos
GC 6506
Isazophos
Isofenphos
Leptophos
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
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:

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 (lR)-isomers
Tralomethrin

Organochlorines

Aldrin
Benzene hexachloride
Chlorbenside
Chlordane
Chlordecone
Chlorfenethol
Chlorobenzilate
Chloroforrn
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

BOTULISM

Gary Wobeser

Avian botulism likely kills more waterbirds than any other disease. Mortality is greatest among prairie-nesting ducks; geese are almost never effected by this disease. Coots, shorebirds and grebes can be severely effected during some outbreaks. The disease occurs throughout the world, but the greatest frequency of occurrence and extent of losses is in western North America.

Botulism is a type of food-poisoning. Birds become poisoned by consuming pre-formed toxin produced by the bacterium *Clostridium botulinum* type C. This bacterium is capable of producing several toxins, but the one of importance is a very potent neurotoxin that blocks nerve transmission at the synapse between nerve endings and muscle fibres, resulting in flaccid paralysis.

The ecology of avian botulism is relatively complex and not understood completely. *Clostridium botulinum* type C is a bacterium that can only grow vegetatively under circumstances where oxygen is absent, i.e. it requires "*anaerobic*" conditions for growth. Its normal habitat for growth is decaying organic matter in which there is no oxygen. When the organism is exposed to unfavourable conditions, such as the presence of oxygen in its surroundings, it forms a very resistant spore that is a dormant or resting stage. Spores are extremely "*tough*", persisting for years in soil until they again find themselves in suitable conditions for anaerobic growth. The organism prefers high temperatures (>30C) for vegetative growth, and toxin production only occurs during the vegetative growth phase.

Spores are common in wetland soils, particularly in marshes with a past history of botulism outbreaks. (In general, spores are >10X more common in botulism-prone marshes than in marshes with no history of the disease). Although *C. botulinum* can grow in a variety of decaying materials, rotting vegetation is not a good substrate. Decaying animal matter is an excellent substrate for growth. The bacterium could grow in decaying invertebrate carcasses (insects, etc), but there is no clear evidence that this is important in outbreaks. Vertebrate carcasses are the ideal substrate for bacterial growth, they provide abundant nutrients for the bacteria, the interior of the decomposing carcass is anaerobic, and the decay process produces the high temperatures preferred for growth.

Animals living in a marsh where spores are common, ingest spores continually. Because the living animal's tissues are well oxygenated, the spores do not "*germinate*" or grow vegetatively as they pass through the intestine; however, if the animal dies for any reason while spores are present, the spores may begin to grow and produce toxin as the carcass decomposes and becomes anaerobic.

Fly maggots and other scavenging invertebrates are very important in the ecology of botulism. These animals feed on decaying carcasses and consume toxin produced by *C. botulinum*; they are not harmed by the toxin but 1-10 maggots leaving a carcass may contain sufficient toxin to poison an adult duck. (To a bird, a maggot simply represents a highly desirable package of nutrients).

Botulism has been compared to a forest fire. Both require a spark to begin, and then a steady supply of fuel and suitable environmental conditions to be maintained and grow.

In most outbreaks, the spark is unknown, because it likely occurred days or weeks prior to recognition that mortality was occurring. There has been considerable interest in looking for physical and chemical characteristics that might be associated with toxin production in the water and sediments; however, no one has been able to identify toxin free in water or to find sufficient toxin in any substrate, other than decaying carcasses, to poison birds. There is considerable evidence that decaying vertebrate carcasses are the spark that starts many outbreaks. The initial carcass might that of any vertebrate (so long as spores are present in its body at the time it dies); it might be a bird that died after colliding with overhead wires and fell into a marsh; birds killed by a hail storm, fish that died because of poor water conditions, or muskrats that died of another disease. (A cow that died in a marsh was identified as the spark that started one outbreak in California). In two studies in California, outbreaks were started among penned ducks by adding a duck carcass to the pens.

There is general agreement that carcasses are the fuel which allows the disease to be maintained and grow. **Botulism is unique among poisonings, because additional toxin may form in the carcass of its victims and potentially poison other birds, whose carcasses may then be suitable substrate for further bacterial growth and toxin production**. This multiplication factor is very important for understanding how botulism can "*spread*" from a very small beginning to involve thousands of birds. A single decaying vertebrate carcass may produce hundreds of maggots laden with toxin. If as few as 1-10 of these maggots can poison another bird, the potential for expansion of the extent of the outbreak with every repetition of the **carcass-maggot cycle** is very large

Outbreaks of botulism typically occur in mid-late summer and wane as the weather cools in autumn. There are likely many factors involved in this timing, including high populations of birds, declining water levels that concentrate birds (and carcasses), abundant flies to produce maggots, and high temperatures for rapid decomposition of carcasses with toxin production. One factor that causes outbreaks to wane in autumn is likely lack of fly activity and, hence, lack of maggots, as the weather cools. Outbreaks sometimes occur very early the following spring on wetlands that had a summer outbreak. These typically are short-lived and involve diving ducks. It is believed that these birds become poisoned by consuming toxin associated with sunken carcasses from the previous summer. These outbreaks likely stop because the temperature is too cool for fly activity, so that no carcass-maggot cycle is established.

Critical factors in determining the spread of botulism once it has begun on a marsh likely include:

- the proportion of carcasses that contain spores that can germinate and produce toxin.
- the proportion of carcasses removed by natural scavengers before toxin and maggots form.
- the amount of contact between live susceptible birds and toxin-laden maggots.
- the proportion of such contacts that result in birds consuming a toxic dose.

Birds with botulism develop progressive paralysis beginning with the wings, then the legs, the nictitating membrane and, finally, the muscles of the neck. Remembering this progression is important, because during outbreaks many poisoned birds may be able to swim and dive and birds with "*limberneck*" (the floppy paralysis of the neck that is sometimes considered characteristic of botulism) may be encountered uncommonly. In mid-summer it is sometimes difficult to differentiate between birds with mild botulism and birds that are flightless because of moulted wing feathers. Birds with botulism have been segregated into three classes: Class I - bright, alert, walking but flightless; Class II - difficulty walking and holding their head erect; Class III - prostrate and almost totally paralysed. Birds with botulism die from a number of causes including drowning (if the neck is paralysed), dehydration, trauma, and paralysis of the respiratory muscles.

Diagnosis of botulism requires demonstration of the presence of toxin within the blood or tissues of birds with appropriate clinical signs. Because the bacterium can grow and produce toxin in carcasses, live sick birds should be tested whenever possible and only very freshly dead carcasses are of value for diagnosis. The standard method of testing has been to inject serum extracted from whole blood into two groups of mice, one group of which had previously received antitoxin to type C toxin. A positive test was considered to occur when the unprotected mice died or became paralysed, while the mice given antitoxin remained healthy. A new ELISA test developed at the National Wildlife Health Centre in Madison, Wisconsin uses whole blood and does not require use of mice. The two methods are of similar sensitivity. Because not all birds in an outbreak will have sufficient toxin in their blood to result in a positive test with either method, at least five birds with typical signs should be submitted to the laboratory. (Blood collected in the field and kept cool, but not frozen, also is suitable).

Management of botulism at the present time consists of surveillance of botulism-prone wetlands and carcass collection and disposal when mortality is recognized. This is most effective when regular surveillance and "*preventive*" carcass collection is done early in the season, prior to the expected first occurrence of an outbreak (as was done in 1996 at Pakowki Lake, Alberta). Although, carcass collection and disposal, to reduce the carcass-maggot cycle, intuitively seems correct, its actual effect on the mortality that occurs in an outbreak has never been assessed. It is clear, that to be effective, carcass collection has to be very intensive and repeated, since newly dead carcasses produce a crop of maggots within about 4 days under summer conditions.

Preventive management is limited at present by lack of understanding of the factors that precipitate and enhance outbreaks. In some areas, an attempt has been made to limit vertebrate mortality that could produce carcasses to trigger outbreaks. For example, overhead powerlines should not be built over botulism-prone marshes and water conditions that will result in fishkills during warm weather should be avoided. Water level manipulation is not possible on most of the largest botulism-prone marshes on the prairies, and has not proven to be a reliable method of preventing outbreaks. Management to enhance removal of carcasses by natural scavengers, or to reduce contact between live birds and carcass material has not been attempted.

A significant proportion (70-90%) of live sick birds in Classes I and II will recover if protected from predators, given water to restore hydration, and treated by injection of antitoxin. However, birds that recover remain susceptible to the disease if they return to the same wetland. (Recovery does not result in any resistance to the toxin). The actual survival of treated birds over the long term has not been determined. Treatment is probably a suitable management technique as part of the response to outbreaks on readily accessible wetlands under close public scrutiny, e.g. Oak Hammock Marsh in Manitoba.

INVESTIGATING AND ASSESSING DISEASE IN AN INDIVIDUAL WETLAND OR OUTBREAK

Trent Bollinger

Most disease in waterfowl goes undetected due to rapid scavenging and decomposition of carcasses, poor visibility of sick and dead birds, infrequency to which most waterfowl habitats are visited by people and the failure to report or investigate reports of sick and dead birds unless large numbers are affected. Outbreaks or epidemics are more frequently detected and often receive considerable attention; however, they are thought to represent only a small portion of the disease which occurs in free-ranging waterfowl. The "tip of the iceberg" is often the analogy used to describe these epidemics and the larger submerged portion of the iceberg represents the low rate mortality and chronic disease which goes undetected. Since disease in waterfowl is rarely detected, recognition of its occurrence warrants investigation. In some cases this may be as simple as recording information on its occurrence and submission of a sample or samples to a diagnostic laboratory to determine a cause. In other situations it should involve a more thorough investigation of the site. Depending on the disease and time of year epidemics may prompt clean-up or control measures.

As part of the waterfowl disease contingency plans for each of the provinces, waterbodies with a history of disease occurrence are identified and these are monitored on regular basis during the summer months. The ability to detect disease on these waterbodies, particularly disease occurring at a low rate, is still a problem for many of the reasons stated above. Without a systematic approach to disease surveillance on these waterbodies is difficult to assess the effectiveness of these monitoring programs. Measurement of population parameters (ie. number affected, number at risk and time) at monitored marshes would be useful in determining the effects of disease on continental flyway populations.

Epidemics or outbreaks are often unpredictable and frequently of short duration. The die-off is often recognized some time after the initiating causes have occurred and there may in fact be no new cases by the time the disease is investigated. The ephemeral nature of die-offs dictates that reports of mortality should be investigated promptly and the investigator should go prepared to collect all the relevant information and samples required to describe the disease. The investigation should be flexible and responsive to each situation.

Each investigation should proceed systematically and attempt to describe the disease occurrence by answering the questions: who, where, when, what, why (W5). We will address these each in turn.

The question of **who** involves determination of the species, sex and age of sick and dead birds. To be most meaningful this requires quantification of the number affected and the number at risk for each of these categories. Some species because of genetic susceptibility, food

preferences, habitat use and so on may be more or less susceptible to specific diseases. High mortality in one species of birds compared to another may also be a reflection of relative abundance of the various species. This cannot be assessed without information on number of birds at risk or at least relative abundance. Age and sex of birds also influence disease susceptibility and is important information to collect during a disease investigation. Measuring disease in populations has been previously discussed and falls under the category of "who".

Where, is also a critical piece of information. Accurate information on where the disease has or is occurring should begin with the initial report. Many of the disease investigations begin with a phone call reporting the disease and considerable time can be saved by detailed information on where the carcasses or sick birds were found, how this site was accessed and what equipment is needed to investigate. Maps of the area in which the mortality has occurred are useful and information should be plotted directly on the map to try and detect spatial patterns. Concentration of carcasses on shorelines opposite to the prevailing winds, adjacent to inlet streams, along mud flats, in agricultural fields, etc. all provide information on potential causes and factors involved in the outbreak. Environmental data such as characteristics of the water, vegetation and so on fall under the category of where. Plotting areas searched, locations of affected and unaffected birds are also useful.

One of the most important pieces of information to collect is "**when**" did the disease occur. Diseases tends to have a seasonal occurrence; for example, botulism tends to occur through the months of July, August and sometimes September. Avian cholera tends to be a disease that occurs during the winter months in the United States but in Canada is observed in spring and fall migrants. The temporal pattern of the mortality is important. One way of assessing this is by plotting the occurrence of new cases over time producing what is called an epidemic curve. Sudden development of new cases and then a rapid subsiding of mortality suggests a point source epidemic which are frequently caused by one time exposure to a highly toxic substance or virulent organism. Waterfowl found dead in an agriculture field in early summer suggests a point source epidemic caused by insecticide poisoning. A slow increase in the number of new cases over time reaching a peak and then subsiding is characteristic of epidemics caused by infectious diseases spreading through a population. Other temporal patterns of disease occurrence can occur and this type of graphical representation can provide information on potential causes and modes of transmission. Relating the time of disease to weather patterns, such as recent storms, hot weather, winds, cold weather, etc. is important in identifying factors which may have precipitated the disease.

Often the disease is detected and investigated some time after the first cases have occurred. Estimates of the date of death of these early cases can sometimes be made based on degrees of decomposition and maggot development. Identification of the cause of mortality in these early cases is important and may differ from the predominant disease currently occurring. For example, several botulism outbreaks have reportedly been initiated by carcasses dead from blue-green algae toxicity, Newcastle's Disease, parasitism, power-line strikes and so on.

The next question to answer is **what** is the disease. This involves a description of clinical signs in affected birds. Signs such as diarrhea, lethargy, paralysis of one wing, paralysis of both legs, etc. are examples of clinical signs. Body condition, such as presence or absence of fat stores and whether there is atrophy or reduction of muscle mass, is also an important characteristic to note. Video tapes or photographs of sick birds can be useful for future study and may reveal signs and lesions not recognized on initial examination.

The lesions or abnormalities in freshly dead birds should be described. Necropsies in the field can provide a preliminary or tentative diagnosis of the cause of disease which may direct the investigation. Complete description of the lesions and identification of the cause usually requires a diagnostic pathology laboratory equipped with bacteriology, virology, toxicology and parasitology facilities. The pathology laboratory should be contacted to ensure proper samples are submitted for diagnosis. In some cases a precise cause of the disease is not identified but with accurate description of clinical signs and pathological lesions this disease or syndrome can be recognized when it occurs at another time or in another place.

Finally there is the question of **why** did the disease or epidemic occur. In some cases this is straight forward ie. the geese fed in fields recently sprayed with pesticide. In other cases the answer to why the disease occurred is complex and is a result of the interaction between disease-causing agents, environmental conditions, and host susceptibility. Why a disease occurs usually requires making observations on the disease over time, keeping accurate records, identifying potential causal factors and proving or disproving their significance.

In summary, disease occurrence is rarely detected and therefore warrants investigation when it does occur. When investigating diseases we try to determine who is involved, where did it occur, when did it occur, what is the disease process and why did the disease occur. All 5 W's are answered in the context of the interaction between the host, the disease agent and the environment.

ASSESSING THE SIGNIFICANCE OF DISEASE

Gary Wobeser

What we have hoped to do in this course is to present the concept that disease in waterfowl comes in many forms and shapes, and that disease is inextricably intertwined with many other factors, including management decisions. Traditionally in waterfowl management, disease has been equated with piles of dead birds and, thus, only those diseases characterized by large die-offs have received much attention. For example, botulism, duck plague and avian cholera were the only diseases described in Owen and Black's 1990 book "*Waterfowl Ecology*". However, large die-offs probably represent only the tip of a much larger ice-berg.

It is perhaps worthwhile discussing why we don't know more about disease in the ecology of waterfowl. Likely the major factor is that most losses to disease are invisible or, at least, difficult to detect. Lead poisoning is a classical example of this phenomenon. We know that ingested lead shot kill waterfowl, and we know that a significant proportion of birds ingest lead shot but, except in unusual circumstances, we don't find many birds dead of lead poisoning; unless someone goes out specifically to find poisoned birds. Biologists in Texas performed a classic field study in reference to the ease with which lead induced mortality can be detected (1). They distributed 100 duck carcasses in a 100 acre marsh, with 50 of the birds in open water to simulate birds that died acutely of avian cholera, and 50 under cover to simulate birds that became sick and subsequently died of lead poisoning. Three hours after the carcasses were placed, a crew of 8 experienced people searched the area for mortality, without knowing that birds had been planted. The searchers found 6 of 50 birds in open water and 0 of 50 birds in cover; for an overall recovery rate of 12%. The researchers concluded, very reasonably, that extensive mortality could be occurring without it being detected. *We should stop at this point to ask ourselves how often we use this intensity of search effort (8 searcher days/100acre of wetland) when we do "surveillance" of marshes for botulism each summer.*

The diseases that we do know most about are those which kill birds directly, and in which a large number of birds die over a short period of time. Under conditions of an epizootic, the normally very efficient scavenging system is overloaded and carcasses persist to be found. But even with these diseases we have very little information about the disease at times of the year when it is not killing masses of birds. Avian cholera is a good example of this. We first recognized that avian cholera was occurring in western Saskatchewan when dead geese were reported by a conservation officer in 1977. Over the 20 years since then, we have always been able to go out to the Kindersley area and, within 1 day, find Snow or Ross' geese dead of avian cholera, but the disease has never been reported again. So that, even when mortality occurs among snow-white birds on flat unvegetated ephemeral sloughs in cultivated land, avian cholera

is invisible. *It is useful to mentally compare the probability of finding dead ducklings in dense nesting cover to that of finding adult snow geese in a summer fallow field along a grid road. This should help to better understand why we know so little about diseases of ducklings and goslings.*

As has been pointed out several times in the course, even when we have a count of dead birds, we can seldom relate this to the population "*at risk*", to actually assess the significance of the event. For example, when we collected 4500 ducks, predominantly Redheads, dead of avian cholera from a moulting lake, as occurred in 1988, what did this mortality mean to the population and what population base should we be using? If we plot the loss against the continental population of Redheads, the numbers may be inconsequential; but to some smaller local population unit; a very significant proportion of the population may have died in this one event.

The simple number of birds that died, even if the count is accurate, (which it usually is not), is inadequate to evaluate the significance of a disease event. For example, we know that thousands of pintails died of botulism over the past 3 years on the prairies. To understand the potential population effects of this loss, it would be much better if we knew how many of the birds were adult females, because the loss of an individual adult female is probably much more significant than loss of a hatching-year male from the same population.

Most diseases do not act by killing birds directly. Instead, as we have tried to show by a number of examples, disease agents usually work by causing sublethal effects and by interacting with other factors such as nutrition, weather, or by acting in combination with other disease agents. This makes it even more difficult to determine the effect of an individual disease agent. For example, a bird may be able to compensate for the energetic costs of parasite X in good years when food is abundant and the weather is benign. If we considered only this year, we might conclude the parasite had no significant impact on the population. However, in the next year when food is scarce and the weather is inclement, the added cost of the parasite may be sufficient to result in widespread reduction in growth rate and even survival. In this year, we would conclude that the parasite had a significant impact on the population.

Most diseases are not distributed randomly or uniformly in the population. This phenomenon is most marked with parasitic diseases, in which the great majority of the population have few or no parasites but a few individuals have very heavy infections. In such cases, most of the population may suffer few effects but a small proportion of birds may be severely effected. In such cases, we might conclude that the disease has no significant effect at the population level but a slight reduction in resistance of the birds, or an increase in the level of exposure, or both, could change the disease pattern significantly. We believe that this is the case in renal coccidiosis in young waterfowl; in most circumstances it is likely of no significance but it may be important in Snow Goose goslings in some colonies.

The above two discussion points lead to a third, i.e, that **disease relationships are not static**; most relationships between disease agents and animals represent a balance that can be shifted by many factors either toward health or toward disease. In order to assess the actual impact of disease we need to look at it over time and in a variety of circumstances.

To be able to better assess the significance of individual diseases and of disease in general we need to: a) improve the detection and diagnosis of disease; b) establish a better data base that includes information on both small and large occurrences of mortality over time.

To understand the impact of some of the most important diseases, it will likely be necessary to use experimental techniques. There are two general ways of doing this:

- by **artificially increasing the occurrence of a disease** by a known amount and measuring the impact. This is the method used by Bellrose (2) to determine the impact of lead shot on ducks. Birds were trapped, dosed with a known number of shot (0, 1,2, or 4 shot) and released. Mortality/survival were followed and compared using band returns. This type of study will likely never be repeated but it did give information on the effects of lead shot that could never have been obtained by any other method.

- by **artificially reducing the occurrence of disease** in some part of the population and then comparing the survival of protected birds with unprotected birds. This type of study, called an *intervention trial*, has been used to study the impact of avian cholera on one group of Snow Geese. Some birds were vaccinated over a period of years on the breeding grounds and their survival has been compared to that of unvaccinated birds. Even though the vaccine probably does give a very good level of protection, the results suggest that birds protected from this one disease have better overall survival that unprotected birds and, thus, that the disease is having an actual population effect.

A similar trial is currently underway by S. Slattery and R. Alisaukos (CWS) to test the effects of parasites on wild Ross' Goose goslings. In this case, drug treatment is being used to reduce the number of parasites in some goslings.

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