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manual

WILD BIRDS AND AVIAN INFLUENZA

An introduction to applied field research and disease sampling techniques

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Centre and right images: Rob Robinson

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An introduction to applied field research and disease sampling techniques

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
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Foreword

Although H5N1 highly pathogenic avian influenza (HPAI) virus has been known for over a decade, the enormous impact of outbreaks in poultry across Asia, Africa and Europe since 2003, as well as deaths in over 200 humans, over 230 million poultry and thousands of wild birds, has made H5N1 HPAI and "bird flu" a part of common daily parlance. However, the expression 'highly pathogenic avian influenza' is a chicken term, and should really not be used to describe the infection in other species (wild ducks, tigers, ferrets, or humans), even though the infection can be highly virulent in many species of animals. We prefer to call these infections AI virus infections or influenza viral infections of avian origin.

In response to the geographic spread of H5N1 and the deaths it has caused in wild bird populations to various degrees, as well as the concern that certain species of wild birds may play a role in the introduction and dissemination of the H5N1 virus along their flyways, FAO has been particularly interested to understand the interactions between wild and domestic birds. Within the Emergency Centre for Transboundary Animal Diseases (ECTAD), FAO has established a Wildlife Disease Programme to promote regional cooperation and action and increase in-country national and regional capacity building through the training and education of biologists, veterinarians, ornithologists and others to better integrate a common understanding of pathogen transmission in affected environments. To support this work, it produced a Manual entitled *Wild Bird Highly Pathogenic Avian Influenza Surveillance – sample collection from healthy, sick and dead birds* in 2006.

There is a wide variety of manuals available to understand the intricacies of the ecology and life cycles of wild bird species, including the wide spectrum of food and foraging habits, social interactions, migration strategies, nesting choices, habitat use. However, it was immediately apparent to FAO and other partners that there is a need for an introductory manual to support field efforts with regard to the study of bird populations and ecological aspects of avian influenza viruses. The topics covered in this Manual address monitoring technologies and sampling techniques, wild bird surveillance, some features of habitat use and migration ecology that are all important aspects of wildlife and disease ecology that need to be better researched.

This Manual is a collaborative effort of FAO, the Agricultural Research Centre for International Development of France (CIRAD), BirdLife International, Percy FitzPatrick Institute of African Ornithology, United States Geological Survey (USGS), Wetlands International, Wildfowl and Wetlands Trust, UK (WWT) and Wildlife Conservation Society (WCS).

The Manual has been brought to life by photographs kindly provided by a number of excellent photographers from around the world. FAO would like to thank Nyambayar Batbayar, Alexandre Caron, CIRAD, Ruth Cromie, Graeme Cumming, Karen M. Cunningham, Robert J. Dusek, Pieter van Eijk, Sasan Fereidouni, Clement Francis, J. Christian Franson, Friedrich-Loeffler Institut, Martin Gilbert, Mark Grantham, Nigel Jarrett, Rebecca Lee, Khanh Lam U Minh, Taej Mundkur, Rishad Naoroji, Kim Nelson, Scott Newman, PDSR/FAO Indonesia, Diann Prosser, Rob Robinson, Giuseppe Rossi, Paul Slota, Kristine Smith, David Stroud, John Takekawa, USGS Western Ecological Research Center, Alyn Walsh, Darrell Whitworth and

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FAO encourages feedback and comments on this Manual.

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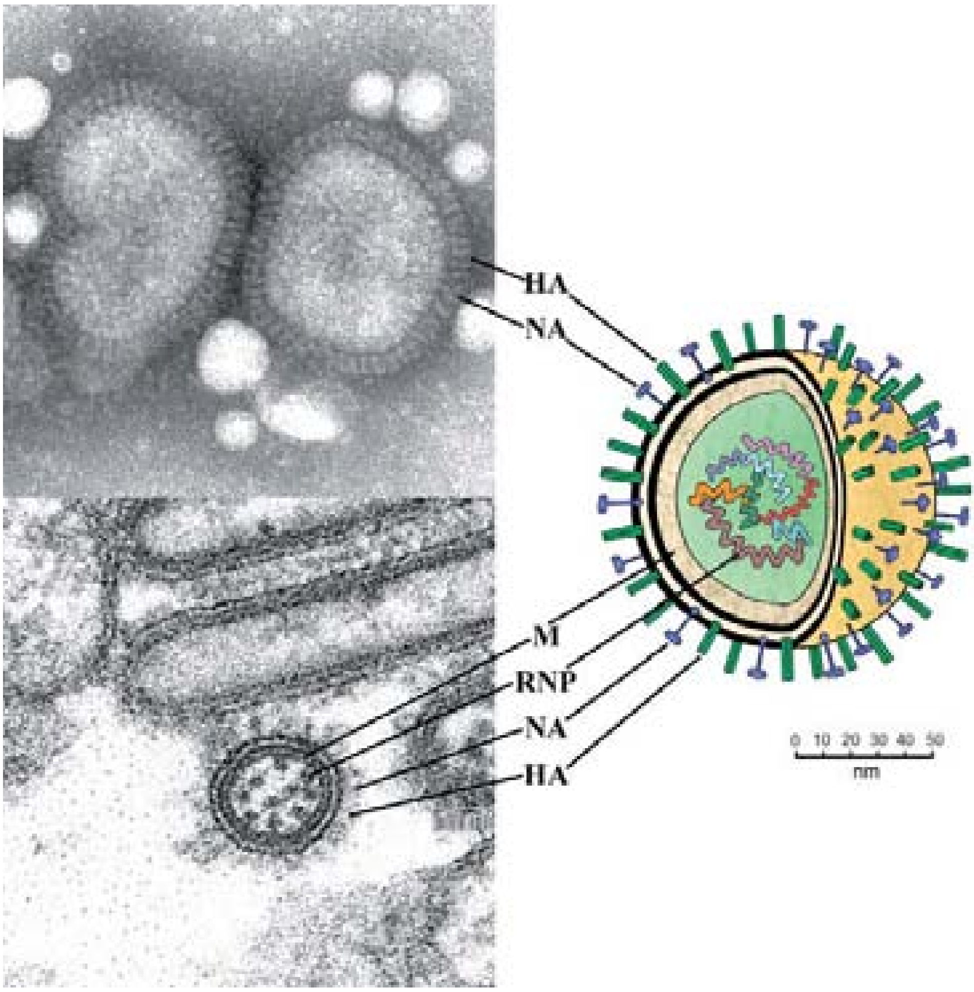
Chapter 1
Avian influenza and the H5N1 virus

ECOLOGY AND BIOCHEMICAL PROPERTIES OF AVIAN INFLUENZA VIRUSES

Avian influenza (AI) is an infectious disease of birds caused by type A influenza viruses of the Orthomyxoviridae family (Figure 1.1). These viruses most commonly infect poultry (e.g. domestic chickens, turkeys, ducks, quails and geese) as well as many types of wild birds. Some AI viruses are also known to infect a variety of mammals, including humans.

FIGURE 1.1
Electron micrographs and diagram of an avian influenza virus



HA- haemagglutinin glycoprotein, M- Capsid, NA-neuraminidase glycoprotein, RNP- ribonucleoprotein

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The different AI virus subtypes are distinguished by the haemagglutinin and neuraminidase antigens (glycoproteins) that cover the virus surface (Figure 1.1). Sixteen different haemagglutinin (H1-H16) and nine different neuraminidase (N1-N9) antigens have been characterised and each viral subtype is identified by the particular antigen combination it possesses (e.g. H5N1 or H3N2). All 16 haemagglutinin and nine neuraminidase antigens have been identified in wild bird populations. Genetically, AI viruses are composed of eight distinct ribonucleic acid (RNA) segments.

A particular AI virus subtype may include a variety of similar but distinct strains (the term "clades" is often used to describe these sub-populations), based on genetic sequences and the clustering – or not – of the isolates. The different strains originate either through genetic mutation as the virus replicates or via recombination (exchange of parts of a segment) or reassortment (exchange of a full segment) of genetic material between different viruses infecting a common host. Specific viral strains (e.g. A/bar-headed goose/Qinghai/5/2005 H5N1) are identified by: 1) influenza type; 2) host species from which the strain was isolated; 3) geographic location; 4) laboratory strain designation; 5) year of isolation[1]; and 6) viral subtype.

AI viruses are classified as low pathogenic (LPAI) or highly pathogenic (HPAI) depending on their virulence in domestic chickens (Figure 1.2). Most AI poultry infections are caused by LPAI strains that may produce a mild disease manifested by a variety of respiratory, enteric or reproductive signs (depending on the strain). Clinical signs may include decreases in activity, food consumption or egg production, coughing and sneezing, ruffled feathers, diarrhoea and/or tremors. Often, few visible clinical signs are noted and some LPAI outbreaks may go entirely undetected unless there is specific laboratory testing for the presence of the virus. Quality assured vaccines, when well applied and used in conjunction with other disease control measures (such as improved hygiene and care, and movement management), are effective in preventing the introduction of AI viruses and their spread within and among domestic flocks.

AI viruses are transmitted via direct contact with an infected bird or indirectly via close exposure to materials contaminated with infected faeces or possibly respiratory secretions. However, AI viruses have limited ability to survive outside the host where persistence in the environment is highly dependent on moisture, temperature and salinity. AI viruses can, however, persist for years in ice in high latitude lakes and have been shown to persist for over one month in other cool, moist habitats. In fact, the viruses are most often encountered in wetland habitats frequented by waterbird species, including Anatidae (ducks, geese and swans) and Charadriidae (shorebirds), which are the most common wild avian hosts of AI viruses.

In wild birds, LPAI infection can affect foraging and migratory performance (van Gils *et al*. 2007), but most infected birds show no obvious clinical signs of disease. Common AI strains and their wild host populations have developed an evolutionary equilibrium over time whereby the virus does not cause serious disease or mortality. Periodically, wild birds, particularly ducks and geese, have been identified as the source of virus introductions to poultry. Reassortment or recombination between LPAI viruses in a common host can, but

[1] Year of isolation does not necessarily correspond to its first appearance.

FIGURE 1.2
Chicken with H5N1 highly pathogenic avian influenza

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does not necessarily, lead to more pronounced virulence. In addition, during viral replication while circulating in domestic flocks, AI viruses also undergo frequent mutations which can give rise to new biological characteristics (i.e. from LPAI to a more virulent or "highly pathogenic" avian influenza or HPAI viruses). Emergent HPAI strains are often more contagious (depending on the density of susceptible hosts) and typically virulent in gallinaceous species, resulting in disease outbreaks with up to 100 percent mortality in unprotected poultry flocks; these are popularly known as "bird flu" or "fowl plague" outbreaks. Though culling of domestic poultry is the most effective means of containing the disease when a

HPAI outbreak occurs, it does depend on early detection and reporting. A compensation plan can often lead to transparency, early reporting, and offset socio-economic losses.

To date, all HPAI outbreaks in poultry have been caused by H5 or H7 strains, but these strains have rarely been found in wild bird populations. However, over the last few years a particularly virulent strain of the H5N1 AI virus has shown the ability to infect poultry and numerous wild birds, as well as wild and domestic cats (Felidae), weasels (Mustelidae), domestic dogs (Canidae) and other mammals, including humans.

The emergence of the zoonotic H5N1 HPAI virus has caused considerable concern among medical and veterinary experts, public health officials, wildlife biologists, wildlife conservationists and, after considerable media attention, the general public. The H5N1 virus that emerged in Asia in late 2003 is particularly alarming because of its high virulence in poultry, ability to infect a variety of hosts, and potential to spread quickly over large geographic areas, presumably via commercial poultry and wild bird trade, and possibly migratory waterbird routes.

It is generally agreed that wild birds serve as the reservoir for LPAI viruses, but the reservoir for the current H5N1 HPAI strains has not yet been identified despite disease sampling from hundreds of thousands of wild healthy migratory and resident birds, including peri-domestic species. The frequent interactions between large numbers of domestic poultry and wild waterbirds in openly grazed rice fields in parts of Southeast Asia and Africa are likely to be sustaining the H5N1 HPAI virus in both the domestic poultry and wildlife sectors.

Fortunately, there is no evidence to date, to indicate that the H5N1 HPAI virus has initiated sustained human-to-human transmission. All evidence suggests close contact with infected domestic birds or their faeces as the principal source for all H5N1 infections in humans. However, there is concern that a mutated or recombinant form of the virus could emerge and acquire improved transmissibility among humans, in which case there is the real potential for a global influenza pandemic.

HISTORY OF THE H5N1 AVIAN INFLUENZA VIRUS

The highly pathogenic strain of the H5N1 AI virus was first isolated and characterised in a domestic goose in the southern Guangdong province of China in 1996 (Table 1.1). The following year, the first H5N1 HPAI outbreak occurred in domestic poultry in Hong Kong, resulting in the culling of over 1.5 million chickens in an effort to contain and eliminate the disease. This outbreak also led to the infection of 18 people (with six fatalities) in what would be the first documented human deaths from the H5N1 virus.

The next outbreak in humans was not detected until February 2003 when two fatal cases of influenza from the H5N1 strain were documented in members of a Hong Kong family that had recently travelled to mainland China. A third member of the family died of severe respiratory disease while in China, but no samples were taken to confirm if the H5N1 virus was responsible.

Suspected H5N1 HPAI virus outbreaks reappeared in Southeast Asia as early as the middle of 2003, but confirmed infections were not reported again until December 2003-January 2004, when captive tigers (*Panthera tigris*) and leopards (*Panthera pardus*) fed on chicken carcasses were diagnosed with the virus in a zoo in Thailand. Soon after, H5N1 HPAI virus outbreaks swept through domestic poultry in eight East and Southeast Asian

TABLE 1.1

Important events in the discovery, detection and spread of the H5N1 highly pathogenic avian influenza virus (January 1996-September 2007)

1996	First isolation of H5N1 subtype in a domestic goose in **China** (Guangdong province).
1997	First H5N1 outbreak in domestic poultry and humans in **China** (Hong Kong SAR).
1998-2002	No documented outbreaks in domestic poultry and humans. Dec 2002: H5N1 kills a variety of captive ducks and other birds in two bird collections in **China** (Hong Kong SAR).
2003	Feb: H5N1 virus reappears with two human cases in a family in **China** (Hong Kong SAR). Mar-Jul: Suspected but undocumented H5N1 outbreaks in **Southeast Asia**. Dec-Jan 2004: Virus kills two captive big cat species (tiger and leopard) in a **Thailand** zoo after being fed chicken carcasses. Dec: First wave of widespread H5N1 outbreaks begins in **Asia** with infections reported on three poultry farms in the **Republic of Korea**.
2004	Jan-Feb: First H5N1 poultry outbreaks in **Viet Nam**, **Thailand**, **Japan**, **Cambodia**, **Lao PDR**, **Indonesia** and **China**, with first human cases reported in **Viet Nam** and **Thailand**. First domestic cat reported infected in **Thailand**. Jun-Aug: Second wave of H5N1 poultry outbreaks begins in **Southeast Asia**, with first cases recorded in **Malaysia**. Jul: Research indicates H5N1 can be lethal in certain wild waterbirds species. Oct: First report of H5N1 in Europe from two Crested Hawk-eagles (*Spizaetus nipalensis*) smuggled into **Belgium** from **Thailand**. Oct: Virus kills 41 captive tigers in a Thailand zoo after being fed chicken carcasses. Dec: Third wave of H5N1 outbreaks begins in **Southeast Asia**.
2005	Apr-May: H5N1 responsible for deaths of over 6,000 migratory birds (Bar-headed Goose, Pallas's Gull, Brown-headed Gull, Ruddy Shelduck, Great Cormorant and other species) at Qinghai Lake, **China**. Jul-Aug: First H5N1 outbreaks detected in **Russia** (**Siberia**), **Kazakhstan**, **Mongolia** and **China** (Tibetan Plateau and Xinjiang) with reports of dead migratory wild birds in the vicinity of some poultry outbreaks, with the exception of Mongolia. Oct: H5N1 outbreaks in **Turkey**, **Croatia** and **Romania** signalled the first detection of the virus in Europe in domestic poultry and wild birds and heralded its spread into 26 European countries by July 2006. Nov: First report in the Persian Gulf states of a single captive Greater Flamingo (*Phoenicopterus roseus*) in **Kuwait**.
2006	Jan-Feb: First human cases of H5N1 outside Southeast Asia - **Turkey** and **Iraq**. Feb: H5N1 detected in commercial poultry in Africa in **Nigeria** and **Egypt**, where the virus spread to eight countries by May. Feb-Jul: Scattered carcasses of H5N1 infected wild birds reported in most **European Union** countries, including **Austria**, **Czech Republic**, **Denmark**, **France**, **Germany**, **Greece**, **Italy**, **Poland**, **Spain**, **Sweden** and the **United Kingdom**, and in **Switzerland**. Apr-Jun: Reports of H5N1 deaths in Barheaded Geese and other birds around Qinghai Lake, **China** Mar: First H5N1 outbreak in humans associated with handling infected dead wild swan in **Azerbaijan**. (To date, this is the only wild bird to human infection)
2007	Jan-Jun: H5N1 detected in poultry in **Ghana** and **Togo** in Africa and **Kuwait** and in captive falcons and poultry in **Saudi Arabia** in West Asia. Jan: H5N1 detected on a commercial turkey farm in the **United Kingdom** and commercial goose farms in **Hungary**. Apr: First outbreak of H5N1 detected in poultry in **Bangladesh**. Jun-Jul: H5N1 detected in over 200 dead wild birds from three countries (**Czech Republic**, **France** and **Germany**) with two of them (**Czech Republic** and **Germany**) experiencing a concurrent outbreak in domestic poultry.

countries (Cambodia, Taiwan Province of China, Indonesia, Japan, the Republic of Korea, the Lao People's Democratic Republic, Thailand and Viet Nam). This wave of outbreaks resulted in the culling of at least 45 million domestic poultry and at least 35 human cases (24 fatal) in Viet Nam and Thailand (up to March 2004).

Subsequent H5N1 HPAI outbreaks in poultry in the northern summer of 2004 and northern winter of 2004/05 remained confined to Southeast Asia, but human cases spread beyond Viet Nam and Thailand to include Cambodia, Indonesia and China. Most human cases involved contact with infected poultry or contaminated materials, but some possible cases of limited human-to-human transmission could not be ruled out.

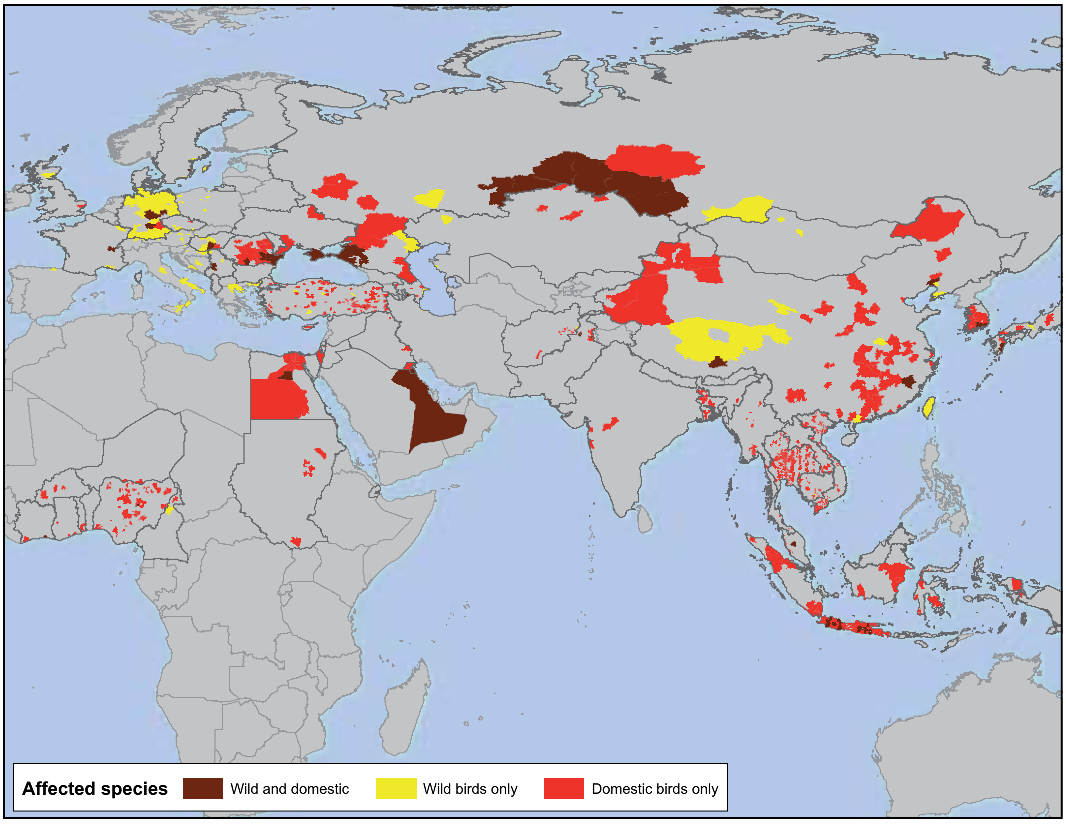
Wild birds were not known to be implicated in the initial H5N1 HPAI outbreaks as the disease emerged in Asian poultry in 2003/04, although there was limited surveillance of wild birds being undertaken at that time. However, in May 2005, an H5N1 virus mortalilty event killed over 6,000 waterbirds (mainly Bar-headed Geese [*Anser indicus*], Great Cormorants [*Phalacrocorax carbo*], Pallas's Gulls [*Larus ichthyaetus*], Brownheaded Gulls [*L. brunnicephalus*] and Ruddy Shelducks [*Tadorna ferruginea*]) at the Qinghai Lake National Nature Reserve in northwest China. Estimates indicate that between 5-10 percent of the entire world's population of Bar-headed Geese were killed during this event. This was the second documented mortality event of wild birds as the result of an AI virus. The only previous event occurred in 1961 when many Common Terns (*Sterna hirundo*) were killed during an H5N3 AI mortality incident in South Africa.

The H5N1 AI-related mortality event at Qinghai Lake and subsequent outbreaks or mortality events in China, Siberia, Kazakhstan and Mongolia (Figure 1.3) in July and August 2005 signalled a significant geographic expansion of the disease. The pattern of disease spread has been suggested as evidence of the possible role of migratory waterbirds in disease transmission, although poultry and wild bird trade routes could also explain some

FIGURE 1.3
Bar-headed Goose (*Anser indicus*) carcass found during an H5N1 AI mortality event in Mongolia in August 2005

CREDIT: MARTIN GILBERT

FIGURE 1.4
Countries in which the H5N1 HPAI virus has been detected in poultry and/or wild birds (updated 5 September 2007)



of the outbreaks (Gauthier-Clerc *et al.* 2007). Outbreaks in some of the domestic flocks in Siberia and Kazakhstan occurred at the same time as reports of mortalities in wild migratory waterbirds in the vicinity of infected poultry farms, but the original source of infections could not be determined. Confirmed H5N1 AI-related mortalities in Mongolia were limited to a Bar-headed Goose and four Whooper Swans (*Cygnus cygnus*) in 2005.

The H5N1 HPAI virus continued its westward expansion during the northern autumn of 2005 and by October it was detected in poultry in Turkey, and subsequently in Croatia and Romania, the first occurrences in Europe. The arrival of the H5N1 HPAI virus in Turkey and Eastern Europe heralded the swift spread of the disease throughout Europe and into the Persian Gulf region by December 2005, and the Middle East and Africa by February/ March 2006.

In January 2006, the first human H5N1 AI infections reported outside East Asia occurred in Turkey. Within a few months human infections were also reported in Iraq, Azerbaijan, Egypt and Djibouti, raising to 10 the number of countries reporting H5N1 virus infections in humans (258 cases, 154 fatal as of 29 November 2006). As in Asia, human cases in most of these countries were associated with handling of infected domestic poultry. However, the first fatality in Azerbaijan in March 2006 was linked to plucking a dead infected swan. This marked the first, and only, known case of H5N1 virus transmission from a wild bird to a human.

TABLE 1.2
Countries affected by the H5N1 avian influenza virus in domestic poultry, free-ranging wild birds, captive wild birds and humans since 1996 (as of 7 September 2007)

Country	Year*	Poultry	Wild birds	Captive birds	Humans
ASIA					
Afghanistan	2006	•	×		
Bangladesh	2007	•			
Cambodia	2004	•		•	×
China**	1996	•	×	•	×
India	2006	•			
Indonesia	2004	•			×
Iran	2006		×		
Iraq	2006	•			×
Israel	2006	•			
Japan	2004	•	×		
Jordan	2006	•			
Kazakhstan	2005	•	×		
Kuwait	2005	•		•	
Korea, Rep of	2003	•	×		
Lao PDR	2004	•			
Malaysia	2004	•	×		
Mongolia	2005		×		
Myanmar	2006	•			
Pakistan	2006	•	×	•	
Saudi Arabia	2007	•		•	
Thailand	2003	•	×		×
Viet Nam	2004	•			×
West Bank and Gaza Strip	2006	•			
AFRICA					
Burkina Faso	2006	•			
Cameroon	2006	•	×		
Côte d'Ivoire	2006	•	×		
Djibouti	2006	•			×
Egypt		•			×
Ghana	2006	•			×
Niger	2006	•			
Nigeria	2006	•	×		
Sudan	2006	•			
Togo	2007	•			

(Continued)

TABLE 1.2 *(Continued)*

Countries affected by the H5N1 avian influenza virus in domestic poultry, free-ranging wild birds, captive wild birds and humans since 1996 (as of 7 September 2007)

Country	Year*	Poultry	Wild birds	Captive birds	Humans
EUROPE					
Albania	2006	•			
Austria	2006		×	•	
Azerbaijan	2006	•	×		×
Bosnia-Herzegovina	2006		×		
Bulgaria	2006		×		
Croatia	2005		×		
Czech Republic	2006	•	×		
Denmark	2006	•	×		
France	2006	•	×		
Georgia	2006		×		
Germany	2006	•	×	•	
Greece	2006		×		
Hungary	2006	•	×		
Italy	2006		×		
Poland	2006		×		
Romania	2005	•	×		
Russian Federation	2005	•	×		
Serbia	2006	•	×		
Slovakia	2006		×		
Slovenia	2006		×		
Spain	2006		×		
Sweden	2006		×	•	
Switzerland	2006		×		
Turkey	2005	•	×		×
Ukraine	2005	•	×		
United Kingdom	2006	•	×		

* The year indicates when the virus was first confirmed - data compiled from various sources, including OIE, WHO and FAO.

** Including Hong Kong and Tibet.

Over a two-month period during the northern summer of 2007, H5N1 was detected in over 200 dead wild birds from three countries (Czech Republic, France and Germany) with two of them (Czech Republic and Germany) experiencing a concurrent outbreak in domestic birds. These mortalities in wild birds involved primarily non-migratory species, and took place at a time of year (June-July) when birds may have been flightless due to moult, and were not migrating into or away from Europe.

As of September 2007, the H5N1 HPAI virus had been confirmed in poultry or wild birds in 59 different countries on three continents (Figure 1.4 and Table 1.2). In Europe, the virus has been detected in both wild birds and poultry in 12 countries (Azerbaijan, Denmark, France, Germany, Hungary, Romania, Russia, Serbia, Sweden, Turkey, Ukraine and the United Kingdom), only in wild birds in 12 countries (Austria, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Greece, Italy, Poland, Slovakia, Slovenia, Spain and Switzerland), and only in poultry in one country (Albania).

By contrast, outbreaks in 10 African countries (Burkina Faso, Cameroon, Côte d'Ivoire, Djibouti, Egypt, Ghana, Niger, Nigeria, Sudan and Togo) have been limited almost entirely to poultry. Only three H5N1 AI cases have been recorded in wild birds: a Sparrow Hawk (*Accipter nisus*)[2] in Côte d'Ivoire and unspecified duck and vulture species in Cameroon and Nigeria respectively.

As the H5N1 virus spread over Eurasia and Africa in 2006, recurrent outbreaks in Southeast Asia suggested that the virus had become endemic in many regions and was still expanding. Wildlife mortality events in China were fewer in number, with some 1,800 wild birds, but over a broader geographic range compared to 2005. Four new countries (Afghanistan, India, Myanmar, and Pakistan) reported presence of the H5N1 HPAI virus in early 2006, raising to 19 the number of Asian countries with confirmed outbreaks in poultry or wild birds. Although Japan had effectively controlled H5N1 HPAI virus outbreaks in poultry and declared itself disease free in the northern summer of 2004, outbreaks continued in most other countries, including Malaysia and the Republic of Korea, which had been able to eliminate the disease earlier but probably experienced reintroductions. In early 2007, a commercial turkey farm in the United Kingdom reported the first domestic turkey outbreak in the country that was possibly linked to importation of frozen turkey meat from Hungary. The H5N1 HPAI virus spread in poultry in Ghana and Togo in Africa and Bangladesh in Asia.

SURVEILLANCE STRATEGIES FOR AVIAN INFLUENZA

The scientific community has acknowledged that the H5N1 HPAI virus is primarily responsible for a poultry disease and that more emphasis on surveillance, prevention and control measures should be addressed at the animal (agricultural) production level to improve husbandry and marketing biosecurity practices in order to halt the risk of human infections and curb further spread in poultry. However, concern remains about the role that wild birds may play in harbouring and transmitting the disease. Most of the information regarding the relationship between wild birds and the H5N1 virus has relied on samples collected from sick or dead birds during mortality events. While this "opportunistic" surveillance has provided important data (e.g. host range and susceptibility), it is a biased collection technique and does not offer insight into identification of the *reservoir role* that wild birds might play in the propagation and spread of the H5N1 virus or other infectious diseases.

[2] Other sources refer to a Yellow-billed Kite (*Milvus migrans parasiticus*) which highlights the problem of wild bird identification in the official reporting channels for avian influenza. Limited involvement by qualified wildlife biologists often results in failure to identify or misidentification of wild birds both in the vicinity of outbreaks and in the wider countryside.

Recently, several surveillance programmes specifically designed to collect samples from healthy free-ranging wild birds have been undertaken by a number of international or national agencies, and non-governmental organisations. However, active surveillance in wild birds presents practical, logistic and financial obstacles that make it a challenge. Given the expected low prevalence of H5N1 AI viruses in healthy wild birds and the often limited resources available for what are costly efforts, it is important to approach active surveillance sampling in a strategic manner with clearly defined goals, sound epidemiological justification and sufficient technical skills and capabilities to perform both field and laboratory activities. The primary goals of effective and active wildlife surveillance programmes for the H5N1 virus should be: 1) to determine which species can host the virus; 2) to determine temporal and spatial variation in disease prevalence; 3) to determine the role of wildlife in the ecology of the disease; and 4) to develop protocols that will reduce the potential for human and poultry exposure to the virus from wildlife sources and vice versa.

Active surveillance programmes for free-ranging healthy wild birds should be targeted at species with the following characteristics: 1) species known to have been infected with the H5N1 AI virus; 2) species known to be epidemiological reservoirs for LPAI viruses; 3) social species that are known to aggregate seasonally at breeding, roosting, migration stopover and non-breeding (wintering) sites; 4) species that potentially share habitats with poultry farms, integrated livestock-aquaculture systems, backyard poultry flocks and croplands such as rice fields; and 5) species whose seasonal movements or migratory patterns may explain disease dispersal and/or emergence. Selection of sampling sites will primarily be dictated by the habitat preferences of the species to be sampled and occurrence of outbreaks in poultry, although other factors such as bird and researcher safety, and project logistics should also be considered (see *Chapter 3*).

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Chapter 2
Wild birds and avian influenza

AVIAN INFLUENZA IN WETLAND BIRD SPECIES

Although the H5N1 AI virus has been detected across a diverse range of free-ranging wild species (over 75 species of wild birds from 10 different avian orders; Table 2.1), it is wetland or aquatic species that are the most frequently recorded. Birds with affinities for wetland habitats make up nearly 60 percent of the wild species infected with the H5N1 virus and also account for the greater proportion of wildlife mortalities.

The term "wetland" encompasses a variety of inland freshwater and marine coastal habitats that share one common feature; soils or substrates that are at least periodically saturated with or covered by water. This simple description belies the fact that wetland systems are quite complex and exhibit a wide range of differences in substrates, salinity, frequency of flooding and vegetation (Ramsar Convention Manual 1997) - important features that determine the bird species inhabiting a particular wetland.

Waterbirds have evolved foraging and breeding strategies to exploit natural wetlands and can be found in virtually all types of wetland; from perennially submerged bays, lakes, ponds and rivers to seasonally flooded marshlands, swamps and tundra bogs, and tidally flooded estuaries, salt marshes and mudflats (Figures 2.1 and 2.2). Human-created and/

FIGURE 2.1
Roosting shorebirds on a tidal flat, Yalu Jiang, China

CREDIT: YUAN XIAO

FIGURE 2.2
Typical wetland habitats frequented by waterbirds

CREDIT: SCOTT NEWMAN

Khorin Tsagaan Nuur, Mongolia

CREDIT: APHALUCK BHATIASEVI

"Wet" poultry farm in Viet Nam

or altered wetlands have also become important wildlife habitats as natural wetlands are increasingly altered and converted to other types of habitat for human use. Wild birds have been quick to adapt to altered wetlands and are commonly seen at water reservoirs, salt ponds, flooded agricultural fields, irrigation ditches, "wet" poultry farm ponds and aquaculture ponds.

Substantial loss of natural wetlands and the attraction of altered wetlands converted to intensive rice farms are factors that may be resulting in concentrating of waterbirds in smaller habitats, thereby increasing their density and increasing the risk of virus transmission, primarily among and between waterfowl and shorebirds that populate these habitats.

As the most frequently detected wild hosts of the H5N1 virus, wetland birds represent an appropriate target for active disease surveillance. Birds such as ducks, geese, swans, gulls, shorebirds, herons, egrets, storks, rails, coots, gallinules, cormorants and grebes are common wetland species (Table 2.1). A review of their general nesting, migration and foraging strategies is helpful for understanding their potential role in the spread of the H5N1 virus. While the ecological strategies described in this Manual are valid for the majority of species in each group, exceptions can and do occur.

TABLE 2.1
List of the avian taxa in which the H5N1 highly pathogenic avian influenza virus has been detected in wild and/or captive populations* (as of September 2007)**

			Number of species H5N1 detected		
Order family	**Common species**	**Habitat preferences**	**Total**	**Wild**	**Captive**
Anseriformes					
Anatidae	Ducks, geese, swans	Wetland, marine	30	11	19
Charadriiformes					
Laridae	Gulls	Marine, wetland	3	3	2
Scolopacidae	Shorebirds	Wetland	1	1	0
Gruiformes					
Rallidae	Rails, coots	Wetland	4	4	0
Pelecaniformes					
Phalacrocoracidae	Cormorants	Marine, wetland	2	2	0
Podicipediformes					
Podicipedidae	Grebes	Wetland, marine	2	2	0
Falconiformes					
Accipitridae	Hawks, eagles	General	7	5	2
Falconidae	Falcons	General	2	1	2
Passeriformes					
Corvidae	Crow, ravens	General	3	3	0
Other	Songbirds	General	12	8	4
Galliformes					
Phasianidae	Pheasants, partridge	General	4	2	2
Columbiformes					
Columbidae	Pigeons, doves	General	2	2	0

* Captive birds include those held in a zoo or sanctuary. Some species may be included both as wild and captive.
** Data source: USGS NHWC website

Waterfowl (Anseriformes)

Ducks, geese and swans (Anatidae family; Figure 2.3), collectively known as waterfowl, are well-studied common hosts for LPAI viruses, and the only bird group in which the viruses have been found all year round in wild populations. A list of the species and numbers of birds counted among the known wild bird fatalities due to the H5N1 virus reveals that waterfowl are, by far, the bird group from which the H5N1 HP and LP AI virus pathotypes have been most commonly recovered. Waterfowl made up the vast majority of wild birds infected during the H5N1 AI mass mortality event in China in 2005/06 and were they also the prevalent group of wild bird species infected during numerous mortality events as the virus spread from east Asia into west Asia and Europe.

Ducks, geese and swans are a familiar group of waterbirds totalling about 150 species distributed worldwide. In general, they are medium to large birds with heavy bodies, long necks relative to body size, webbed feet and, in most species, a broad, blunt bill – a distinct combination of external features that make them among the most conspicuous and easily recognisable of all the wetland avifauna. Waterfowl have a long history of exploitation by humans both as wild game and domesticated poultry. A few species, most notably the Mallard *(Anas platyrhynchos)* and Greylag Goose *(Anser anser)*, have been raised as domestic birds for thousands of years.

Waterfowl are gregarious and many boreal species form sizable flocks that converge on traditional wetland foraging areas during the northern autumn and winter seasons. In contrast to their gregarious nature outside the breeding season, waterfowl are primarily solitary nesters, although some species such as the Bar-headed Goose nest in colonies of tens to thousands of birds. Most waterfowl nest on the ground in vegetated areas near the water's edge or in the immediate vicinity of water. However, some species breed in shallow submerged habitats by building up vegetation to form a dry nest mound surrounded by water, while other species construct floating nest platforms anchored to emergent vegetation. A number of duck species nest in cavities excavated in tree trunks by other species.

In general, waterfowl are monogamous although the duration of the pair bond differs among groups. In most ducks, pair bonds are temporary and females are responsible for all incubation and brood-rearing duties. By contrast, male swans and true geese share brood raising duties, and long-term, often lifetime, pair bonds are common in these groups.

Waterfowl chicks are highly precocial (i.e. well-developed, active and alert at hatching) and capable of following their parent(s) to water and foraging independently within hours of hatching. Female ducks attend their young until they are able to fly, while geese and swans form family units that may remain intact until the following breeding season.

All waterfowl undergo a brief post-breeding flightless period each year when the flight feathers are shed (moulted) simultaneously. During the moulting period, large numbers of flightless waterfowl often congregate in wetland habitats relatively safe from predators. The complete moult occurs near the breeding grounds during the chick-rearing period for all female waterfowl and males of those species in which both sexes participate in brood-rearing.

Differences in morphology and behaviour allow waterfowl to partition foraging habitats. Waterfowl are commonly characterized as "dabblers", "divers" and "grazers" depending on the particular foraging technique they employ. Many waterfowl species feed on aquatic

FIGURE 2.3

Representative species from the three subfamilies of the Anatidae family

CREDIT: ALYN WALSH

Wigeon (Anas penelope)

CREDIT: ROB ROBINSON

Bar-headed Goose (Anser indicus)

CREDIT: ROB ROBINSON

Mute Swan (Cygnus olor)

invertebrates and plants that they filter from water or mud as it passes through rows of horny plates (lamellae) lining their bill. Swans, shelducks and "dabbling" ducks forage at or just below the surface (how deep depends on the length of their neck) to feed at the bottom of shallow waters. "Diving" ducks, as the name implies, dive below the surface to forage in deeper substrates that are inaccessible to the "dabblers". Mergansers are an exception among the "diving" ducks, feeding on fish in the water column. "Grazing" waterfowl are characterised by upland foraging geese and ducks adapted for feeding on terrestrial plants and grain. "Grazers" also include several species of African "geese" which are not true geese at all, but grazing ducks.

Shorebirds (Charadriiformes)

Shorebirds or waders (Figures 2.4, 2.5 and 2,6) belong to several families in the Charadriiformes order, a large and diverse avian order that also includes gulls, terns and auks. After waterfowl, shorebirds are perhaps the most common hosts of LPAI viruses, although for the species sampled, the viruses appear seasonally and have only been detected in wild shorebird populations during the northern spring and autumn.

Despite the high overall frequency of LPAI viruses in some shorebirds, the H5N1 HPAI virus has thus far been detected in only one species, the Green Sandpiper *(Tringa ochropus)* in the Scolopacidae family. Furthermore, shorebirds do not appear to transmit or spread H5N1. Even though they share considerable geospatial and temporal overlap with waterfowl on Asian migratory routes, they have not transported the virus to Australia where they spend the southern summer in large numbers (and to where northern hemisphere breeding species of migratory Anatidae do not normally migrate in any numbers).

FIGURE 2.4

Eurasian Curlew (*Numenius arquata*), family Scolopacidae (Charadriiformes order)

CREDIT: CLEMENT FRANCIS

FIGURE 2.5
Common Redshank (*Tringa totanus*), family Scolopacidae (Charadriiformes order)

CREDIT: CLEMENT FRANCIS

FIGURE 2.6
Little Ringed Plover (*Charadrius dubius*), family Charadriidae (Charadriiformes order)

CREDIT: CLEMENT FRANCIS

Shorebirds are small to medium-sized birds with relatively long bills and unwebbed feet adapted for wading in mudflats and shallow waters along the margins of wetlands and rocky shores. They are also common in man-made wetlands and agricultural fields. Collectively, the shorebirds include familiar species such as sandpipers, stilts, avocets, oystercatchers, snipe and plovers. Like waterfowl, shorebirds are quite gregarious outside the breeding season when large migrating and non-breeding flocks gather in traditional wetland foraging and roosting areas.

Structural adaptations have allowed shorebirds to exploit the wide diversity of prey available in productive wetland habitats. The bills and legs of shorebirds are often their most conspicuous features and provide the best clue as to their particular foraging niche. Long-legged species such as stilts wade into deeper waters than the relatively shorter-legged species. The elongated, slender bill is adapted to probing for aquatic invertebrates on or in the damp, soft wetland substrates.

Shorebirds are generally solitary breeders (although few species do nest in large colonies), nesting on the ground in marshy tundra, taiga and grasslands, often well inland. Nests are usually simple scrapes lined with pebbles and bits of vegetation. Shorebird chicks are precocial and usually leave the nest shortly after hatching.

Gulls (Charadriiformes)

Gulls (Figure 2.7) are another familiar and rather homogenous group of medium to large-bodied waterbirds distributed in coastal, pelagic and inland freshwater habitats worldwide. The family including gulls (Laridae) is one of many within the Charadriiformes order. LPAI viruses are seasonally common in many Charadriiform species, including the gulls, and the H5N1 virus has been isolated in three gull species, including two, the Brown-headed Gull and Pallas's Gull affected during the first wild bird outbreak in China in 2005.

FIGURE 2.7
Western Gull (*Larus occidentalis*), family Laridae (Charadriiformes order)

CREDIT: DARRELL WHITWORTH

Gulls in general, and the larger species in particular, are resourceful birds that demonstrate complex behaviour and a highly-developed social structure. They are also very adaptable and many species are quite tolerant of humans. Some gulls congregate in populated areas where several species have increased markedly as they have adapted to exploit human sources of food. In fact, gulls scavenging in refuse piles and areas neighbouring domestic poultry farms provide a potential interface for contact with AI viruses. In the wild, gulls are generalist foragers that feed primarily on fish and aquatic invertebrates. However, the larger, more aggressive species are also opportunistic scavengers and kleptoparasites, and will even prey on unattended chicks of their own species.

Although primarily thought of as coastal and marine species, hence the popular term "sea gull", several gull species breed well inland on interior lakes and marshes. Gulls are primarily ground-nesting colonial species, with colonies ranging in size from tens to thousands of birds. Colonies are usually found in the immediate vicinity of water, often on cliffs, islands or other areas which offer protection from terrestrial predators. Nest sites are usually scrapes on the ground lined with varying amounts of dried vegetation. Gull chicks are quite active and mobile soon after hatching, although they are fed and protected by the parents at least until fledging.

The similar and closely related terns (Sternidae) may also be a target for disease surveillance, as Common Terns were the first species known to suffer a high mortality event as the result of an HPAI infection in 1961. Most terns, however, have a specialised diet that is likely to decrease their risk of exposure to the H5N1 virus because they prey almost exclusively on small fish they capture just below the surface of the water by making shallow dives from the wing. Marsh Terns (*Chlidonias* spp.) feed on small fish and invertebrates in freshwater and coastal wetlands.

Herons, egrets and storks (Ciconiiformes)

Herons (Figure 2.8), egrets and storks are medium-sized to large wading birds that are among the most conspicuous of all the wetland avifauna. They are distributed worldwide in a variety of wetland types, but most species have affinities for freshwater and brackish habitats in tropical to temperate latitudes. Although not generally recognised as common hosts of AI viruses, the H5N1 virus has been found in at least four heron or egret species and two stork species.

These closely related groups share several physical features to match their similar foraging and breeding ecologies. Like shorebirds, their long, slender neck and legs, and unwebbed feet are obvious adaptations for feeding in wetland habitats. Herons, egrets and storks are primarily carnivorous birds that wade through shallow water in search of a variety of prey including fish, amphibians, crustaceans, insects and even some small mammals and birds. They stalk with deliberate almost imperceptible movements, but strike quickly by extending the long neck to spear approaching prey with their long, sharp bills.

Most species breed in conspicuous colonies, constructing large stick nests in the upper branches of trees in and around wetlands, although the White Stork *(Ciconia ciconia)* of Eurasia will build nests on rooftops and other artificial structures. Chicks are altricial (i.e. hatch blind and helpless) and require continuous parental care for several weeks after hatching.

FIGURE 2.8
Grey Heron (*Ardea cinerea*), Ardeidae family (Ciconiiformes order)

CREDIT: CLEMENT FRANCIS

Grebes (Podicipediformes)

Grebes (Podicipedidae family; Figure 2.9) are small to medium-sized diving birds that are probably the most aquatic of all the species described here. In fact, grebes are quite awkward on land and are rarely, if ever, encountered out of water, except during migratory flights. This is another group not usually recognised as a common host of AI viruses, although the H5N1 virus has been found in at least two species, the Little Grebe *(Tachybaptus ruficollis)* and the Great Crested Grebe *(Podiceps cristatus)*.

Although some species migrate to coastal waters after the breeding season, grebes breed exclusively in freshwater wetlands. Their loose colonies of floating nest platforms anchored to emergent vegetation range in number from a few to hundreds of nests. Both parents participate in raising the precocial chicks which are often carried on the backs of the parents as they swim.

Grebes are often observed swimming with just the head and neck exposed, a feat they accomplish by pressing or releasing their feathers against the body to adjust buoyancy. Aided by lobed membranes on each toe that are characteristic of the family, all grebes are accomplished divers. The grebe diet consists of fish and aquatic invertebrates they obtain while diving. They also habitually consume their own feathers.

FIGURE 2.9
Great Crested Grebe (*Podiceps cristatus*), (Podicipediformes order)

CREDIT: TAEJ MUNDKUR

Coots, gallinules, rails and crakes (Gruiformes)

The members of the Rallidae family, including the coots and rails (Figures 2.10 and 2.11), gallinules, moorhens and crakes are perhaps the least familiar of the wetland birds described here. With the exception of the gregarious coots, most species are solitary, retiring birds which skulk in or along the margins of heavily vegetated wetlands, quickly disappearing into cover at the first sign of danger. Most species are highly vocal and much more likely to be heard than seen.

The family can be divided into two "natural" groups, the aquatic coots and gallinules and the more terrestrial marsh-dwelling rails and crakes. Species such as the widespread Coot *(Fulica atra)* and Common Moorhen *(Gallinula chloropus)* appear to be more vulnerable to the H5N1 virus, although at least one crake species has also been infected.

In keeping with their more aquatic habits, coots and gallinules construct floating nest platforms anchored to emergent vegetation. By contrast, rail nests are concealed in the thick vegetation along the wetland margins, sometimes over water. All Rallidae species are generalist feeders, consuming whatever food happens to be available at any given time, including aquatic plants and invertebrates. Rails and crakes tend to forage along the damp wetland margins, using their long toes for walking across marshy vegetation. Coots and gallinules feed in the shallow water by diving (coots) or tipping-up (gallinules) to feed on aquatic invertebrates and plants.

FIGURE 2.10
Coot (*Fulica atra*), Gruiformes order

CREDIT: ROB ROBINSON

FIGURE 2.11
Water Rail (*Rallus aquaticus*), Gruiformes order

CREDIT: PIETER VAN EIJK

Cormorants (Pelecaniformes)

Cormorants (Figure 2.12) are a homogenous family (Phalacrocoracidae) of medium-sized to large diving birds related to the pelicans. Cormorants are considered occasional hosts of AI viruses, and the H5N1 virus sub-type has been isolated in at least two species, including

FIGURE 2.12
Great Cormorant (*Phalacrocorax carbo*), Pelicaniformes order

CREDIT: CLEMENT FRANCIS

the widespread Great Cormorant *(Phalacrocorax carbo)* which can be found in coastal and inland wetlands throughout much of Eurasia, Africa and Australia. Interestingly, cormorants are often infected with Newcastle Disease virus (paramyxoviridae)[3], which causes a common widespread poultry disease, despite limited or no known interaction among these groups.

Although primarily marine and coastal birds, several cormorant species breed well inland in freshwater wetlands. Cormorants are colonial breeders, nesting in often large colonies on cliffs and offshore rocks in coastal areas or in the branches of trees at inland or coastal wetlands. Chicks are altricial and require continuous parental care for several weeks after hatching.

All cormorants share predominately dark plumages, relatively long necks and hooked bills. They use their webbed feet for propulsion while diving to capture fish which make up the bulk of their diet. Although waterbirds, cormorants lack waterproof plumage and scores of roosting birds are often seen with wings extended to dry in the sun.

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3 In its most virulent form, velogenic viscerotropic Newcastle disease in poultry, the disease can resemble HPAI clinically and require laboratory analysis to discriminate between causative agents.

FIGURE 2.13
Common Buzzard (*Buteo buteo*), Accipitridae family (Falconiformes order)

CREDIT: CLEMENT FRANCIS

FIGURE 2.14
American Kestrel (*Falco sparverius*), Falconidae family (Falconiformes order)

CREDIT: ROBERT J DUSEK

FIGURE 2.15
Tawny Eagle (*Aquila rapax*), Accipitridae family (Falconiformes order)

CREDIT: CLEMENT FRANCIS

Raptors (Falconiformes)

Many species of raptors, the collective term for diurnal birds of prey such as hawks, eagles, falcons and condors (Accipitridae family) (Figures 2.13, 2.14 and 2.15), have been fatally affected by H5N1 virus. Although not generally considered "wetland" birds, their role as predators and scavengers of other bird species may leave raptors vulnerable to AI viruses through consumption and exposure. It is believed that raptors contract the disease through direct contact with infected tissues as they scavenge the carcasses of poultry and wild birds that have died from H5N1, or prey upon infected birds weakened by the virus.

Raptors are a widespread and conspicuous group of birds distributed in a variety of habitats worldwide. Characterised by their strong talons, sharp hooked bill and keen eyesight, obvious adaptations for a predatory lifestyle, raptors consume a wide variety of prey, including insects, fish, amphibians, reptiles, birds and mammals. Raptors range greatly in size from small falcons with wingspans of less than 30 cm to vultures and condors with wingspans of over 3 m. Unlike most other bird groups, raptors exhibit an often marked sexual size dimorphism, with females up to twice as large as males.

Raptors are generally solitary nesters that construct nests in a variety of habitats including trees, cliffs, natural cavities and sometimes on the ground. Raptors are generally monogamous, with both sexes providing prolonged care for their altricial chicks which do not achieve sexual maturity for 1-3 years.

"BRIDGE" SPECIES

Several bird groups without particularly strong ties to wetland habitats, but with a high tolerance for human-altered habitats, have also been known to become infected fatally from H5N1 (Table 2.1). Prevalent among these are several species of songbirds or perching birds (Passeriformes) such as crows (Corvidae family; Figure 2.16), sparrows (Passeridae family; Figure 2.17), mynas (Sturnidae family; Figure 2.18) and the ubiquitous feral pigeon *(Columba livia)* of the Columbiformes order. Corvids, sparrows and pigeons have broad and diverse habitat preferences, but all are familiar birds that have adapted to exploit anthro-

FIGURE 2.16
Large-billed Crow (*Corvus macrorhynchos*), Corvidae family, Passeriformes order

CREDIT: CLEMENT FRANCIS

FIGURE 2.17
House Sparrow (*Passer domesticus*), Passeridae family (Passeriformes order)

CREDIT: CLEMENT FRANCIS

FIGURE 2.18

Indian Myna (*Acridotheres tristis*), Sturnidae family (Passeriformes order)

CREDIT: CLEMENT FRANCIS

pogenic food sources. Their close association with humans often results in close contact with domestic poultry, especially at open poultry farms where food is readily available. Thus, these species may serve as links between wild birds in natural habitats and domestic poultry, acting as a "bridge" in the transmission of AI viruses from poultry to wildlife or vice versa.

Potential "bridge" species warrant specific surveillance and monitoring efforts at HPAI poultry outbreaks and wildlife mortality events to determine their potential for contracting the disease and possible role in transmitting the virus to or from wild habitats.

MIGRATORY BIRDS AND SPREAD OF THE H5N1 VIRUS

Many bird species travel long distances between their breeding grounds and non-breeding areas. Waterfowl are perhaps the most familiar of these seasonal migrants, but for many northern hemisphere breeding bird species, including shorebirds, songbirds, raptors and many others, at least a portion of, if not the entire population makes seasonal migrations. As natural reservoirs or known hosts for AI viruses, the movements of these species can play an important role in the maintenance and spread of LPAI viruses and may also have a role in the spread of the H5N1 virus.

Migration between breeding and non-breeding (wintering) grounds is a well-documented phenomenon that enables migratory species to exploit seasonally abundant food supplies in habitats that are highly productive during the breeding season, but less productive, frozen or barren during other times of the year. The extent of migratory movements can vary greatly both among and within species. In fact, certain segments of a population may stay in a hospitable area all year as permanent "residents" if conditions permit.

Some species like shorebirds, have very long trans-equatorial annual migrations; they breed in high latitudes of the arctic during the northern summer, then travel to more hospitable middle or southern latitudes as far south as South America, South Africa and Australasia in the northern autumn and winter. Migratory routes of birds are grouped together as "flyways" (Figure 2.19) to assist international management and conservation efforts. A flyway can be defined as "the entire range of a migratory bird species (or groups or related species or distinct populations of a single species) through which it moves on an annual basis from the breeding grounds to the non-breeding areas, including intermediate resting and feeding places as well as the area within which the birds migrate" (*see* Boere and Stroud 2006 for further explanation).

Other groups, such as the northern hemisphere ducks that breed at the higher latitudes, may migrate only as far south as the equator; for example, the Northern Pintail (*Anas acuta*), a common and widespread duck which breeds in the northern areas of Europe and Asia and across most of Canada, Alaska and the mid-western United States (Figure 2.20), migrates south to East, South and Southeast Asia, West and East Africa and in North America southward to northern South America.

Some species may use different flyway routes for their mainly southward (northern autumn) and northward (northern spring) migrations, and different populations of the same species may use distinct flyways to arrive at separate non-breeding areas.

Northern hemisphere stereotypes regarding migration in waterfowl and many other waterbird species do not apply to southern hemisphere species. South African and Australian waterfowl tend to be nomadic, their movements dictated by available food supplies and rainfall, rather than truly migratory. However, a few southern hemisphere species regularly migrate north from Australian breeding grounds to Southeast Asia.

While the role of some migratory species in the propagation and spread of strains of LPAI has been long established, their role in the spread of the H5N1 HPAI virus is less clear. During the early H5N1 HPAI outbreaks in domestic poultry in Southeast Asia in 2003/04, there was no strong evidence that wild birds could become infected, then move long distances and shed the virus as they moved. During this period, the spread of the virus through domestic poultry, including the domesticated Mallard Duck *(A. platyrhynchos)*, was mostly attributed to movement of animals through trade, and most cases of H5N1 in wild birds coincided with nearby poultry outbreaks. Wet markets and trade involving caged wild birds are mechanisms for disease spread over short, medium or long distances. Raptors and passerines are popular species commonly trafficked in the international bird market (both legal and illicit). In fact, in 2004, raptors smuggled into Belgium were the first H5N1 HPAI infected birds detected in Europe.

However, the situation changed when the H5N1 AI virus spread into western Asia and Europe in 2005/06. Small localised wildlife cases and outbreaks were recorded in several countries where stringent poultry biosecurity measures were in place. Likely because of the biosecurity and hygienic measures, nominal spill over of the virus into commercial poultry operations occurred. The discoveries of sick, moribund and dead migratory birds infected with the H5N1 AI virus in scattered locations across western Europe suggested incursion of the disease via wildlife movements, hypothesised as abnormal local movements in response to severe cold weather. Studies reporting the virus in apparently healthy migratory birds

FIGURE 2.19

General "flyways" used by migratory shorebird species that move between northern summer breeding grounds and wintering areas and connect the northern and southern hemispheres

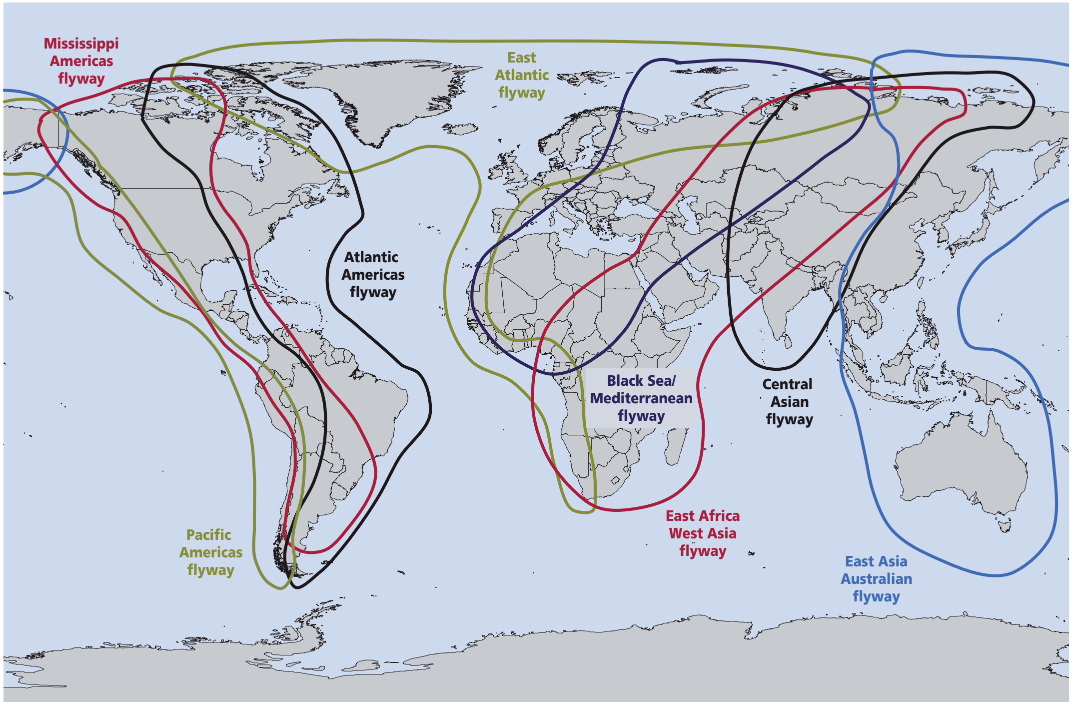
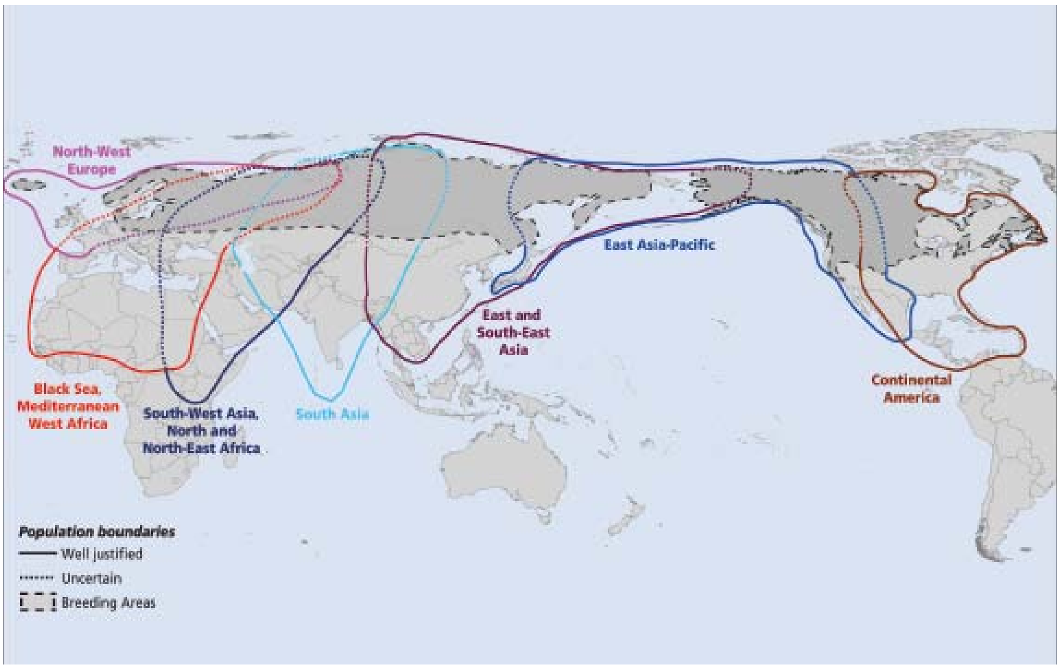


FIGURE 2.20

Major flyways of the Northern Pintail (*Anas acuta*) duck



are limited, but do suggest the possibility that wildlife movements may serve as a mechanism in the introduction of the virus, with husbandry and poultry commercialisation more responsible for disease spread. However, it has yet to be demonstrated that infected wild birds made long distance movements concurrently shedding the H5N1 virus during long distance movements. More information is needed to understand the role of migratory birds in this context.

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Chapter 3
Wild bird capture techniques

For millennia, humans have relied on wild birds as a source of food, clothing, and social and religious manifestations of culture, art and sport. While their mobility, wariness of humans and diverse habitats often make live capture of wild birds a challenge, a multitude of trapping techniques and devices have been developed over the centuries. Most of the live capture techniques utilise bait, decoys, recorded calls or lures to attract birds to trapping sites, but a few active techniques in which the trapper actually pursues the bird have been developed and may be useful in some situations. Thus, there are few, if any, bird species which cannot be captured.

Capture techniques specifically designed for wild birds such as waterfowl, shorebirds and other wetland species are of primary interest because current knowledge indicates these are the species that serve as the primary reservoirs of low pathogenic AI viruses. However, practical techniques for capturing passerines, raptors and other bird groups vulnerable to the virus are also important. Extensive reviews of capture techniques for a wide range of bird groups can be found in Bub (1991), McClure (1984) and Schemnitz (2005).

The health and well-being of the birds should be the primary concern during all phases of capture. The following principles should be adhered to ensure birds are captured correctly, safely and with minimum disturbance:

- Wild bird capture is an activity that is strictly controlled in most countries; those engaged in capture activities should always be aware of and comply with local and national laws regarding these activities and obtain all the required local, state, provincial and federal permits well in advance.
- Capture techniques and equipment which expose birds to foreseeable risk of injury should be avoided at all costs.
- Those conducting capture efforts should take all necessary precautions to avoid disturbing nesting birds at breeding sites or enhancing vulnerability to nest site predation following human intrusion.
- Monitor weather forecasts prior to conducting capture efforts to ensure birds are not captured during extreme climatic conditions when they may be at increased risk of hypothermia or hyperthermia.
- Always have a sufficient number of experienced personnel (at least four) available before undertaking any capture operation.
- Check operative traps and nets at appropriate time intervals; birds should not remain in traps or nets any longer than is necessary. This is capture technique and weather dependent, and could be as short as every 15 minutes to twice a day.
- Close or dismantle traps and nets that are inoperative and not checked regularly.

CORRAL TRAPS (ROUND-UPS)

The two- to three-week period after breeding when waterfowl, grebes and coots simultaneously moult all their flight feathers is an opportune time for capturing these species. During this flightless period, birds can often be "rounded up" by herding or driving them between barriers which funnel the birds into capture pens constructed near the moulting grounds.

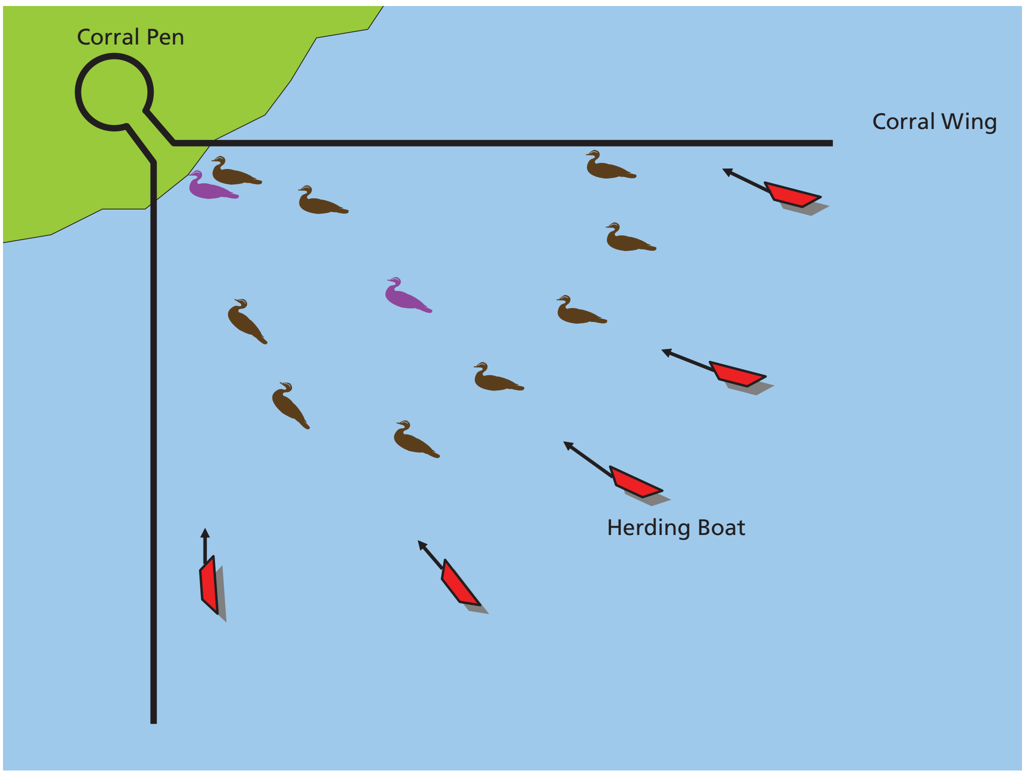
The basic drive trap design used by the Wildfowl & Wetlands Trust (WWT) consists of a capture pen or "corral" with two long barriers or "wings" extending some distance from the mouth of the corral (Figure 3.1). Birds may be on the water or on land when they are initially driven between the corral wings by the capture team, but if the wings extend out into the water they should eventually funnel the birds into a corral constructed on flat dry ground.

Corral trap construction

Specific features of the trap will depend on the size of the species to be captured. In the following instructions, a range of specifications for the corral pen(s) and wings are given both for smaller birds (ducks, grebes and coots) and larger waterfowl (geese and swans).

- The corral pen and wings should be marked out with 1.5-2.0 m wooden posts or metal fence stakes firmly driven into the ground and spaced approximately 1 m apart; a round corral is usually best, but the shape may differ if conditions warrant.

FIGURE 3.1
Basic set-up of a corral trap



- The diameter of the corral is dependent on the number of birds to be caught and may vary from less than 2 m up to 30 m, or more (Figure 3.2).
- Side pens may also be constructed to ensure that no single pen contains too many individual birds; this is particularly important for the welfare of birds during the capture process.
- The corral wings should be erected in straight lines over flat ground or water and should not snag on branches, brambles or other vegetation because this may damage the net and cause birds to become entangled.
- The width of the wings at the entrance to the corral can be as narrow as 0.5-1.0 m for small numbers of ducks or up to 50 m when capturing large numbers of geese or swans.
- Attach black nylon netting or other appropriate material to the corral and wing posts; use a material that will not injure birds when they run up against the corral pen or wing walls.
- The nylon netting (or other wall material) should be stapled at the top, middle and bottom of wooden posts; metal stakes can be run through the top, middle and bottom of the netting.
- When attaching the netting to the posts make sure the net is taut and the bottom 0.1 m of the net curves toward the inside of the corral to prevent birds escaping beneath the net during the 'drive'.
- The height of the corral should be 1.0 m for ducks and 1.5-2.0 m for geese and swans, but corral wings can be 1.0 m high for all three groups.

FIGURE 3.2
Corral trap pen

CREDIT: PAUL SLOTA

- Hessian (or any other fabric) should be tied to the bottom 0.5 -1.0 m of the corral to prevent birds catching their claws on the netting.
- If the ground is wet or cold, clean straw should be spread on the ground in the corral to a depth of 3-15 cm.

It should be noted that the details of corral construction described above apply to instances when the trap can be erected before the attempted drive. In some cases (e.g. on the tundra), it will often be impossible to predict where the final capture will occur and the corral will need to be constructed after the birds have been herded and surrounded. In such instances, less precise corral specifications are acceptable, from both a bird welfare and an effectiveness perspective.

Herding birds into a corral trap

Depending on the location of the capture efforts, birds can be driven between the corral wings and 'funnelled' into the corral either by rowing in small boats, wading through shallow water or walking behind them. General instructions for 'herding' birds into the corral are described below:

- The number of "herders" required will depend on the number of birds to be caught, the size of the corral enclosure and habitat. A minimum of four herders will be required.
- The herders should form a line with the birds between themselves and the funnel shaped corral opening (Figure 3.3); in cases where the final capture site is uncertain, the herders should form a circle and drive birds towards a central point, then construct the corral near the herded birds and move them into it.

FIGURE 3.3
Technique for herding flightless waterfowl

CREDIT: RUTH CROMIE

- Using coordinated movements, the herders should then drive the birds as a group toward the corral opening (or central point).
- Birds should be herded at a steady pace so that they do not panic and scatter in all directions or charge at speed into the corral causing the walls of the pen to collapse.
- Hand nets or poles can be used to direct the birds' movements and catch any birds which try to escape through the line (although it is better to let a single bird escape rather than break the line and risk losing the entire flock); waving a hand-net will persuade a bird to move away, while pointing the net to the right or to the left, may persuade birds to move in the desired direction.
- Once all birds have entered the corral, the mouth of the corral should be carefully closed (making sure no birds are caught in the door) and the designated bird extractor should position him/herself inside the pen and in front of the exit.

BAITED TRAPS

Drive traps for waterfowl can only be used near moulting areas when birds undergo their annual wing moult, so other capture techniques must be employed outside the flightless moulting period. Baited traps are an effective technique for capturing a wide array of wild birds, including waterfowl and many ground-feeding terrestrial species. However, because hunting often occurs where waterfowl and other game birds aggregate, it is advisable to locate bait traps within "sanctuaries" (when practical) to avoid attracting birds to areas where high levels of lead shot may be ingested.

Many of the baited trap designs utilise self-contained wire cages or enclosures supported by posts that are baited with appropriate food stuffs for the target species. For most waterfowl, typical baits include wheat, corn kernels, whole rice or other grains. These traps may go by several different names (e.g., cloverleaf traps, drift traps), but two designs particularly useful for waterfowl are baited funnel traps and baited dive-in traps.

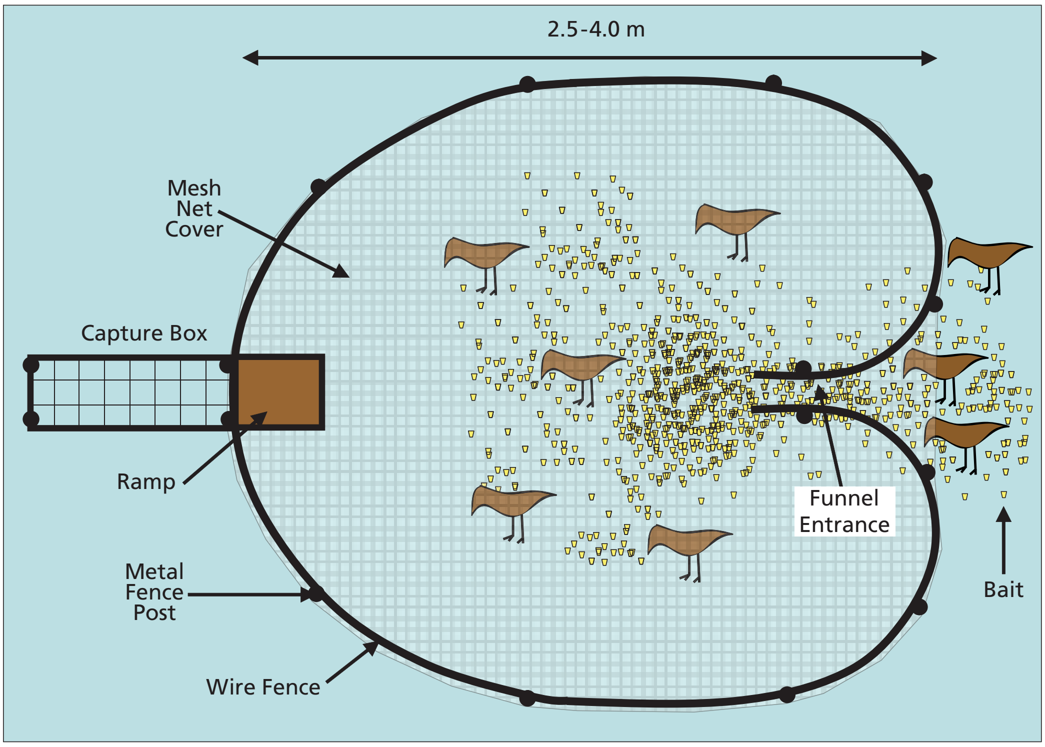
Funnel trap

A baited funnel trap can be deployed or constructed on land or in water shallow enough for foraging by dabbling ducks, coots and waders, usually <25-30 cm, although the design will function in deeper water provided those handling the birds can reach the trap site in waders or a boat. The basic funnel trap design consists of a wire cage or enclosure with one or more funnel shaped entrances which the birds can enter, but have difficulty exiting (Figure 3.4). Fine mesh netting can be placed over enclosure traps to prevent birds escaping over the wire fence when handlers approach.

Funnel trap construction

- An appropriate trapping site should be selected (preferably a site already frequented by the target species) and bait type (catered for the targeted species) spread over the site for several days before the trap is constructed.
- The funnel trap enclosure should be marked out with 1.5-2.0 m posts firmly driven into the ground or shallow wetland substrate; many different trap sizes and shapes with one to many entrances have been designed (Figures 3.5, 3.6 and 3.7).

FIGURE 3.4
Basic set-up of a baited funnel trap



- The size of the enclosure or trap should be appropriate for the number and size of the target species.
- The funnel entrance(s) should be just wide enough to allow birds to pass through the opening, or push through the opening if the material is slightly flexible; the larger the entrance the greater the likelihood of birds escaping.
- Put the wire enclosure fencing around the post border; use fencing with a lattice pattern that will not allow birds to get stuck while trying to escape.
- Attach the wire fencing to the posts with plastic or soft wire ties, making sure the fence extends all the way down to the ground or substrate; cut and adjust the tie ends so they cannot cut or scratch birds.
- If possible, attach the wire fence to the posts prior to placement at the site as this may facilitate construction of the trap; for some smaller diameter traps, posts may not be necessary at all.
- The nylon netting (or other cover material) should be attached with ties to the top of the wire fencing; if needed a wooden "tent" pole can be placed in the middle of the enclosure to hold up the net cover.
- Create a doorway in the enclosure at the opposite end of the trap from the funnel entrance that allows birds to enter a capture box or net for easy removal from the trap.
- Bait heavily inside the trap but lightly around the funnel entrance to entice birds into the enclosure.

FIGURE 3.5
Wire funnel enclosure for waterfowl in a shallow marsh

CREDIT: DARRELL WHITWORTH

FIGURE 3.6
Wire funnel trap for waterfowl in a shallow lake

CREDIT: DIANN PROSSER

The funnel trap entrance(s) can be easily closed by removing the ties from the posts and securely joining the ends of the wire fence together. In general, traps should be baited and opened in the late evening, checked first thing in the morning, and left wide open (so birds can become accustomed to entering and exiting the trap) for the rest of the day. When checking larger traps, one handler should enter the enclosure through the funnel entrance

FIGURE 3.7
Self-contained wire funnel trap for shorebirds

CREDIT: SCOTT NEWMAN

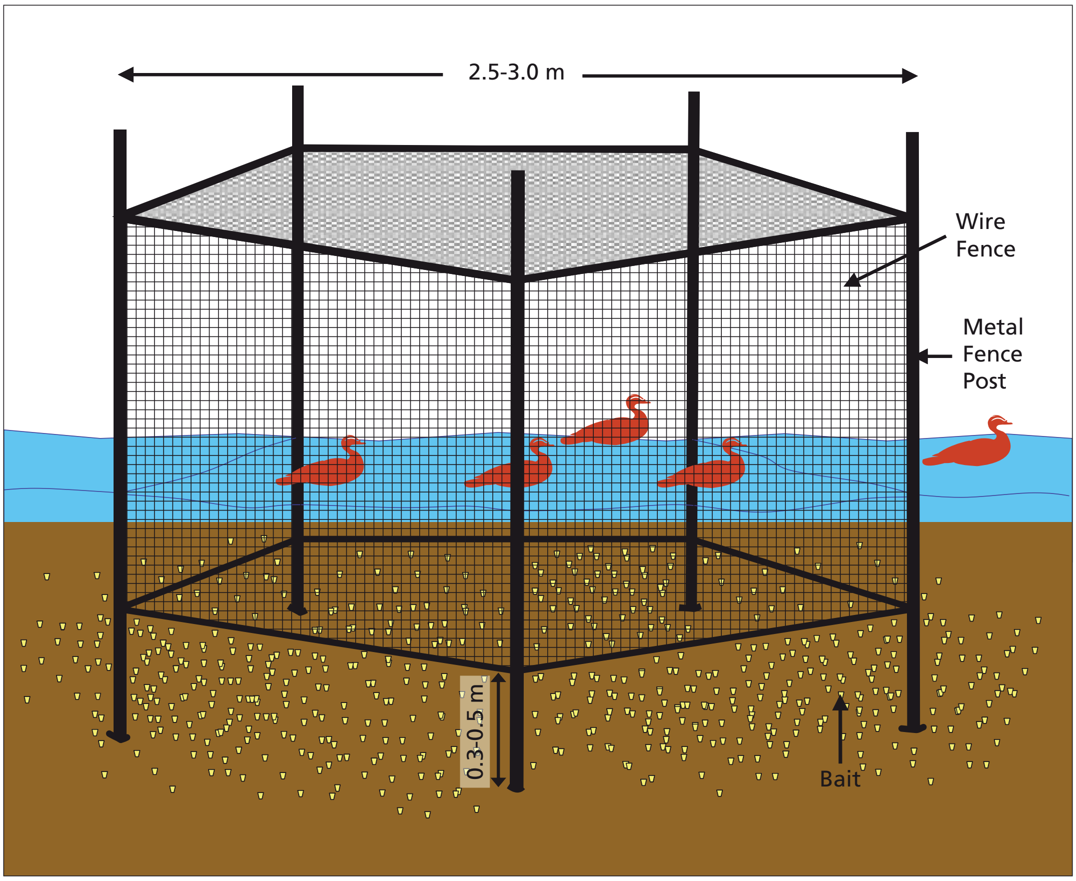
and drive birds through the open doorway into a holding box or net. In smaller traps, birds can be removed by a handler outside the enclosure with a hand-held keep net. Birds can be removed individually and processed at the capture site or transferred to a travel container and transported to a nearby processing site.

Dive-in traps

As the name implies, baited dive-in traps are effective for capturing aquatic diving birds, primarily diving ducks. Dive-in traps can be constructed in relatively shallow water (<1.25 m) habitats that are frequented by diving birds and accessible to handlers in small boats or wearing chest waders. The basic dive-in trap design is similar to the funnel trap enclosure; however, in the case of dive-in traps, the wire fence is raised (0.3-0.5 m) slightly off the wetland bottom allowing the birds to dive under and into the enclosure (Figure 3.8).

Dive-in traps are only effective in wetland habitats, but may be used in permanent water bodies of adequate depth or tidally influenced wetlands. Some familiarity with tide levels will be necessary when deploying traps in tidal wetlands. Dive-in traps may be constructed during low tides when the trap site may be completely exposed, but will need to be checked whenever the tide rises to inundate the site and birds move in to feed. Because diving ducks have some difficulty taking off directly from the water, fine mesh netting placed over traps may not be necessary. If netting is used to cover the trap, it should be removed from unattended traps to avoid drowning birds at high tides.

FIGURE 3.8
Basic set-up of a baited dive-in trap



Dive-in trap construction

Many of the issues involved in the construction of dive-in traps are similar to those for funnel traps:

- An appropriate trapping site should be selected (preferably a site already visited by the target species) and bait spread over the site for several days before the trap is constructed.
- The dive-in trap enclosure should be marked out with 1.5-2.0 m posts firmly driven into wetland substrate; circular dive-in traps are most common (Figure 3.9), but other shapes may be optimal in certain circumstances.
- As for funnel traps, the diameter of the enclosure should be appropriate for the number and size of the target species to be captured.
- Put the wire fencing in place around the fence post border; be sure to use wire fencing with a lattice size that will not allow birds to get stuck while trying to escape.
- Attach the wire fencing to the posts with plastic or soft wire ties, raising the fence approximately 0.3-0.5 m off the substrate all the way around the enclosure; cut and adjust the tie ends so they cannot cut or scratch birds.
- If possible, attach the wire fence to the posts prior to placement at the site as this may facilitate construction of the trap.

FIGURE 3.9
Dive-in trap set up in a tidal wetland

CREDIT: DARRELL WHITWORTH

- Nylon netting (or other cover material) may or may not be needed to prevent birds escaping over the top of the enclosure; if netting is needed it should be attached with ties to the top of the wire fencing and supported in the middle with a "tent" pole.
- Bait heavily inside the trap but lightly around the funnel entrance to entice birds into the enclosure.

Dive-in traps are generally baited in the late evening and checked first thing in the morning, although tidal fluctuations will affect schedules for traps in tidal zones. Birds should be removed by reaching over the enclosure fence with a hand-held net and dipping the birds out of the trap. Holding boxes floated on buoys can be used to transport birds to the shoreline.

CANNON NETS

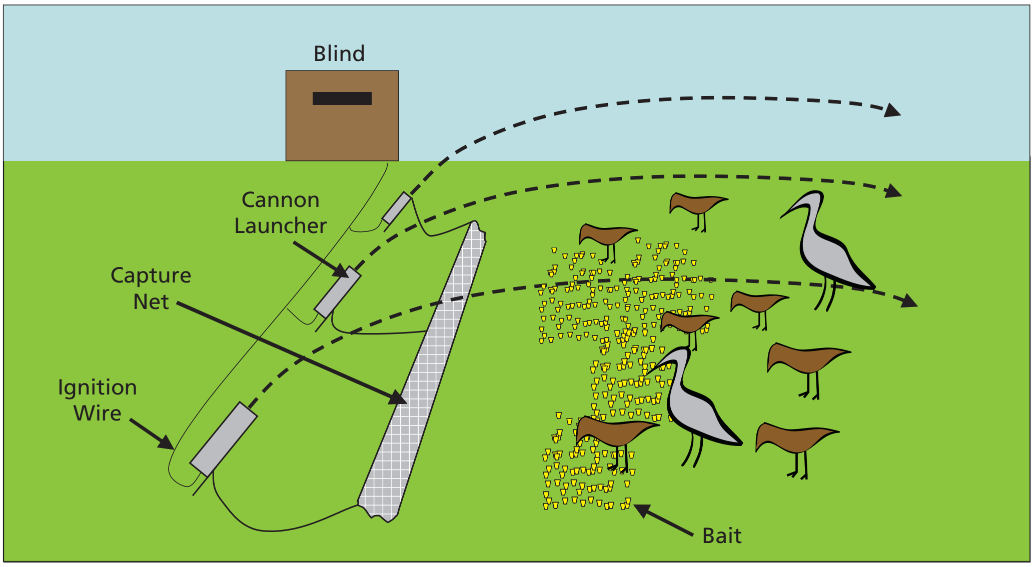
Birds that congregate in large numbers at roosting or feeding sites can be captured with large mesh nets attached to projectiles that are propelled over the roosting or feeding flocks by explosive charges (Figure 3.10). However, as might be expected with high velocity projectiles launched near dense bird congregations, there is a significant risk of injury or mortality to wild birds and humans if this technique is used by inexperienced operators. Because cannon-netting requires a high level of technical expertise, it should not be attempted without assistance from experienced personnel. Details of cannon-netting procedures are best obtained from experienced practitioners and specific training manuals (e.g. Appleton, undated), however, some general guidelines in the use and application of the technique are provided.

Cannon nets have been used to capture many species of waterfowl, wading birds such as herons and egrets, upland game birds, gulls and shorebirds. Some scouting is usually

FIGURE 3.10
Launching the cannon-net

CREDIT: ALYN WALSH

FIGURE 3.11
Basic set-up at a cannon-netting site



necessary to identify suitable capture sites where predictable roosting or feeding flocks congregate in open and dry upland or very shallow (a few centimetres deep at most) wetland habitats. Bait and decoys can also be used to attract waterfowl and other target species to suitable capture sites.

The cannon net setup (Figure 3.11) is usually prepared and the capture team in place (out of view in a blind near the capture site) well in advance of the expected arrival of the birds; if birds are to be lured to capture areas with bait or decoys, the site should be prepared several days to a week before the capture effort. Specially designed boxes or platforms for storing, transporting and launching pre-folded nets can greatly facilitate preparation of the cannon netting site.

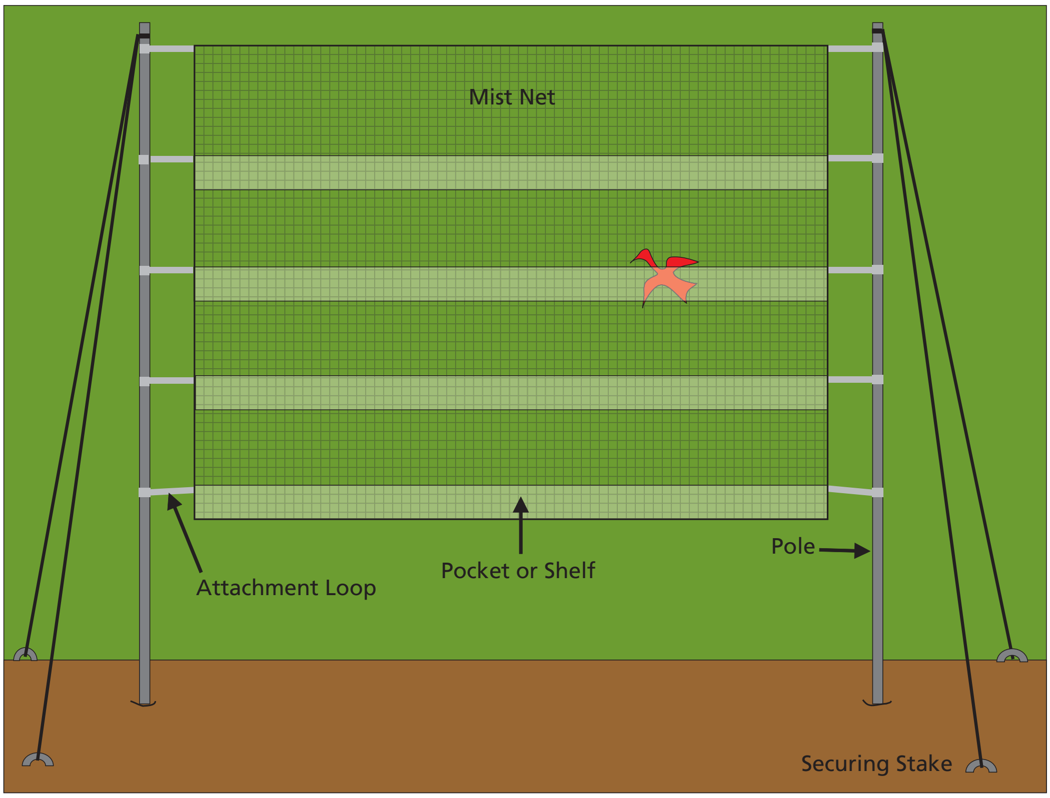
MIST-NETTING

Mist-netting is perhaps the most versatile and widely used method for catching small to medium-sized wild birds such as passerines and shorebirds. The basic principle of mist-netting is simple; an inconspicuous mesh net is erected vertically on poles and deployed in areas of high activity to intercept birds as they go about their normal daily routines (Figure 3.12).

Mist nets and mounting systems

Mist nets are available in many different measures, materials, mesh sizes, colours and strand thickness. Dark-coloured nylon nets are most commonly used, but the optimal features for a mist net will depend on the target species and habitat characteristics at the netting site. Lighter coloured nets may be available from some vendors and should be considered if they better blend into the habitat at the netting site. Short nets are more practical in heavy cover, while longer nets can be used in more open habitats. Optimal mesh size is directly

FIGURE 3.12
Basic set-up of a mist-net



related to the size of the target species; smaller mesh for smaller species and larger mesh for larger species. Nets with finer strands are less visible but more fragile than nets with coarser strands, although the more durable coarse nets may be adequate for species netted at night or in other low light conditions.

When properly positioned, mist nets are inconspicuous even to the birds' keen vision, and unsuspecting birds may strike the net at considerable speed. However, the mist net is designed to "give" and gently decelerate the bird when it impacts the net. Almost all mist nets have a series of 3-4 shelves or pockets running horizontally along the length of the net into which the bird drops when it strikes the net.

The mounting poles are another important part of the mist net package and should be chosen carefully. Poles should be light-weight, portable, strong and drably coloured to blend in with the habitat at the netting site. The pole surface should be smooth enough to allow the net attachment loops to slide cleanly on and off the pole. Sectioned poles are convenient for storage and transport.

Mist-netting sites

Choice of an appropriate mist-netting site is vital for capture success. Obviously, mist net sites should be located in areas the target species are known to frequent, preferably in large numbers. Thus, some knowledge of the target species' daily movements and activity patterns is essential before deploying nets. Identifying the target species' nesting areas, feeding areas, roosting sites and the preferred flight paths between them is an important step in ensuring capture success.

Fine mesh mist nets are relatively inconspicuous when deployed, but choice of a netting site that helps conceal the net is advisable. Avoid erecting mist nets on sites where the outline of the net is clearly revealed against a monotonous background such as the sky, open water or uniformly coloured fields. Shaded sites are always preferable to sunlit areas. A clearing in a vegetated area with a dark but variegated background is an optimal netting site.

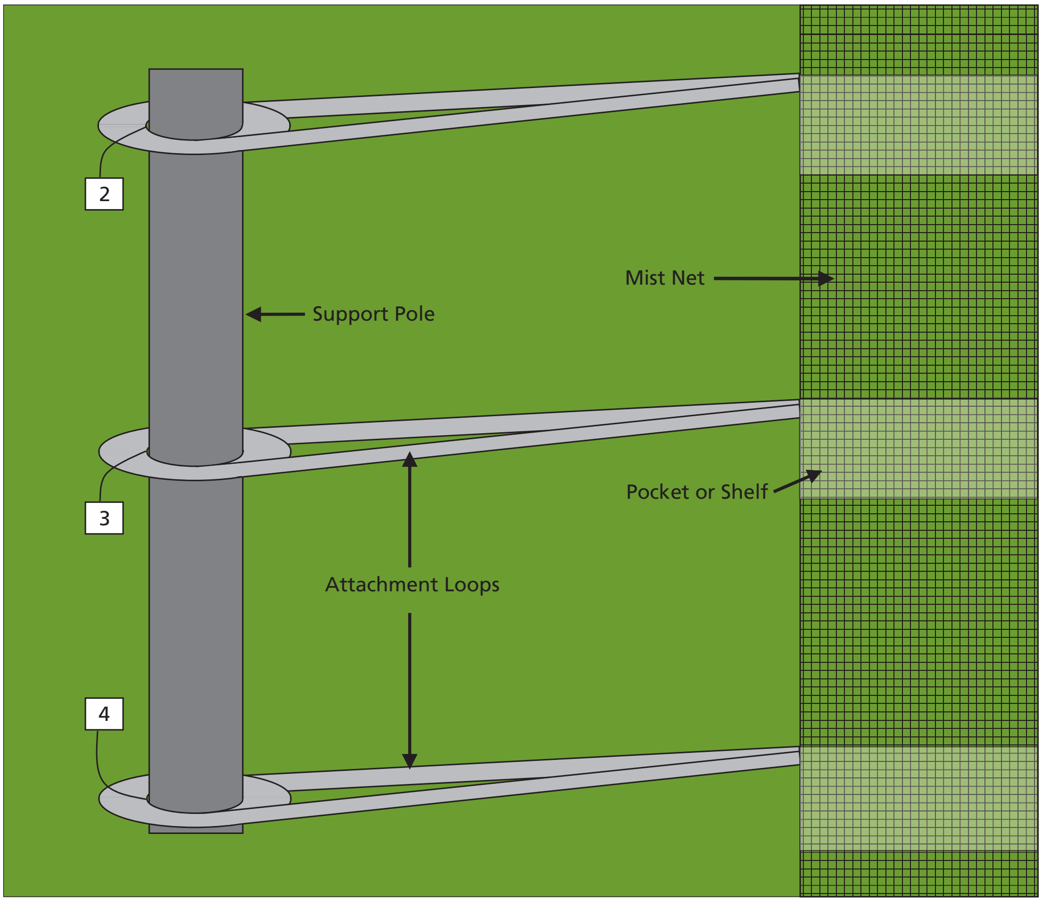
Many species are most active at dawn and dusk, so these are prime mist-netting periods. Fortunately, the weak early morning and late evening light comes from oblique angles and throws long shadows that help to conceal mist nets. Great care is needed when setting nets for waterbirds, which may be present in large numbers, because the potential for a large catch exists. It is essential that the number of nets be limited to that which the field crew can effectively deal with should a large number of birds be caught.

Erecting mist nets

Having chosen a suitable mist-netting site, the net is erected as follows:

- Find the pole attachment loops at one end of the net and number them from top to bottom; be sure to keep the mist net taut and off the ground to avoid catching on rocks and vegetation.
- Form a loop within each pole attachment loop and slide them all in order over one of the poles (Figure 3.13).
- Push the pointed end of the support pole into the ground; do not pound with a mallet because this will damage the pole.

FIGURE 3.13
Correct method of placing the mist net loops on the support pole



Tags 2-4 indicate numbering of pole attachments loops

- Take the second pole and repeat the first two steps with the other end of the mist net.
- Pull the net taut and push the second pole into the ground.
- Use a mallet to drive the four securing stakes into the ground then tie off each of the securing lines so the mist net is held firmly in place; securing lines can be tied to fixed objects (e.g., rocks or bushes) if the ground is rocky and stakes cannot be used.
- The erect mist net should be taut enough that the net does not sag excessively when birds are in the net (this is particularly important when trapping over dense vegetation and water), but not so taut that birds bounce out when they strike the net.
- When mist-netting over water it is advisable to use an object with a weight similar to that of the species likely to be caught to test the extent of sag in the net.
- The tautness of the mist net can be controlled by adjusting tension on the securing lines.
- Clear away any twigs or branches that might catch in the fine mesh and interfere with net function.

The simplicity and versatility of the basic mist net design has allowed modifications for capturing an endless variety of birds. Some of the more novel designs include mist nets deployed on pulley systems and suspended high in the forest canopy, floating nets mounted on tethered buoys or boats, and submerged nets strung across narrow channels. Deploying multiple nets in formation (e.g., "L" or "V" shaped arrays) may increase capture rates.

Using mist nets

- Remain silent and concealed when monitoring mist nets.
- Approach nets quietly to avoid panicking captured birds which are likely to become seriously entangled while struggling to escape.
- An open mist net should never be left unattended for more than a few minutes, under normal conditions 15-20 minutes maximum; if nets cannot be checked frequently close them by sliding the loops together, furling the net and securing it with twist ties.
- Never use mist nets in the rain. Birds netted in rainy conditions are vulnerable to hypothermia.
- Mist-netting in windy conditions is problematic because the net becomes more visible as it blows in the wind; wind also allows birds striking the net to avoid capture as the pocket does not form properly in the billowing net and may also cause injuries (e.g. muscle strains) to captured birds.
- Keep an eye out for avian and terrestrial predators which may be attracted to mist net sites by distressed or struggling birds.
- Maintain mist nets in good condition and properly dispose of used and damaged nets; old nets should preferably be incinerated.
- Recorded calls and decoys have been used to attract birds to netting sites.

Extracting birds from a mist net

Extracting entangled birds from a mist net (Figure 3.14) can be a challenge; however, with patience and experience, even seemingly inextricable birds can be removed without injury or resorting to cutting the mist net. Each entangled bird may pose a different set of problems, but the following guidelines will usually facilitate quicker removal:

- Regularly monitor mist nets and try to remove a bird as soon as possible after it hits the net; the more time the bird spends struggling to escape, the more tangled it will become.
- Determine from which side the bird entered the net; this is accomplished by finding the bird's bare belly.
- Immediately immobilise the bird, especially the wings and feet, to prevent struggling while extracting the bird from the net; this is done by wrapping your index and middle finger around either side of the birds neck while cradling the body with the palm of your hand and other fingers (i.e. small passerines), taking care not to squeeze the bird too tightly. Large birds may take two people.
- In almost all cases, the feet should be untangled first then immobilised to prevent the bird becoming entangled again; always hold the bird by the upper leg (tibia) and never by the lower part of the leg (tarsus).

FIGURE 3.14
Reed Warbler (*Acrocephalus scirpaceus*) captured in the pocket of a mist net

CREDIT: PIETER VAN EIJK

- Lift the bird out of the pocket and gently pull it away from the net; often the net will simply fall away, but if not inspect the bird to determine how best to proceed.
- In most cases, it is easier to free the tail and one wing, then re-examine the situation to determine if the head or other wing should be freed next.
- If it is clear a bird is hopelessly entangled, do not hesitate to begin cutting individual strands of the net to free it; usually cutting one well chosen strand is enough to free a bird.
- In the worst cases, the net wraps tightly around the bird's closed wing or tongue; in such instances it is best to request assistance from experienced handlers and snip individual strands until the bird is released.
- Take care to avoid the bird pecking or scratching you with its claws while you extract it from the net. Some birds like parakeets *(Psittacula spp)*, shrikes *(Lanius spp)*, herons *(Ardea spp)*, falcons *(Falco spp)* and hawks *(Accipiter spp)* need to be handled with special care as they will often try to peck or scratch.

MISCELLANEOUS CAPTURE METHODS

In the following section, we briefly describe capture methods which have proven useful for bird groups that may be difficult or impossible to capture with the above techniques. In general, these miscellaneous capture techniques have much lower capture rates (number of birds captured per unit time) than the previously described methods; in fact, in most cases the technique is used to trap individual birds rather than large groups. However, these capture methods may be the only effective means of conducting active disease surveillance for some species known to contract AI viruses. More detailed descriptions of these techniques can be found in the references cited at the end of this chapter.

Raptors require special capture and trapping techniques specifically designed for these species. **Bal-chatri traps** consist of small wire cages of various sizes and shapes containing live bait (a rodent or small bird) and covered with numerous small nooses or slip knots tied from fine fishing line. Raptors attacking the enclosed prey are ensnared when the feet contact the nooses.

Bal-chatri traps are portable and can be quickly deployed when raptors are sighted in the vicinity, but must be weighted or tethered to prevent larger birds from flying off with the trap. The size and shape of the wire cage and strength of fishing line employed depend on the size of the raptor targeted. Nooses should be tied in 3-5 cm loops. Do not hesitate to cut nooses when extracting raptors from bal-chatri traps because the nooses can be easily repaired or replaced.

Several variations of the bal-chatri trap that utilise **noose carpets** have been developed, including: 1) a noose carpet tethered on top of an owl decoy to capture those passerine and raptor species that mob intruding owls; 2) noose carpets placed on baited feeding stations to capture ground-feeding species; 3) noose carpets placed near the entrance of a nest.

Dho-gaza nets exploit the tendency of raptors and many other species to mob intruding owls. A fine mesh net suspended above an owl decoy is effective for capturing these species as they dive on the perceived threat. The net should be tautly suspended above the decoy, but very lightly held in place at the four corners with clothes pins or similar sensitive triggers that release when the attacking bird strikes, allowing the net to envelop the attacking bird.

A dho-gaza net is most effective when placed near a raptor nest where it can be suspended from poles or surrounding vegetation. Decoys should be rendered as realistic as possible (mounted specimens are optimal) by tethering them in a manner (e.g. mounted on a spring) that allows some movement. If plastic decoys are used, attaching a few feathers may help attract the target birds' attention.

Numerous variations of the basic **drop trap** have been developed. The simplest models use bait or other lures to attract birds to an area where a manual or automatic trigger drops a cage, door or net. As with other baited traps, trapping areas should be baited for several days (at least) before traps are deployed and set. The variety of species that can be captured with drop traps is limited only by the ingenuity and patience of the trapper.

Night-lighting techniques utilise bright lights to attract or disorient nocturnally active species which can be captured passively in fixed nets or actively pursued with hand-held nets. A variety of aquatic species including waterfowl, alcids, shearwaters and cormorants have been captured by night-lighting from boats.

Many types of **nest trapping** methods have been developed, but the capture of breeding birds from nest sites is generally discouraged as disturbance at nest sites and colonies is likely to cause abandonment or nest failure.

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Chapter 4
Bird handling and ringing techniques

Disease surveillance and other studies related to the H5N1 AI virus will inevitably involve the capture and handling of large numbers of wild birds. Depending on the objectives of the study, birds may be subjected to a variety of research techniques, including ringing (or banding), biometric measurements, sample collection for laboratory diagnosis (see *Chapter 5*), and radio-tagging or other marking techniques (see *Chapters 6* and *7*). All these techniques require the handling and restraint of wild birds, thus instruction in safe and effective handling techniques is essential.

The health and well-being of captured birds should be the primary concern during all phases of handling. Proper handing techniques will minimise stress and maximise the chances that the bird can returns to its pre-capture state with a minimum alterations in behaviour; a goal which ensures the welfare of the bird and high data quality. Several simple guidelines can be taken to ensure that birds are handled safely and with minimum disturbance:

- Always be aware of and comply with local and national laws regarding handling and ringing activities. Obtain all the required permits well in advance.
- Use approved restraint techniques and follow the handling guidelines described in this Manual; consult with experienced wildlife veterinarians and biologists if modifications to the restraining and handling techniques are required.
- Always have at least one other person on hand, one of them with bird-handling experience, during handling and ringing procedures. Even if the bird can be restrained and ringed by one person, a second person for data recording and other essential tasks will speed the process and result in less time in captivity and therefore, less stress.
- Maintain a calm and quiet environment at the bird handling site.
- Conditions at the bird processing site should be appropriate for the environmental conditions; in cold, wet conditions, birds should be kept warm and dry, while in hot, sunny conditions, birds should be processed in a sheltered, shaded and cool site.
- Processing stations should be located as near as possible to the capture site to avoid holding birds for transportation any longer than is absolutely necessary.
- AI disease surveillance involves the handling of bird species known or suspected of being H5N1 virus carriers; thus appropriate precautions should be taken to avoid the mechanical transmission of pathogens between birds and sampling sites (consult FAO 2006).
- The use of personal protective equipment (PPE) appropriate for the level of risk is strongly advised even when clinical signs of disease are not evident in birds in the region (consult FAO 2006).

BIRD HANDLING AND RESTRAINT

The variety of birds likely to be captured and handled during disease surveillance and other AI related studies is so broad that no single handling technique is adequate for all birds. However, some general handling practices are applicable regardless of the species or size of the bird.

- Safe handling is achieved by controlling the bird's head, feet, legs and wings; however, you should never move these appendages into awkward or unnatural positions that may injure the bird.
- Use the proper amount of restraint; birds need to be grasped firmly enough to prevent them from struggling, but gently enough to avoid putting too much pressure on the bird's body and restricting its respiration.
- Protecting handlers from injury is also important; be sure to securely restrain the head and talons of those bird species (e.g. raptors or herons) that may lunge at the handler's face and eyes; handlers should wear appropriate protective clothing for the task, including goggles or eye protection, long-sleeved shirts and leather gloves, where necessary.
- Do not hesitate to request assistance if a bird struggles excessively or is otherwise difficult to handle; if the bird is overly agitated it is likely to overheat or undergo exertional muscular damage (myopathy). Consider putting it in a darkened holding container/crate to calm down; in extreme cases, the bird should be released.
- Never grab or seize at a bird (especially the wings, legs or tail) if it escapes from the hand; if indoors, corner the bird and capture it under a net or towel before regaining a hold, if outdoors it is better to let the bird escape rather than risk injury.
- Lightly wrapping the bird in a clean, dry cloth towel can be an effective form of restraint; alternatively, gently covering the bird's head with a breathable cloth towel can eliminate stressful visual stimuli often calming the bird.
- Consider other physical and chemical restraining aides; hoods, restraining jackets or even anaesthesia may be warranted, particularly when working with large or aggressive species.
- Be on the lookout for signs of distress (gasping, laboured, or open-mouthed breathing) or physical injury to the bird.

Proper handling and restraining techniques improve quickly as the handler gains experience with a variety of birds. Inexperienced handlers need to be advised and supervised in proper handling techniques because they may tend to exert too much pressure when restraining the bird out of fear it might escape. Applying too much pressure can restrict the bird's breathing or heart function. Gasping is an obvious sign that too much pressure is being used and the holder should immediately loosen the grip. Other inexperienced handlers may be afraid of harming a bird and not grasp it firmly enough, when in fact, birds are more likely to be injured while struggling to escape from light restraint.

Some of the most practical handling and restraining techniques for birds of various sizes are described below.

Small birds

In general, small birds such as passerines and many shorebirds can be efficiently handled by one person, using one hand to restrain the bird while the other hand is free to perform relatively simple tasks such as ringing or biometric measures. However, to perform delicate tasks such as cloacal/tracheal swabbing, blood sampling and attachment of telemetric or data-logging devices requires two people; one to restrain the bird, the second to perform the procedures.

The most useful one-handed restraining technique is known as the **ringer's hold** (Figure 4.1):

- Use the non-dominant hand to grasp the bird (e.g. if you are right-handed, hold the bird in your left hand), leaving the dominant hand and free for ringing, biometric measures and other tasks.
- Firmly but gently grasp the bird with its back and closed wings against the palm of the hand.
- Hold the head between the index and middle finger while the ring and little finger are closed around the body of the bird.
- For ringing, the leg can be held between the thumb and either the index, middle or ring finger, whichever is most comfortable for the bird and the handler.
- If the handling protocol involves manipulating the wing to perform blood sampling, moult scores or chord measurements, the wing can be held open by gripping the upper wing (humerus) between the thumb and tip of the index finger.

FIGURE 4.1
The ringer's hold for handling small birds

Note: Most manipulations of the wing should be performed using the ringer's hold, by holding the humerus, which is closer to the body near the shoulder joint. In this image, the ringer is holding the base of the primary feather to assess moult of the primary wing feathers by extending the wing.

The **reverse ringer's hold** is similar to the ringer's hold and may be a more comfortable method for grasping the leg during ringing, although it is not convenient for taking biometric measurements:

- Firmly but gently grasp the bird with its back and closed wings against the palm of the hand, but with the head facing downward toward the handler's wrist.
- Hold the tail between thumb and index finger.
- Wrap the other fingers gently but firmly across the bird's chest.
- For ringing, the leg can be held between the thumb and the index finger.

Medium-sized birds

In most cases, medium-sized birds should be restrained by one handler using two hands, while another person conducts ringing and other procedures. Two-handed restraining techniques approved by the WWT are particularly suited to waterfowl (ducks and small geese) and species such as gulls, grebes, coots, cormorants and larger shorebirds.

The **two-handed grip** (Figure 4.2) is the most natural two-handed restraining hold:

- Firmly but gently grasp the bird with the hands placed either side of the bird so that the wings are held against the bird's body by the handler's palms.
- The thumbs should be placed on the bird's backbone at the level of the scapulae or shoulder and the fingers curled around the breast and abdomen, with the legs tucked up against the underside of the bird.
- The bird's body can be held horizontally (with the head facing away from the handler) or tilted vertically (head up) with the legs facing forward for ringing.

FIGURE 4.2
The two-handed grip for handling medium-sized birds

CREDIT: NIGEL JARRETT

FIGURE 4.3
The reverse two-handed grip for handling medium-sized birds

CREDIT: REBECCA LEE

The **reverse two-handed grip** (Figure 4.3) can be used to restrain a bird belly-up in the handler's lap or on a table while delicate procedures such as blood sampling and swabbing are conducted; however, birds should not be held belly-up for long periods as this may interfere with proper respiration:

- With the bird belly-up, firmly but gently grasp the bird with the hands placed either side of the bird so that the wings are held between the bird's body and the palms of the handler.
- The thumbs should be placed on the bird's breast near the sternum and the fingers curled around the back; if need be, the index and middle fingers can be used to hold the bird's legs.
- The bird can be restrained horizontally on the table or with the head tilted slightly upward for ringing and other procedures.

Both of these grips can be modified if the handling protocol involves manipulating the wing to perform blood sampling, moult scores or wing chord measurements:

- Gently remove one wing from under the handler's palm and extend it away the bird's body.
- Hold the wing open by gripping the upper wing (humerus near the scapula) between the thumb and index finger (**two-handed grip**) or thumb and base of the index finger (**reverse two-handed grip**).

Very experienced handlers may be able to restrain medium-sized birds with one hand using a waterfowl handling technique known as the **one-handed ringer's grip** (Figure 4.4), although if another person is available, the other techniques are recommended:

FIGURE 4.4
The one-handed ringer's grip for handling and ringing medium-sized birds

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- Starting from the two-handed grip, the handler should use the dominant hand to place the bird snugly against his/her torso.
- Switch hands so that the non-dominant hand restrains the bird against the handler's body with the head of the bird facing either forward or back; one wing is pinned against the handler's torso and the other against the palm of the handler with the fingers curled under the bird's abdomen.
- From this position, the fingers of the restraining hand can be used to hold the legs while the dominant hand is free to perform ringing and other tasks.

Large birds

Large birds such as geese and swans, and awkward long-legged and long-necked species such herons, egrets and storks can be quite difficult to handle and should only be restrained by experienced and handlers. When possible, these species should be restrained by at least two handlers; one to hold the body and wings and another to restrain the head and legs.

The only practical technique for restraining large birds is the **underarm hold** (Figure 4.5):

FIGURE 4.5
The underarm hold for restraining larger birds

CREDIT: REBECCA LEE

- The body of the bird is held under the handler's left arm with the wings held against the bird's body with pressure from the handler's torso and left elbow and forearm.
- In most cases, the bird's head can be held behind the handler because this will prevent it from lunging at the handler's face and eyes.
- Place the left hand under the bird's abdomen and the right hand across the bird's back to help restrain the legs and wings, respectively.
- Another handler can restrain the bird's head and legs to prevent injuries caused while struggling to escape.
- Certain species may require special handling techniques; for example, pelicans cannot breathe through the nares, thus the bill must be held open when restraining the head to allow the bird to breathe.

FIGURE 4.6
Velcro jacket used to restrain large birds during handling

CREDIT: TAEJ MUNDKUR

PHYSICAL AND CHEMICAL RESTRAINING AIDS

A variety of equipment can be used to physically immobilise birds. Covering the bird's head with a cloth towel, bag or hood to eliminate visual stimuli is a simple but often effective means of calming a bird and preventing injuries to handlers. Hoods or some other form of head covering are essential when handling aggressive or sharp-billed species such as herons and egrets, and are advisable when handling gulls and cormorants. Lightly wrapping birds in a cloth towel or placing them into a snug-fitting plastic or heavy paper tube can effectively immobilise the wings of small and medium-sized birds. Velcro jackets have been specifically designed for restraining larger geese and swans (Figure 4.6; Rees 2006).

Special care should be exercised when handling raptors, because even the smaller species possess sharp beaks and extremely powerful talons that can injure the unwary handler. Hoods and long thick leather gloves are required gear when handling raptors. A cloth cloak or "aba" that permits biometric measurements and blood sampling has been specifically designed for immobilising the body and legs of raptors and other large birds (Maechtle 1998).

Chemical restraint with anaesthesia is an option that should only be considered in two instances: 1) to alleviate pain during invasive marking procedures; and 2) when handling aggressive or sensitive species for which other restraining techniques are ineffective. Anaesthesia should always be administered under controlled conditions by trained wildlife veterinarians.

BIRD WELFARE

As the well-known saying goes, "prevention is the best medicine". Careful planning and execution of capture activities and adherence to proper handling guidelines will help prevent most injuries or unnecessary stressors to birds. However, there is always the risk of distress or injury when handling wild birds and handlers should always be aware of the principles of animal welfare and be alert for signs of a bird suffering. Preferably an appropriately trained veterinary clinician will be available to examine and treat any injured or distressed bird, but, at the very minimum, a basic first aid kit should be included in the equipment list of every field study involving the handling of wild birds. Some of the most frequent maladies and treatments are described below.

Scratches, cuts and abrasions may be unavoidable during capture and confinement. Simple treatment by rinsing the injury with clean water or sterile saline before releasing the bird should suffice for most minor injuries. More serious injuries such as **deep cuts, sprains and fractures** should be brought to the attention of the attending veterinarian. In no instance should a seriously injured bird be released into the wild without first being examined and treated by a veterinarian.

Some birds unable to cope with the stress of capture and handling may suffer a physiological (**shock**) or neurological (**inertia**) reaction that leaves them in evident distress. The signs of shock and inertia are generally similar; birds become unresponsive to external stimuli to the point that they appear "frozen", although shock may also be accompanied by rapid breathing that is not evident in inertia. Birds should be allowed to recover in a quiet, sheltered and well-ventilated area, well away from any human activity. Limiting time in captivity, maintaining a calm and quiet captive environment, and working at a site appropriate for the environmental conditions will help prevent shock and inertia.

Capturing, transporting and handling birds during extreme temperatures, rain or foul weather may leave them vulnerable to chilling (**hypothermia**) or heat stress (**hyperthermia**). Hypothermia can occur in cold conditions when feathers become wet and lose their insulating properties. Signs of hypothermia include shivering, lethargy and skin that is cold to the touch. Birds suffering from hypothermia should be dried and placed near a heat source such as a heating lamp or a hot water bottle (non-insulated). Hypothermia can be prevented by avoiding capture and handling in cold/wet conditions and making sure bird plumages are kept dry while being handled or held in captivity. Holding birds in dry airy crates, at sufficiently low density and away from human disturbance usually allows them to preen themselves dry. Handlers should avoid use of petroleum-based lotions (e.g. common hand-creams and moisturisers) that may cause plumage to lose its insulating properties.

Hyperthermia can occur in hot conditions when birds are held in direct sunlight, at high ambient temperatures, or in overcrowded crates without adequate ventilation or water. Hyperthermia may also occur if birds are subject to a prolonged chase during capture. Signs of hyperthermia include panting, wings held away from the body, lethargy, seizures or prostration. Birds suffering from hyperthermia should not be handled, but should be placed in a well-ventilated box/crate, moved to a cool, shaded area and provided with abundant drinking and swimming water. It may be beneficial to mist the bird with water or apply alcohol or water to the bird's feet to accelerate heat dissipation. Hyperthermia can be prevented by avoiding capture and handling in hot conditions and not overcrowding holding pens/crates.

Injuries caused by improper capture and handling techniques such as **fractures, brachial (wing) paralysis** and **capture myopathy** are common and in all cases, avoidable. Never carry a bird by the wings or legs alone and do not hyperextend the wings or legs while restraining a bird. Do not keep long-legged birds in cramped conditions that prevent standing. Avoid prolonged chases or forceful restraint of struggling birds that may overtax birds during capture and handling.

RINGING (BANDING)

The ringing (or banding in some countries) of wild birds for scientific purposes has provided a wealth of information revealing the life histories and movements of many different species. Metal leg rings (bands) are the oldest and most widespread ringing methods, and the uniquely numbered rings allow for individual identification of any marked bird. Ringing is advisable whenever a bird is captured and released back into the wild, and is essential during disease surveillance programmes to prevent repeated sampling of recaptured birds that would bias results. However the repeated sampling of marked birds assists in tracking changes in disease status.

Several national or regional agencies have been formed to regulate and coordinate bird ringing activities worldwide. Organisations such as EURING[4], AFRING[5] and the US Bird Banding Laboratory[6] can usually provide detailed information regarding all aspects of ringing in their region, including permitting procedures, obtaining rings, the proper size ring for species of interest and basic ringing equipment. Ringing agencies are also responsible for collecting and collating data for all the birds marked or recaptured in their jurisdiction. Timely submission of ringing data is vital to maintaining a complete and up-to-date history for each marked bird.

EQUIPMENT LIST FOR RINGING AND BIOMETRIC MEASURES

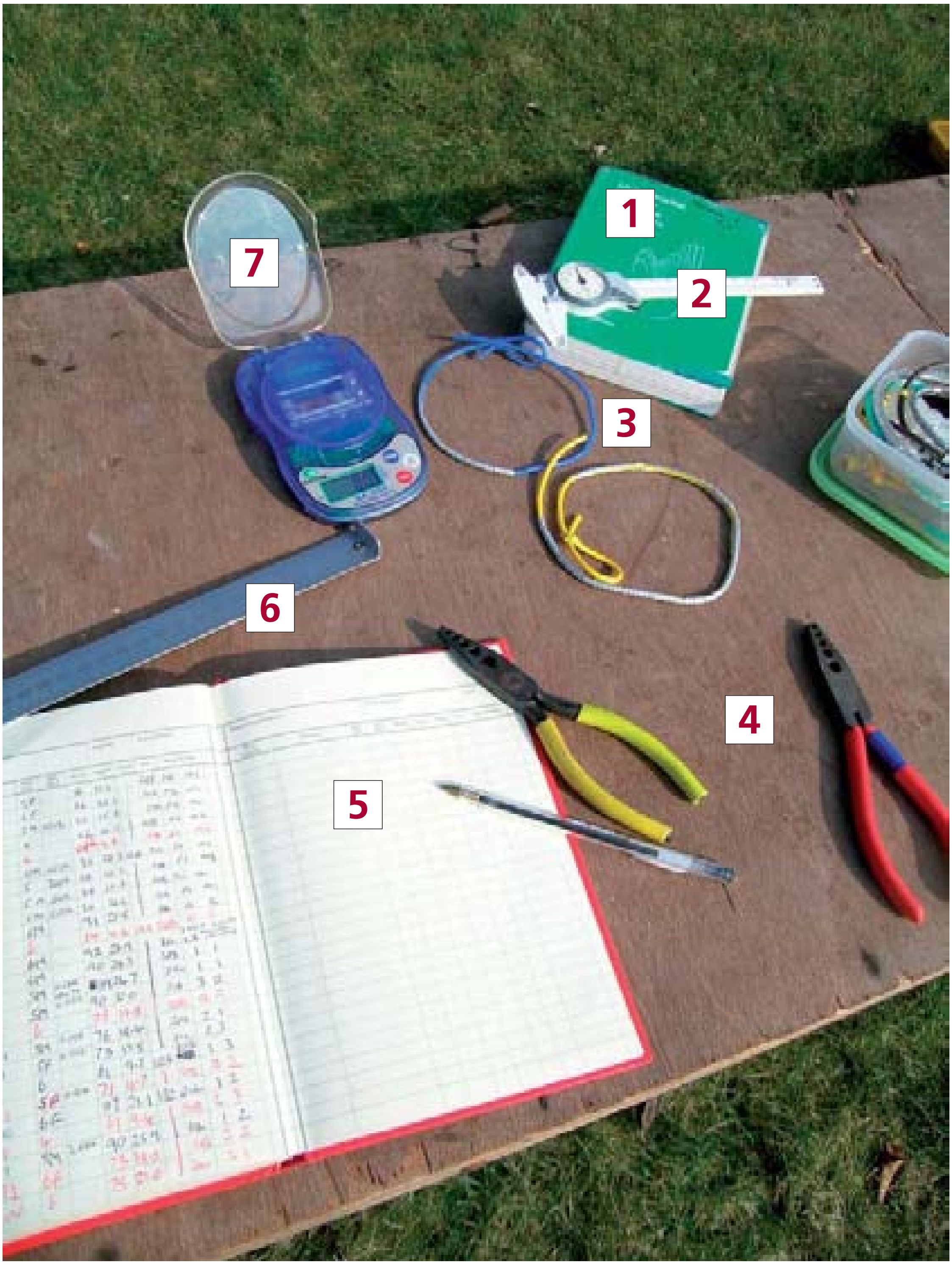
1. Leg rings sized to fit the species of interest
2. Ringing pliers and needle-nose pliers
3. Data notebook and pens/pencils
4. Vernier callipers
5. Stopped wing ruler (preferably metal)
6. Tail ruler (preferably metal)
7. Bird Guides
8. Weighing scale
9. Weighing bags
10. Wire or nylon fishing line

4 http://www.euring.org/

5 http://web.uct.ac.za/depts/stats/adu/safring-index.htm

6 http://www.pwrc.usgs.gov/bbl/

FIGURE 4.7
Basic equipment for ringing and biometric measures



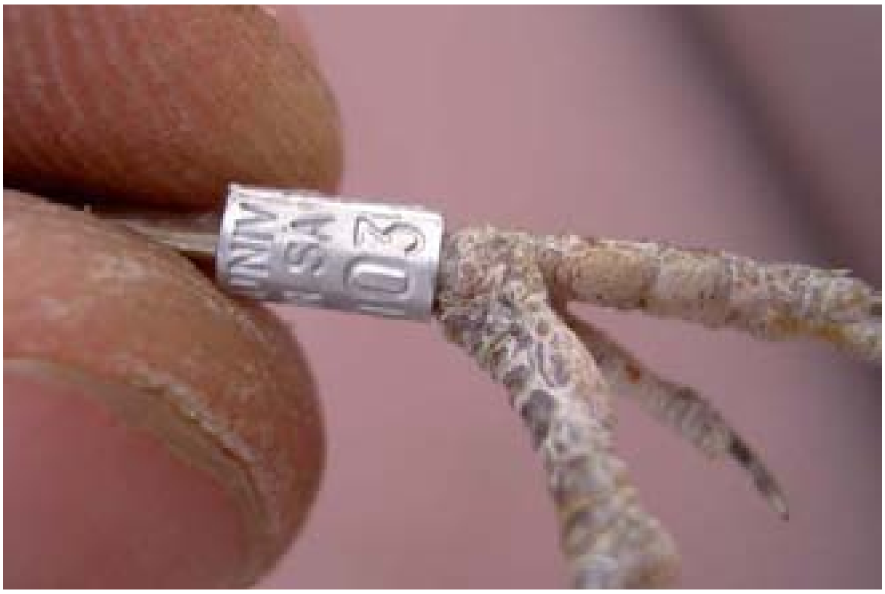
CREDIT: MARK GRANTHAM

1) Bird guide, 2) Vernier callipers, 3) leg rings, 4) ringing pliers, 5) data notebook and pen, 6) stopped wing ruler, 7) weighing scale

Ringing a bird

Leg rings are available in a variety of different sizes (inside diameter from <2 mm to over 30 mm) and materials to accommodate any bird species. Rings should have an inside diameter slightly larger than the maximum diameter of the bird's tarsus, but be careful because tarsus width can vary by sex and age within a species. Common aluminium rings are sufficient for most terrestrial bird species, but rings composed of alloys such as monel, incoloy, stainless steel or titanium may be better for long-lived or aquatic species in which ring wear is an issue. Coloured, anodised metal rings are available to facilitate sighting, but may require

FIGURE 4.8
Aluminium leg ring on the tarsus of a passerine bird



CREDIT: GIUSEPPE ROSSI

FIGURE 4.9
Coloured plastic leg rings on the tarsus (lower leg) and tibio-tarsus (upper leg) of a Black-tailed Godwit (*Limosa limosa)*

CREDIT: ROB ROBINSON

special permits. Consult the regional ringing agency for information on acquiring rings of the proper size and material for the species of interest.

Leg rings are almost always placed on the bird's tarsus (the long bone immediately above the toes) in most passerines and waterbirds (Figure 4.8), but are often placed on the tibio-tarsus (above the "knee") on some long-legged waders (Figure 4.9). No convention exists as to which leg should be ringed or the orientation of the ring numbers on the standing or perched bird. Ring placement is greatly facilitated by the use of **ringing pliers**, which are basically long-nosed pliers with holes of various sizes that correspond to the outside ring diameters. The proper ringing procedures for most situations are as follows:

- Remove the ring from the string by using a pair of **needle-nose pliers** to open the ring enough that it just fits over the tarsus of the bird; the less the ring is opened to fit over the tarsus, the easier it will be to close.
- Using whichever restraining hold is best suited to the bird, extend the bird's leg and slip the ring over the narrowest point of the tarsus.
- Holding the ring in place with the fingers, slip the appropriately sized hole of the ringing pliers around the ring so that the gap in the ring is aligned with the open end of the pliers (Figure 4.10).
- Gently squeeze the pliers so that the ring closes and it can no longer be removed from the tarsus.
- Rotate the ring in the pliers so that the butt ends are now within the same closed half of the pliers' hole (Figure 4.11), then again apply pressure to fully close the ring; this step may need to be repeated several times before the ring is properly closed.
- Record the ring number and other pertinent observations in a notebook; this information should be recorded prior to completing closure of the ring on the bird and is facilitated by the use of standard forms/ headings to ensure that all essential data is recorded.

FIGURE 4.10

Phase 1 of proper alignment of the leg ring in the pliers during ring closure: align the gap in the ring with the open end of the pliers and apply pressure to partially close the ring around the tarsus

FIGURE 4.11
Phase 2 of proper alignment of the leg ring in the pliers during ring closure: rotate the ring in the pliers so that the butt ends are within the same closed half of the pliers' hole and apply pressure to fully close the ring

CREDIT: DARRELL WHITWORTH

When properly closed the ring should be loose enough to slide freely and spin around the tarsus, but tight enough that it cannot slide over the leg joint or foot, or get caught on vegetation. The ends of the ring should butt squarely and tightly, without projecting corners or edges to abrade the leg. Stiffer rings of stainless steel or other alloys may require considerably more pressure to completely close compared to aluminium rings.

On occasion, too much pressure may be applied when closing the ring and the ends will overlap. Overlapping rings should be removed and replaced before the bird is released. Removing bands is always tricky, but necessary, as the sharp edges may scrape and abrade the bird's leg. To remove a poorly fitted ring:

- Insert two pieces of wire or nylon fishing line between the bird's tarsus and the ring.
- The wire or line should be long enough that it can be easily tied into loops that can be gripped by the handler, and strong enough that the loops will not break when pulling the ring open.
- Insert a pencil into each loop and carefully pull the loops apart, thereby opening the ring.
- To avoid injury to the bird while pulling the loops apart, keep the bird's leg stationary and maintain steady even pressure on both loops as the ring opens; at all costs avoid jerky pulling motions which are likely to put undo pressure on the wire/line and the bird's leg.

BIOMETRIC MEASUREMENTS

For many bird species, the sex or age of a captured individual may not always be immediately evident with a simple visual inspection. However, subtle but significant differences in morphology are often useful for differentiating between sexes and age classes. Thus, recording biometric measurements in conjunction with bird ringing is a common practice, and can have important applications in disease sampling studies for determining differential infection or exposure rates based on sex or age. Weight, culmen length and depth, tarsus length, wing length and tail length are among the most commonly recorded biometric measures. Additional data such as the presence of incubation (brood) patches and the moult stage also provide important data revealing the breeding or physiological status of the bird when captured.

Weight

Bird weight can be determined using **electronic, beam or spring scales** (or **balances**), although spring scales (e.g., Pesola scales) are often the most practical for use in field situations. Have a number of different-sized scales available to cover the range of birds likely to be captured. Birds should be placed in cloth bags or other containers for weighing. When using spring scales the bird is suspended from the scale (Figure 4.12) to obtain the gross weight (bird + bag). The weight of the bag or container should be measured after each use and subtracted from the gross weight to obtain the bird weight (gross weight – bag weight = bird weight). Always record the gross weight, bag weight and bird weight in the field notebook.

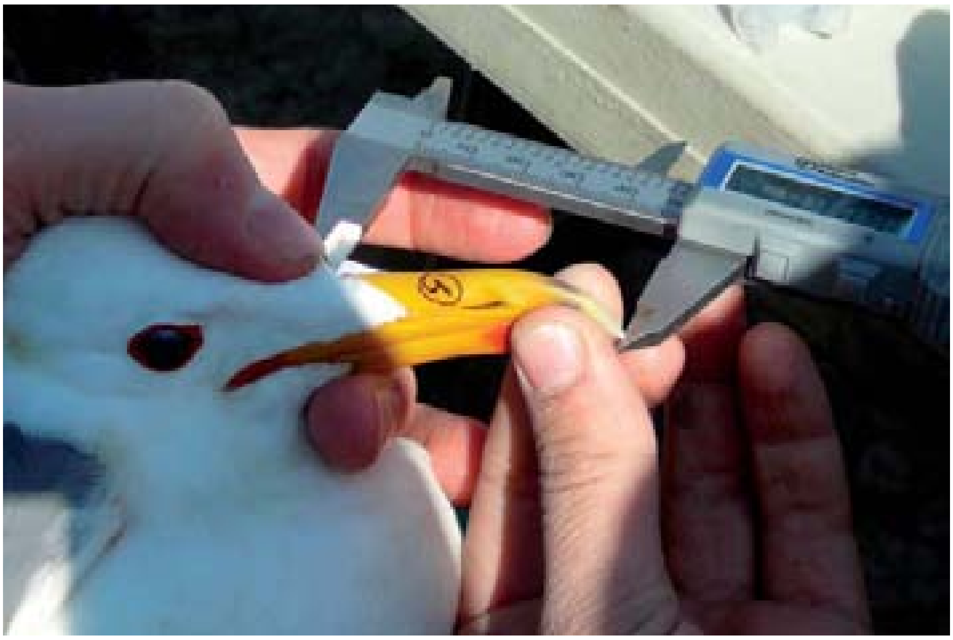
Culmen length and depth

Culmen (bill) length and depth are measured using sliding **Vernier callipers**. Depending on the bird species, three different measures of culmen length may be taken: 1) tip of the bill to the base of the skull (passerines); 2) bill tip to the cere (birds of prey); and 3) bill tip to feathering at base of bill (Anatids, waders and other long-billed birds). Record the method used in field notes.

FIGURE 4.12
Weighing a bird with a spring scale

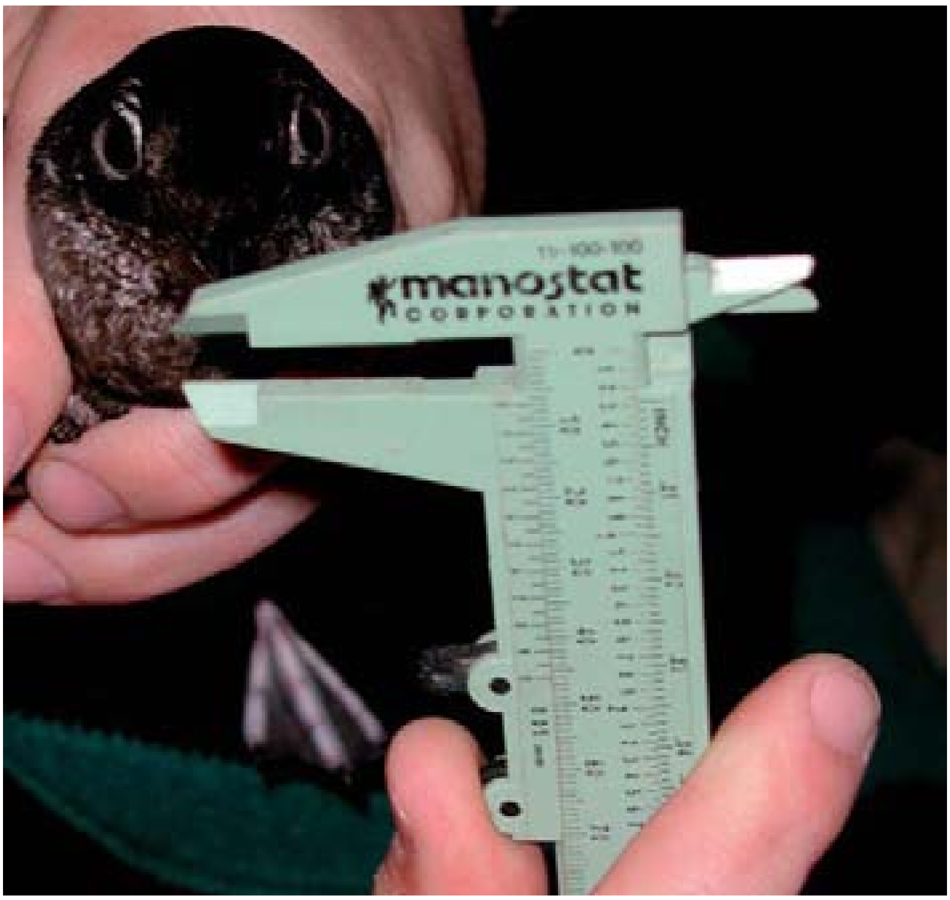
CREDIT: GIUSEPPE ROSSI

FIGURE 4.13
Measuring culmen length with Vernier callipers



CREDIT: USGS WESTERN ECOLOGICAL RESEARCH CENTER

FIGURE 4.14
Measuring culmen depth with Vernier callipers



CREDIT: SCOTT NEWMAN

To measure culmen length:

- Open the callipers so that the opening is wider than the length of the bill.
- Gently place the outer calliper jaw against the base of the bill where the measurement starts (base of skull, cere or feathering).
- Slide the inner calliper jaw until it just contacts the distal tip of the bill (Figure 4.13).
- Record culmen length to the nearest 0.1 mm in the field notebook.

To measure culmen depth:

- Open the callipers so that the opening is wider than the depth of the bill.
- Place the inner jaw of the callipers against the base of lower mandible.
- Slide the outer calliper jaw inward until it just touches the upper mandible, either at the base of the bill where the feathering starts or at the proximal edge of the nostril (Figure 4.14).
- Record the culmen depth to the nearest 0.1 mm and where the measurement was taken (feathering or nostril) in the field notes.

Tarsus length

Tarsus length is a measure of the length of the tarsometatarsal bone and also requires the use of **Vernier callipers**. To measure tarsus length:

- Open the callipers so that the opening is wider than the length of the tarsus.
- Place the inner jaw of the callipers into the notch of the intertarsal joint at the back of the bird's leg.
- Bend the bird's foot downward at a 90° angle to the tarsometatarsal bone and slide the outer calliper jaw inward until it just touches the point where the foot bends (Figure 4.15).
- Record the tarsus length to the nearest 0.1 mm in the field notes.

FIGURE 4.15
Measuring tarsus length with Vernier callipers

CREDIT: USGS WESTERN ECOLOGICAL RESEARCH CENTER

Wing length

Wing length is defined as the distance from the distal portion of the carpus to the tip of the longest primary feather. By convention, the wing length is measured with the wing chord flattened and straightened, a practice which yields the maximum and most consistent

FIGURE 4.16

Measuring wing length with a stopped wing ruler

CREDIT: SCOTT NEWMAN

FIGURE 4.17

Measuring a bird's tail length with a normal ruler

CREDIT: ROB ROBINSON

results. A **stopped wing ruler** (blocked off at the 0 mm mark) is needed for wing chord measurements. To measure wing length:

- Slide the stopped wing ruler under the wing and press the carpal joint gently but firmly against the stop.
- Flatten the wing against the ruler by gently pressing down on the covert feathers near the base of the primaries (Figure 4.16).
- Use the index finger to gently straighten the longest primary feathers along the ruler.
- Record the wing length to the nearest 1 mm in the field notes.

Tail length

Tail length is defined as the distance from the base to the tip of the longest tail feathers (rectrices). Measurement of tail length requires little more than a normal ruler. To measure tail length:

- Slide the tail ruler between rectrices and undertail coverts until it reaches the base of the two central tail feathers.
- Use the index finger to gently flatten and straighten the tail feathers along the ruler (Figure 4.17).
- Record the length of the longest tail feather to the nearest 1 mm in the field notes.

Brood patches

During the breeding season, many birds develop a bare patch on the abdomen where downy feathers are shed just before the onset of incubation. This brood (or incubation) patch permits the efficient transfer of body heat from the incubating parent to the developing eggs. Not all species develop brood patches: ducks for instance do not. Brood patches

FIGURE 4.18
Brood patch examination for a Xantus's Murrelet (*Synthliboramphus hypoleucus*)

usually develop in both females and males if incubation duties are shared, but if incubation is performed solely by one sex, usually only that sex develops a brood patch. Some bird species develop just one brood patch located medially on the abdomen, while other species develop two bilateral brood patches.

If captures are conducted during the breeding season, birds known to develop brood patches should be inspected for their presence.

- For species with thinner, fine plumage (e.g. passerines): hold the bird (ringer's hold) belly-up near the handlers face with the bird's head away from the handler, and gently blow across the bird's abdomen to lift the body covert feathers and expose the brood patch.
- In aquatic species with thicker, dense plumage: hold the bird (reverse two-handed grip) belly-up with the bird's head away from the handler and use the thumbs to gently part the body covert feathers on the abdomen to expose the brood patch (Figure 4.18).

Moult scores

Feathers are essential to the survival of birds, which spend considerable time preening to maintain their plumage in good condition. Nevertheless, wear and tear over time causes feathers to deteriorate. Thus, all birds undergo regular periods when they shed old feathers and replace them with new ones during a process called moult (Figure 4.19). Moult patterns differ by species; some birds moult annually, others less frequently and others more frequently.

FIGURE 4.19
Wing moult (note the blue sheaths at base of primary feathers) on an Egyptian Goose (*Alopochen aegyptiacus*)

CREDIT: GRAEME CUMMING

Growth of new feathers is an energetically expensive process and birds may be physiologically stressed or compromised during moult; thus, recording the presence of moult in captured birds is important for determining periods in which they may be weakened and more vulnerable to disease. Rather involved schemes have been devised for characterising the progress of moult, but these are beyond the scope of this Manual. Those seeking more detailed information on moult should consult Ginn and Melville (1983) or Jenni and Winkler (1994).

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Chapter 5
Disease sampling procedures

Circumstantial evidence suggests that wild birds may play a role in the transmission and spread of the H5N1 HPAI virus. Yet, despite the fact that disease surveillance programmes in Europe, Asia, Africa and the Americas have collected samples (2004-2007) from several hundred thousand wild, apparently healthy birds, there is still no irrefutable evidence demonstrating that wild birds are acting as H5N1 HPAI viral reservoirs capable of travelling long distances and shedding the virus. Thus far, the H5N1 virus has been isolated primarily in sick, moribund or dead wild birds.

As the H5N1 HPAI virus continues to sporadically reappear in poultry farms, active disease surveillance programmes will become increasingly important for determining if wild birds are indeed acting as vectors in the transmission and geographic spread of the virus. Fortunately, H5N1 disease sampling in wild birds involves minimally invasive techniques that can be quickly learned following training in the basic procedures. These techniques are relatively straightforward and can be completed in just a few minutes with little or no detrimental effects to the bird. This means that active disease surveillance can be incorporated into most studies where wild birds are captured and handled. In addition, collection of fresh faeces of peridomestic and wild species can be a relatively simple and cheap process of collecting samples for the detection of avian influenza viruses, especially where catching of wild birds is not feasible.

Proper specimen collection is essential for providing samples that ensure reliable isolation and identification of any pathogens present. This chapter presents a brief description of the most practical disease sampling techniques used for the H5N1 AI virus in free-ranging wild birds. Please note that while these sampling techniques are for live, apparently healthy free-ranging birds, the use of personal protective equipment (PPE) appropriate to the level of risk level is recommended whenever wild birds are handled, because apparently healthy birds may be infected without exhibiting clinical signs of H5N1 infection. Clean PPE should be used at each sampling site to prevent the spread of disease among wild bird populations and between wild populations and domestic flocks. Good biosecurity practices should be followed, where the same PPE should not be used for sampling wild and domestic bird populations or between collection sites or between poultry holdings.

In countries where no outbreaks have been recorded, minimal PPE measures may include gloves, a mask and proper post-handling hygiene. However, working with sick and dead birds at suspected disease outbreak sites requires full PPE (including latex or vinyl gloves, a mask, goggles and coveralls or medical gown) and special handling and sampling procedures described in FAO (2006). If free-ranging birds captured during active surveillance programmes exhibit clinical signs (see below) of suspect infectious disease (i.e. H5N1 infection), immediately stop all bird handling activity and contact the appropriate governmental, veterinary or wildlife agencies in the country.

Possible clinical signs of H5N1 HPAI include (but are not limited to): diarrhoea; regurgitation; sneezing; emaciation; open sores; discharges from the mouth, nose, ear or vent; swelling or discolouration of head tissues including the conjunctiva; behavioural/neurological abnormalities (falling over, head tilt, head and neck twisting, seizures, circling, paralysis); and feather abnormalities in chickens. Some susceptible wild bird species would also show some of these signs but their presence or severity will vary greatly. These clinical signs are not specific for H5N1 infection, but suggest the presence of serious clinical disease that needs to be investigated and diagnosed in a timely manner.

The disease sampling techniques are presented with the following assumptions:

- all investigations will be performed by appropriately trained personnel;
- each bird sampled is correctly identified by an appropriately trained individual, and information relative to the bird (species, and when possible, sex and age) is properly recorded; if uncertain take a photo (See guidelines for taking good quality photographs in Annex A).
- proper human health and biosafety precautions will be adhered to (see FAO 2006);
- consent from the responsible local, state and federal veterinary and wildlife agencies has been obtained prior to any investigation;
- disease outbreak investigations should be conducted in collaboration with the responsible government agencies, and with FAO and OIE representatives when appropriate.

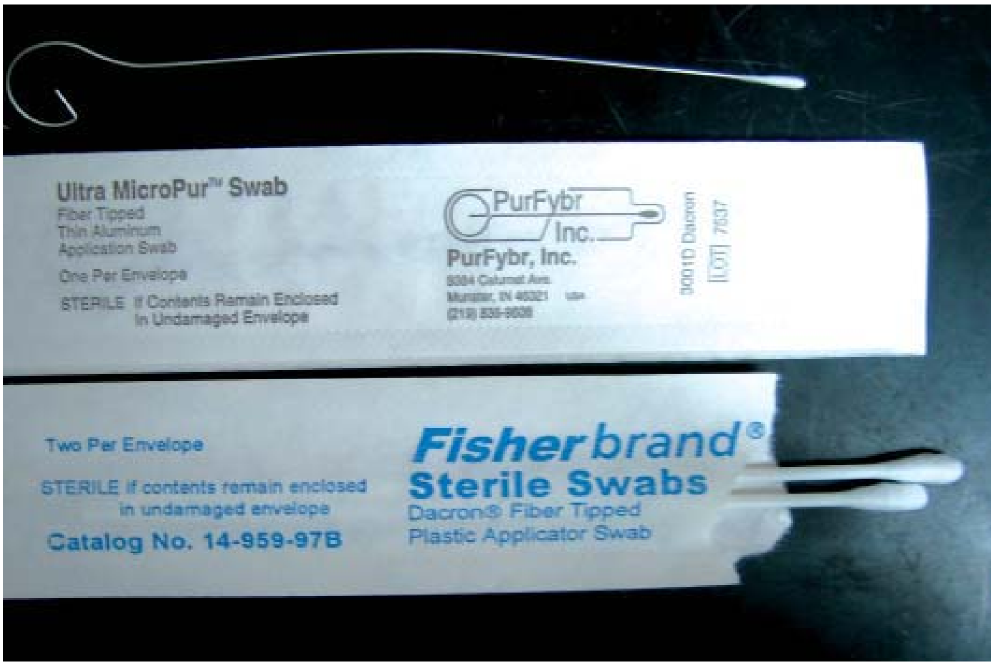
TRACHEAL AND CLOACAL SWABS

Swabs taken from the cloaca (vent) or trachea can be used for viral cultures or reverse-transcription polymerase chain reaction (RT-PCR) to test for the presence of many viral pathogens, including AI viruses. While non-pathogenic AI viruses replicate primarily in the avian intestinal tract, recent strains of H5N1 HPAI viruses have been detected both from cloacal and tracheal/oropharyngeal samples. Research has revealed that, unlike other AI viruses, the H5N1 HPAI subtype replicates to higher levels and for longer periods in the res-

EQUIPMENT LIST FOR TRACHEAL AND CLOACAL SWAB SAMPLES

1. Personal protective equipment (PPE)
2. Rayon or Dacron-tipped swabs
3. 2-2.5 ml screw-top cryovials
4. Viral transport medium (VTM)
5. Scissors/pliers
6. 70% alcohol solution
7. Cooler and ice and/or liquid nitrogen to store samples
8. Cryovial labels and pencils or indelible markers
9. Data forms
10. Headlamps or pen-lights

FIGURE 5.1
Dacron tip sterile swab for tracheal, oropharyngeal and cloacal samples



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piratory tract compared to the gastrointestinal tract (Sturm-Ramirez *et al.* 2004, Hulse-Post *et al.* 2005). Furthermore, after experimental exposure, higher concentrations of the virus have been found in tracheal samples than in cloacal samples on any given day. Therefore, tracheal and cloacal swabs are currently the preferred samples for H5N1 surveillance in wild birds.

Swabbing techniques require Dacron or rayon-tipped swabs (Figure 5.1); avoid using swabs with cotton-tipped or wood stems as they may inhibit genetic detection or viral growth (due to inherent RNAse activity of cotton or wood cellulose). Wire-stemmed swabs may also be used, especially for very small-sized birds. Cryovials containing a viral transport medium (VTM) will also be needed to store and transport the samples. Cryovials and cryolabels that are appropriate for the intended storage temperatures should be chosen, because some are certified only for use in dry ice and are unsuitable for use in liquid nitrogen.

VTM can be prepared locally at a laboratory (see instructions at WHO website[7]) or purchased as kits from commercial dealers (e.g. TBD Universal Viral Transport Media or Cellmatics Viral Transport Pack[8]). VTM should be stored at a low temperature (<4°C) in the field before use.

Rapid detection tests using tracheal swabs to detect the presence of a type A virus (in the case of AI, any of the possible 144 sub-type combinations) are available for use in the field, but these tests are relatively insensitive and require a substantial viral titre to return a positive result; thus the value of a negative test may be low (i.e. infection is present but

7 http://www.who.int/csr/resources/publications/surveillance/WHO_CDS_EPR_ARO_2006_1/en/index.html
8 http://www.bd.com/support/locations.asp

not of sufficiently high level for the diagnostic strip to show up as a positive). However, a positive test in conjunction with a clinical scenario consistent with H5N1 AI infection warrants immediate notification of appropriate authorities, although an actual H5N1 diagnosis requires confirmation by laboratory tests.

Swabbing procedures

Other than the sampling site, the equipment and techniques for tracheal and cloacal swabs are similar. Tracheal swabs may not be possible for small birds (passerines) with narrow tracheal openings. In such cases, an oropharyngeal swab should be conducted. Be sure to use a swab size appropriate for the bird.

- Open the swab package from the stem end, being sure not touch the swab tip with anything prior to or after sampling
- **Tracheal swabs** are collected from the air passage (trachea) at the back of the bird's mouth. To gain access to the opening of the trachea, it is often useful to gently pull the tongue forward, exposing the trachea at the rear end of the tongue. Wait until the bird breathes and the cartilage protecting the trachea is open before inserting the swab and gently touching the sides and back of the trachea (Figure 5.2); moving the tongue forward can help expose the trachea.
- **Oropharyngeal swabs** are conducted by gently rolling the swab tip around the inside of the bird's mouth and behind the tongue (Figure 5.3).
- **Cloacal swabs** are collected by inserting the entire tip of the swab into the cloaca and swabbing with two to four circular motions while applying gentle pressure against the mucosal surfaces (Figure 5.4); gently shake any large faecal residues from the swab before placing it in the cryovial.

FIGURE 5.2
Proper location for a tracheal swab

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Arrow points to tracheal opening

FIGURE 5.3
Proper procedure for an oropharyngeal swab

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FIGURE 5.4
Proper procedure for a cloacal swab

CREDIT: TAEJ MUNDKUR

- Carefully remove the swab, open the cryovial and place the swab tip in the VTM about ¾ of the way toward the bottom of the vial; avoid overfilling the cryovial as the contents may expand and leak during the freezing process.
- Cut or break the swab stem so that the swab tip remains in the VTM and close the vial (Figure 5.5); if wire-stemmed swabs are used, these can be cut with wire cutters.
- If scissors or wire cutters are used to cut the swab stem, disinfect them after each use by cleaning the blades with a 70% alcohol solution.

FIGURE 5.5
Proper placement of swab sample in the viral transport medium

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FIGURE 5.6
Labelled cryovial with date, species, sample type and an ID number specific to each sampled individual that refers to a database containing all the information for that bird

CREDIT: SCOTT NEWMAN

- Label each cryovial sample with the date, species, sample type (tracheal or cloacal), and an ID number specific to each sampled individual that refers to a database containing all the information for that bird (Figure 5.6); labels should be written with a material that will not dissolve when wet, then placed in liquid nitrogen (Figure 5.7) or ethanol, or stored at temperatures below -70° C.

Check with the supplier of the VTM to determine the proper storage methods for that medium. If using a VTM that requires refrigeration or freezing, store samples in a sealable plastic bag on ice at or below 4° C, or in liquid nitrogen. It is important to maintain the "cold chain" during the entire storage and transport process as loss of the cold chain can result in samples being rendered non-diagnostic. Commercially available kits that inactivate the virus and are stable at room temperature may be a convenient back-up option for remote field sites where cold chain storage for transport media cannot be guaranteed. If samples cannot be transported to the laboratory within 24-48 hours, longer-term storage in liquid nitrogen or in a freezer at temperatures less than -70° C will be needed.

FIGURE 5.7
Liquid nitrogen container used to freeze and preserve samples when working at remote locations

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BLOOD SAMPLING

Serological testing of blood samples indicates prior exposure to the virus by detecting antibodies in the blood rather than viral antigens or specific genetic targets. Blood samples can be collected by different methods depending on the size of the bird. For small birds (e.g. passerines and small waders) blood should be collected from the jugular vein (right side of neck; Figure 5.8) using a 0.3-0.5 ml insulin syringe with a 0.33 mm hypodermic gauge (22-30G) needle depending on the size of the bird. For larger birds (e.g. ducks, coots, gulls and herons), blood can be collected from the jugular vein or medial metatarsal (leg) vein (Figure 5.9) using a 1-2 ml syringe and 23-27 gauge hypodermic needle. Sampling from the brachial (wing) vein is also an option for some larger birds.

In general, it is safe to collect 0.3-0.6 cc of blood for every 100 g of body mass from live birds (total volume collected should not exceed one percent of body mass), although it is a good practice to collect only enough blood needed for conducting the required tests.

The optimal venipuncture site (location where the hypodermic needle penetrates the vein) will vary depending upon the species being sampled. Intuitively, venipuncture techniques are easier on larger birds with larger veins, but the techniques become easier for all species as one gains experience. After the proper amount of blood is collected, a gauze square should be pressed against the venipuncture site as the needle is removed from the bird, and pressure applied to the venipuncture site for 30 seconds. This will prevent the bird from developing a painful haematoma (blood clot) under the skin which may affect movement of the wing or leg.

To reduce the risk of haemolysis, it is advisable to remove the needle from the syringe (for non-mounted syringes) when transferring blood into the tube by gently expelling the blood against the inside wall of the vial.

FIGURE 5.8
Blood sampling procedure from the jugular vein

CREDIT: TAEJ MUNDKUR

FIGURE 5.9
Blood sampling procedure from the medial metatarsal vein

CREDIT: DIANN PROSSER

- When sampling from the jugular or brachial vein, expose the venipuncture site by using alcohol to wet the feathers, then separate the feathers with the fingers.
- Sample collection from the brachial or medial metatarsal vein is best accomplished by holding the vein off (applying pressure to the vein), proximal (towards the heart) to the desired venipuncture site to temporarily block blood flow and make the vein easier to locate.
- Sample collection from the jugular vein is most easily accomplished by holding off the vein on the right side of the neck, at the level of the clavicle.

EQUIPMENT LIST FOR BLOOD SAMPLING

1. Personal protective equipment (PPE)
2. Hypodermic or butterfly needles of various sizes (22-30 gauge)
3. Syringes of various sizes (1cc -12 cc)
4. Red top (serum) and green top (plasma) separator tubes
5. Portable centrifuge (if available)
6. 70% alcohol solution and cotton gauze
7. Cryovials
8. Sterile pipettes
9. Indelible marker and cryovial/separator tube labels
10. Cooler, ice and/or liquid nitrogen to store cryovials
11. Previously designed data sheets
12. Sharps Container

- Prior to inserting the needle into the bird, pull the plunger back to release the vacuum in the syringe, and then press it all the way forward so there is no air in the syringe.
- Carefully insert the hypodermic needle under the skin and into the vein with the bevel pointing upward so that the needle opening faces the inside and not the wall of the vein; for jugular vein sampling, the needle can be bent slightly to form a curve that eases insertion into the vein.
- Once assured that the hypodermic needle is in the vein, pull back very gently on the syringe plunger to draw blood.
- Any bird, regardless of size, may experience stress, cold or other factors that can cause vasoconstriction and impede the flow of blood; in conditions where blood does not flow smoothly, gentle digital massage above the venipuncture site may aid sample collection.
- After blood is collected, cover the venipuncture site with gauze and apply digital pressure until bleeding stops, usually 30-60 seconds.
- Dispose of used hypodermics and other veterinary-related wastes in appropriate and safe containers.
- Immediately transfer blood from the syringe to a **serum** (red top) or **plasma** (green top) separator tube to prepare samples for centrifugation.
- **Plasma tubes** should be immediately refrigerated or kept in a cool water bath before they are spun down in the centrifuge.
- **Serum samples** should be allowed to clot at room temperature (22-25 °C) before refrigeration; clotting can be facilitated by slightly inclining the tubes.
- Spin down blood samples in a centrifuge after collection to separate the fractions for later laboratory analyses; separation of **serum** samples is aided by refrigerating samples for several hours and carefully ringing the sample with a sterile, round "stick" to free the clot from the vial.
- After centrifugation, transfer **serum** and **plasma** samples to cryovials (preferably screw top cryovials with rubber o-rings) with a sterile transfer pipette; if pipettes are not available the samples can be carefully poured into the cryovials.
- Label each cryovial sample with the date, species, sample type (plasma or serum), and an individual ID number.

Choice of serum and/or plasma separator tubes will depend on the laboratory assays to be performed and should be confirmed with the laboratory before conducting field work. Cryovials with the separated serum or plasma fractions can be kept in a zip-lock bag for storage and transport. Samples can be stored on ice at 4° C if they can be shipped to the laboratory within 24-48 hours. Otherwise, store samples on dry ice, in liquid nitrogen or in a -70° C freezer.

If an electric centrifuge is not available during field work, consider use of a battery powered or hand-powered crank centrifuge, or send non-centrifuged blood samples to the laboratory if they can be shipped to the laboratory within 24-48 hours and maintained at 4° C. Transport samples on ice blocks by placing tubes in zip-lock bags and wrapping the bag in a cloth towel before placing in the cooler. Plasma and serum tubes with whole blood samples should not be frozen or come into direct contact with ice as this may damage the red blood cells causing haemolysis, which may interfere with diagnostic results.

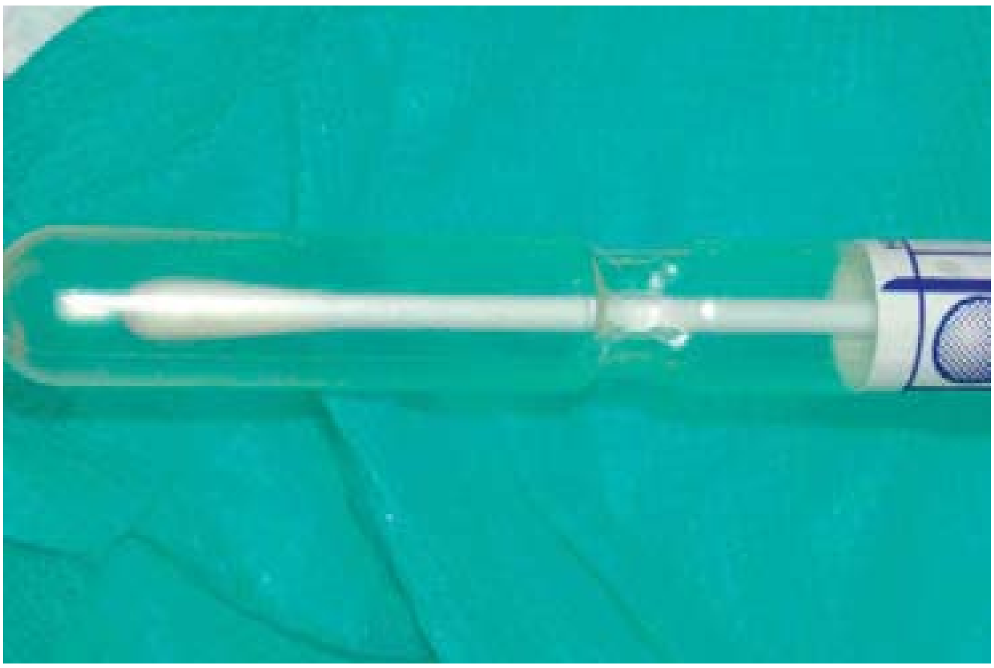
FAECAL SAMPLING

The collection of fresh faeces of peridomestic and wild species for the detection of avian influenza viruses can be a relatively simple process and an inexpensive way of collecting large number of samples, especially where catching of birds is not feasible. Faecal samples are also called "environmental samples" in some countries (such as in the United States of America).

The following guidelines should be followed for collection of faecal samples from a single individual or from a flock of birds:

- Observe the bird(s) from a distance and carefully note the area where it (they) are gathered. Birds may roost on the ground, within the poultry farms, in fields or around wetland areas, on wires, posts or on roofs or other structures on which they will defecate.
- Identify the species of birds that are to be sampled and to ensure that the bird(s) are roosting as either single species flocks or at least in mixed flocks where it is possible to be sure of the species from which faeces is subsequently collected. For example mixed goose flocks are problematic as the faeces may be difficult to separate. But a single goose species mixed with gulls should not create any problem as there would be no risk of misidentifying the faeces, based on size, colour and content.
- Walking rapidly towards a group of roosting birds normally causes them to move or fly away and in the process some individuals will defecate.
- Try to minimize the opportunity for resampling the same individual by limiting the number of faecal samples collected from each flock and ensuring that samples are collected evenly across the area where a single species flock had been observed.

FIGURE 5.10
Sterile swab to be used for faecal sampling



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EQUIPMENT LIST FOR FAECES SAMPLING

1. Personal Protective Equipment (PPE)
2. Sterile Rayon or Dacron-tipped swabs
3. Prelabelled cryovials with transport medium
4. Indelible marker and cryovial labels
5. Cooler, ice and/or liquid nitrogen to store cryovials
6. Previously designed data sheets

- Only collect fresh faecal samples, ideally those that are still moist. Dried and powdery faeces usually indicate old specimens and should not be collected as they are of poor diagnostic value. High temperatures may inactivate viruses within in a few hours.
- Collect the faeces using a sterile swab (Figure 5.10) and place in a prelabelled vial with transport medium. If the swab is going to be placed in the viral transport medium, collect the faeces using a Rayon or Dacron-tipped swab.
- Resist the temptation to scoop faeces into the tube. It is better to roll the swab over the faeces and shake off excess matter.
- Where possible, try to sample the underside, or shaded side (since direct sunlight may reduce viral survival)
- Developing of a photo file of faeces of different bird species can assist in improving sample collection. A scale for identifying the size of the faeces is useful to include in the photos.

REFERENCES AND INFORMATION SOURCES

European Commission, DG SANCO. 2006. Guidelines on the implementation of survey programmes for avian influenza in poultry and wild birds to be carried out in the Member States in 2007. (also available on http://ec.europa.eu/food/animal/diseases/controlmeasures/avian/surveillance4_en.pdf).

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Sturm-Ramirez, K.M., Ellis, T., Bousfield, B., Bissett, L., Dyrting, K., Rehg, J.E., Poon, L., Guan, Y., Pieris, M. & Webster, R.G. 2004. Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. *Journal of Virology*, 78: 4892-4901.

Chapter 6
Avian surveys and monitoring

A more complete understanding of the role that wild birds play in the ecology of wildlife diseases require baseline studies of those species likely to host, transmit or spread pathogens. Baseline studies of wild bird populations will generally fall into three categories: inventory and monitoring, movement patterns and behavioural studies. Initial studies will likely focus on inventory and monitoring with specific objectives that include: 1) an inventory of all the bird species in an area of interest; 2) determining the abundance or density of the species present; and 3) monitoring seasonal changes in species composition and numbers. When applied to understanding the emergence of infectious diseases such as H5N1 AI, these techniques serve to provide an early warning system for detection of higher than expected mortality rates in wild bird populations.

Species inventories and population monitoring are common tasks of biologists, and a variety of avian survey and monitoring techniques are available. While each technique has its advantages, the most appropriate technique will depend on the specific objectives of the study, the size of the study area, characteristics of the species and habitat of interest, and the logistic and financial feasibility of implementing the study. This Manual provides a brief review of some the practical techniques used to survey and monitor avian populations, with special emphasis on those techniques applicable to waterbirds, shorebirds and other species known or suspected of hosting, transmitting or spreading the H5N1 virus.

Various approaches can be employed to assess wild bird species composition and abundance over an area of interest, from total counts of all animals present (a complete census) to sampling strategies that provide population estimates that can be extrapolated over the entire study area. One important precept applies regardless of the technique employed: it is essential that all techniques are properly described and surveys are conducted by qualified personnel using standard methods that are consistent over time. Observers will undoubtedly encounter a variety of species, conditions and habitats during surveys, but counts are of little use if the species identification is dubious and the survey methodology varies from one day to the next or among sites. Thus, observers should be able to identify most, if not all, of the species likely to be encountered during a survey, including closely-related species that may be nearly identical, and different sexes and age groups within a species.

COMPLETE CENSUSES

The goal of a complete census is to conduct a total count of all the animals present over a specified area to obtain an unbiased estimate of abundance without statistical inferences or underlying assumptions. A reliable census is conditional on the assumption that all individuals present in an area can be recorded; therefore, censuses are most useful for conspicuous species occupying discrete and well-defined habitats. Some situations in which a reliable census may be possible include complete counts of herons and cormorants nesting in trees

FIGURE 6.1
Bird survey with a telescope

CREDIT: TAEJ MUNDKUR

along a wetland margin, waterbirds frequenting small open wetlands, or shorebirds at high tide roost sites in estuaries.

However, in many situations, such as where waterbirds are very numerous or tightly grouped or where time is limited, it may be necessary to estimate the number of individuals rather than to count every individual. Experienced counters can accurately estimate 10, 20, 50, 100 or more birds almost instantaneously, and scan through flocks counting in these units with a tally counter. It is preferable to estimate in small units (10 is probably the most commonly used unit); units of 100 or more are generally used for birds in flight or on nests (for colonial nesting species), and when time is limited.

A complete census is more practical when targeted at large and conspicuous species such as swans or geese and is the preferred method especially where there are active networks of participants to undertake the work. This kind of approach is promoted for periodic special census of swans by organisations such as Wetlands International/IUCN/SSC Swan Specialist Group at the regional level (*see for* e.g. Worden *et al.* 2006). For large-scale coordinated census of waterbirds, such as under the annual International Waterbird Census coordinated by Wetlands International (Delany 2005a, 2005b), all the birds of a selection of appropriate species, at a selection of suitable sites are covered, in a series of "look-see surveys" (*sensu* Bibby *et al*. 1998).

Achieving the ambitious goals of a conventional census count will often involve considerable logistic preparations. A large census area will usually need to be divided into smaller units that can be conveniently surveyed over time or by multiple field personnel at the same time. In the latter case, the survey team requires proper training in census techniques, species identification, accurate number counting or estimation, and use of field equipment (e.g. spotting scopes, Global Positioning System - GPS). In either case, the survey period should also be considered. Observers need enough time to thoroughly examine each survey unit, but not so much time that individuals of the target species move between survey units and are counted more than once.

The census area also needs to be accurately mapped and the entire area completely surveyed. Individual survey units should be easily discernable in the field because poorly defined unit boundaries may result in missing or double counting individuals. All habitats in the survey area which are suitable to the target species must be searched. Incomplete coverage (e.g. neglecting areas considered less suitable to the target species) may miss some individuals and introduce biases in the survey data.

Photographic or video images provide an efficient census technique that has been used increasingly in recent years. This involves producing a set of photograph or video images covering the entire area of interest (and all the animals within) which can be counted at a later time. Photographic and video surveys are usually conducted from aircraft, but any platform which provides unobstructed views of the survey area is suitable for conducting a census.

Photographic surveys must be conducted at a distance (or altitude) that produces images with sufficient resolution to permit species identification and distinguish individual birds in sometimes dense flocks or colonies, but not so close that the spatial relationship among images is lost. Concurrent ground- or boat-based surveys are advisable when conducting aerial photographic or video surveys to verify species identification and examine other potential biases.

SAMPLE PLOTS

In many studies, the time and effort required to conduct a complete and accurate census is prohibitive, usually because the area of interest is too large to adequately survey in a reasonable amount of time. In such cases, sample plots can provide data indicating species diversity and the abundance of each species within the study area. Sample plots are most amenable to ground-based observers because time is less of a limiting factor than in boat-based or aerial surveys, allowing for greater search effort dedicated to ensuring accurate counts and proper species identification.

Sample plots need not be limited to counts of actual birds and cannot be used for that purpose where birds move between sample plots during counts. Sample plots are most useful when the target species (or objects) are relatively immobile over the survey period, for example wading birds attending discrete roost sites. Specific applications of sample plots to AI-related wildlife investigations may include estimating waterbird nest densities or the number of carcasses at an H5N1 outbreak site.

The selection of sample plots should be carefully considered when designing a study because plot location can have a strong influence on population estimates. Consideration must be given to factors such as bird behaviour and heterogeneous habitats which may result in non-random animal distributions that require stratified sampling techniques. Details of more sophisticated sample plot design and analysis techniques are beyond the scope of this Manual, but Bibby *et al*. (1998, 2000) provide useful references[9].

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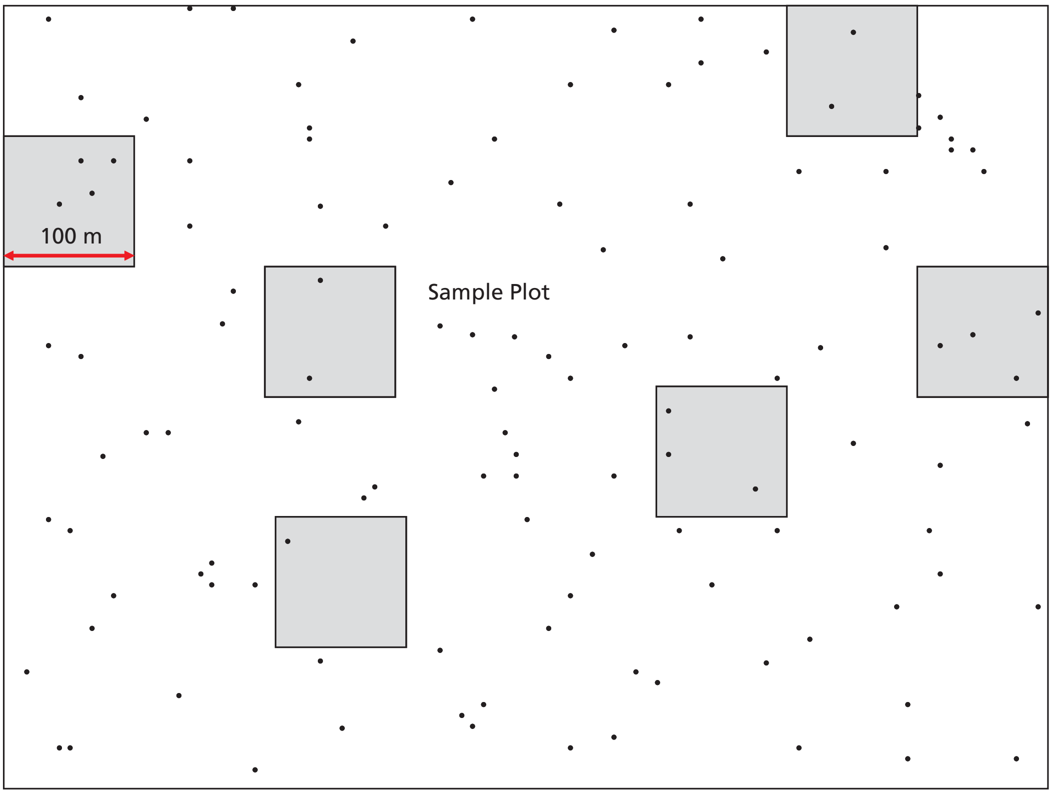
[9] A free download is available at http://conservation.bp.com/advice/field.asp#fsm.

FIGURE 6.2

Use of sample plots for estimating avian density and abundance



Note: Dots represent individual birds.

In the simplest applications, complete counts of all animals *(n)* in sample plots of known size *(a)* are conducted and the plot density is calculated as $d = n / a$. The average density *(D)* from all the plots can be calculated and extrapolated over the entire study area *(A)* to provide an estimate of total animal abundance *(N = D / A)*, although more sophisticated means of determining average density by examining variability in sample plots may be desirable.

Figure 6.2 illustrates a simplified example of the use of sample plots to determine waterbird nest density and abundance.

Actual density in this hypothetical population of 120 nests distributed over 0.48 km^2 is 250 nests km^{-2}. A total of 16 nests are detected in the six randomly chosen 100 m^2 plots for an average density of 267 nests km^{-2} (16 nests / 0.06 km^2) and an abundance estimate of 128 nests (267 nests km^{-2} x 0.48 km^2) over the entire study area.

The accuracy of density estimates will increase as survey effort (the number or size of the plots) increases. In the above example, sampling a single 100 m^2 plot could result in densities ranging from 0 to 800 nests km^{-2}. The size and number of sample plots will depend on the effort required to detect individuals of the target species. Intuitively, more or larger plots can be established for species that are easier to detect and require less search time per individual, thus moving closer to the conditions of a complete census.

Sample plots need not be square (quadrats), although regularly shaped plots (e.g. square or circular) are usually easier to demarcate and search. If plots are to be repeatedly surveyed, boundaries should be marked and coordinates recorded with a GPS unit.

STRIP TRANSECTS

Strip transects are one of the most commonly used survey techniques for determining avian species composition and density. Essentially, strip transects are modified versions of a sample plot in which the observer performs counts while traveling along a fixed transect line instead of searching over an entire plot.

Transects are randomly located, often within stratified sub-areas of the total study area, to obtain representative samples of the species and numbers of each species present. If density estimates are desired, the counts are limited to objects within a fixed distance of the transect line. In such cases, the sampled plot becomes a rectangular strip extending a specified distance on either side of the transect line.

Strip transects have been adapted for a variety of species and habitats that have direct applications to AI-related studies. Aerial and boat-based strip transect methodologies have been specifically developed for conspicuous aquatic species and these techniques have become the preferred survey method in large open water habitats. Aerial strip transects can be established to assess the distribution and abundance of waterfowl over broad geographic areas where waterfowl habitat overlaps with poultry production, agricultural fields and other potential H5N1 outbreak zones. Over smaller scales, ground-based strip transects established along the interface between waterbird habitats and poultry operations can identify particular species likely to bridge these habitats.

As for sample plots, the density from a strip transect plot can be extrapolated over the study area to obtain an abundance estimate. Figure 6.3 illustrates a simplified example of a 50 m strip transect (extending 50 m on each side of the line).

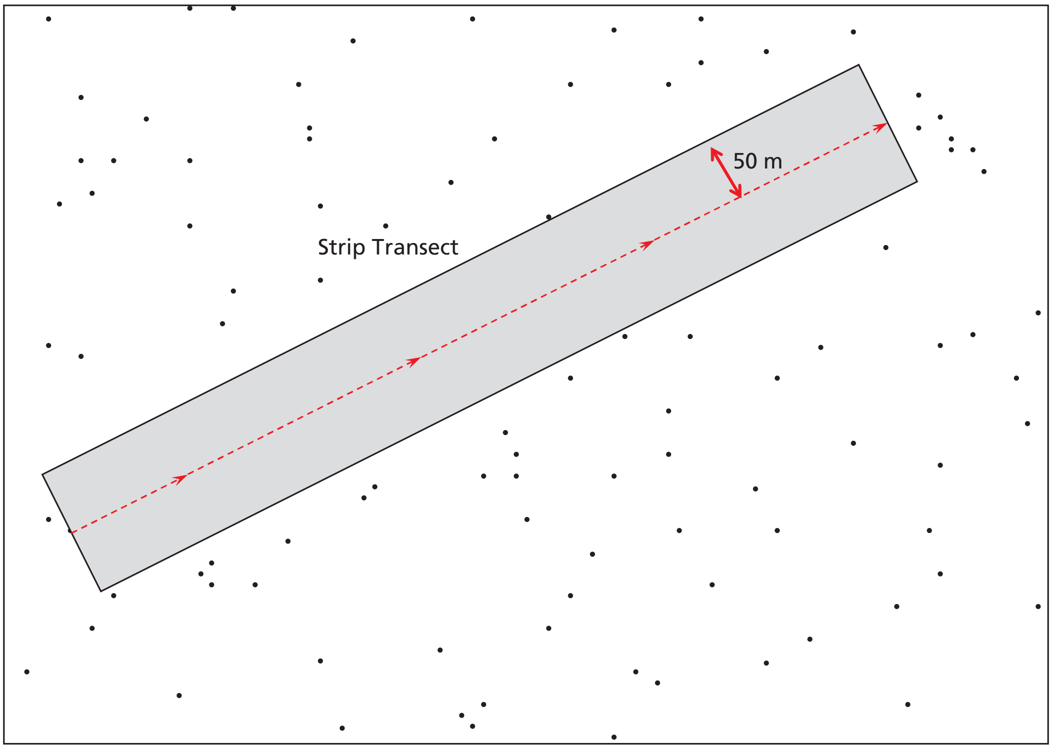
As in the previous example, actual density is 250 animals km^{-2}. A total of 17 animals are detected within the 700 m long by 100 m wide transect for a density of 243 animals km^{-2} (17 animals / 0.07 km^2) and an abundance estimate of 117 animals (243 animals km^{-2} x 0.48 km^2) over the entire study area.

In practice, strip transect methodology is rarely as simple as the above example suggests, and several factors must be considered before surveys can be conducted. If density estimates are desirable, choice of the appropriate strip transect width is a compromise between maximising detection probability for the target species and surveying as large an area as possible. Intuitively, detection probability (and strip transect width) increases for large, conspicuous species in more open habitats. Obviously, it is senseless to establish a 400 m wide strip transect to count tiny sandpipers foraging in a vegetated wetland, just as it is inefficient to use a 50 m strip transect to survey large and conspicuous swans on a lake.

Like sample plots, density estimates from strip transect surveys operate on the assumption that all animals within the plot are detected, thus surveys are best conducted in open habitats where visibility is unobstructed. However, unlike sample plots, the observer does not usually leave the transect line to search the plot, thus complete detection of all animals in the plot may be difficult to achieve. Binoculars (image-stabilised models are best) are commonly used during ground- and boat-based strip transect surveys to aid visual detection and species identification, but visual aids are of little use during aerial surveys.

The ability to make quick and accurate assessments of bird locations in relation to survey boundaries is imperative for reliable density estimates. Errors in estimating bird location

FIGURE 6.3
Use of strip transects for estimating avian density and abundance



Note: Dots represent individual birds.

relative to the transect line can have a considerable effect on density estimates. In the illustrated example (Figure 6.2), counting three individuals located just outside the boundary results in a density of 287 animals km^{-2}, while excluding three just inside the boundary yields 200 animals km^{-2}.

Consistent assessments of bird location in relation to the boundary require that aerial surveys be conducted at the same altitude and boat-based observers are stationed at similar heights above the water (and these parameters are accurately recorded). Aids to distance estimation, such as range finders or markings on airplane windows or wing struts, are helpful for calibrating the observer's eye during the training period, but reliance on these aids often distracts from the primary task of identifying and counting birds.

Strip transects can be conducted by observers on the ground, in boats or in aircraft. Aerial surveys offer far greater spatial coverage (and incur much higher costs) compared to ground- and boat-based surveys, although the extended range sometimes comes at the expense of accuracy, as the speed of the aircraft limits observation time and may make accurate counts and species identification more challenging. In fact, performing a good aerial survey requires specific training and experience.

If biases among survey platforms are suspected, concurrent counts using different survey methods are advisable (triangulation of the data and information). For example, observers on aerial surveys may be more likely to miss single birds or birds of a particular species. Ground-based surveys ("ground truthing") conducted concurrently with aerial surveys can often detect these biases and, if biases are consistent over a number of replicates, a "correction factor" based on the average ratio of counts between the survey types can be determined to account for birds likely missed by aerial observers.

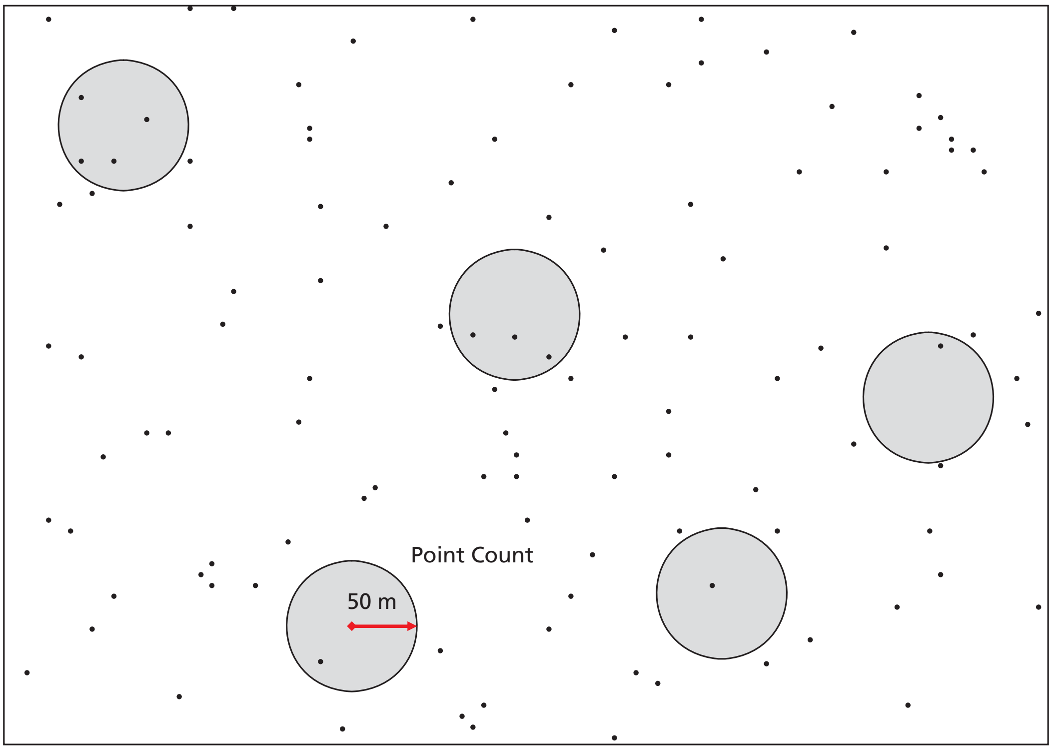
POINT COUNTS

Point counts are another of the most commonly used survey techniques for determining avian species composition and abundance. Point counts are essentially strip transects of zero length in which the observer performs the count in a 360° arc around a fixed survey station. Survey stations are randomly located throughout the study area to obtain representative samples of the species and numbers of each species present. If density estimates are desired from point counts, the counts are limited to objects within a fixed radius from the survey point. In such cases, the sampled plot becomes a circular plot of specified radius from the survey point (Figure 6.4).

As related survey techniques, many of the issues discussed for strip transects also apply to point counts. However, some important differences should be noted. Unlike strip transect surveys, point counts are usually conducted for a pre-determined and fixed period time, usually after allowing for the avian population to come to "rest" before the survey begins. Point counts are limited to ground- and boat-based surveys because observers must remain at the fixed count station.

Point count surveys have been developed for a variety of species and habitats which may not be effectively surveyed with other survey techniques. Point counts are especially useful in difficult terrain where it is not be possible to establish practical transects or per-

FIGURE 6.4
Use of point counts for estimating avian density and abundance



Note: Dots represent individual birds.

form counts while travelling along the transect line; for example ground-based surveys of wetland birds in shallow marshy habitat with soft substrates, or surveys in steep terraced agricultural fields.

Because point count observers are sedentary, they may be more likely to detect shy species that would otherwise hide and escape detection when mobile and conspicuous strip transect observers approach. Thus, point counts can be used to inventory shy and retiring "bridge" species in the immediate vicinity of poultry farms and disease outbreak sites.

Point counts based on vocal cues have been developed for situations where visual cues are limited, such as nocturnal surveys or heavily vegetated habitats. For some species, vocal cues may be the only reliable means of detection; for example, most counts of secretive rails in heavily vegetated marshes have relied on vocal cues for determining their presence and abundance. However, distances from the point count station are often difficult to determine from vocal cues, making density estimates problematic.

DISTANCE SAMPLING

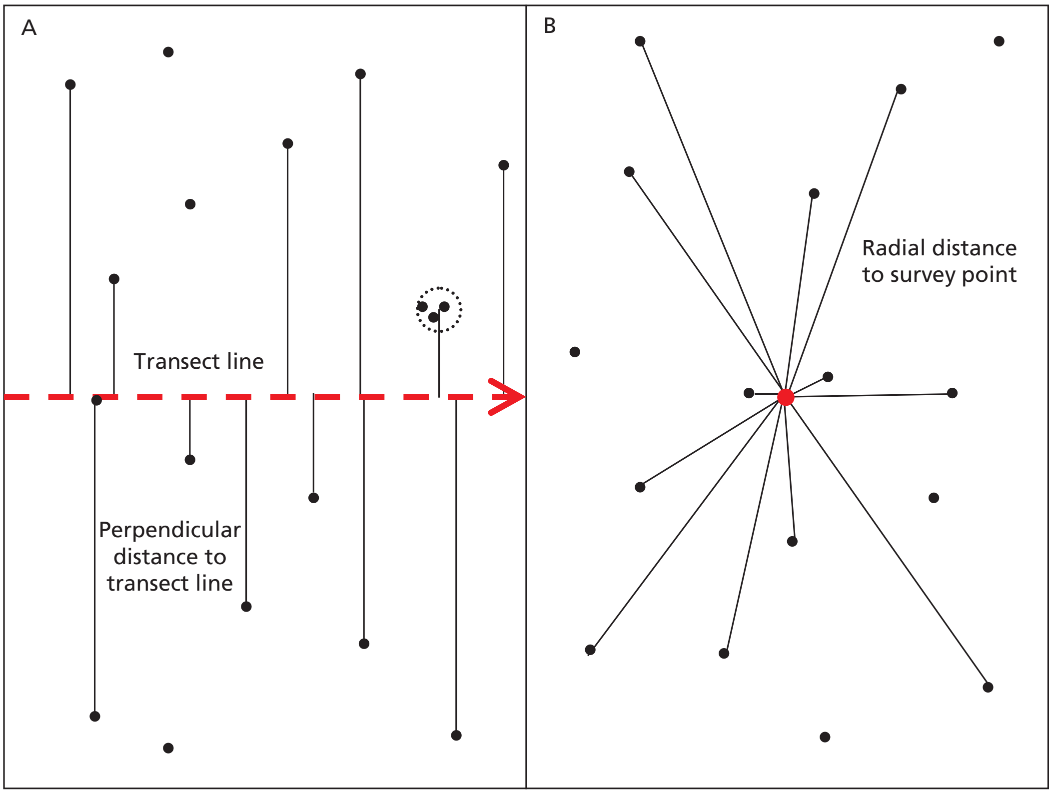
Several studies have demonstrated that a significant proportion of animals within a defined plot are overlooked during strip transect and point counts, particularly those located at distance from the transect line or survey point. Distance sampling offers an alternative to these techniques that takes into account the decreasing probability of detecting animals as distance from the observer increases. In theory, distance sampling provides more reliable density estimates and should be considered when reliable absolute density or abundance estimates (as opposed to relative measures) are important objectives of the study.

Distance sampling survey techniques are similar to strip transect and point counts, with one major exception; distance data (recorded as perpendicular distances from the transect line or radial distances from point count station) are recorded for each animal (or group of animals) observed (Figure 6.5).

Unlike strip transect or point counts, distance sampling does not assume that all individuals within a defined area are detected, but three assumptions need to be satisfied before distance sampling methodology can be used: 1) all objects on the line or point must be detected; 2) objects must be detected at their initial location, prior to any movement in response to the observer; and 3) distances must be measured accurately. In addition, a sufficient sample of observations is needed to model the detection function adequately. However, if the above assumptions and sample requirements can be met, then it is likely that distance sampling will yield more reliable population estimates than analogous estimates from strip transects and point counts.

The computer software program DISTANCE (Thomas *et al.* 1998) uses distance data to generate a detection function that models the decreasing probability of detecting an object as distance increases. DISTANCE is a very user-friendly program and offers a variety of input and analysis options, although a detailed review of distance sampling methodology is beyond the scope of this Manual. An excellent introduction to distance sampling by Buckland *et al.* (2001) provides background information and discussion of relevant issues such as model selection, data grouping and truncation, counting groups versus individuals and much more.

FIGURE 6.5
Use of distance sampling for estimating avian density and abundance



Note: Dots represent individual birds

CAPTURE-MARK-RECAPTURE

Capture-mark-recapture (CMR) studies have a long history of use for estimating population abundance, and a considerable body of literature has been dedicated to the use of CMR models. The basic theory underlying CMR modelling, in its simplest form, can be summarised as follows. Within a closed population of animals *(N)*, two samples (*n1* and *n2*), are captured, marked and released at times 1 and 2, such that the number of marked animals recaptured at time 2 (*m2*) can be accurately determined. Intuitively, the proportion of marked animals recaptured in the second sample (*m2 / n2*) should equal the proportion of the total animals captured at time 1 in the total population (*n1 / N*), or alternatively *N* = *n1 n2 / m2* , where *N* equals the total population size.

This basic model, the Lincoln-Petersen model, makes several assumptions that very few natural populations can meet. However, a number of modifications on this basic theme have been developed to permit CMR analyses even when the basic assumptions above are violated.

An in-depth discussion of all the different models is beyond the scope of this Manual, but references to several useful reviews are included at the end of the chapter for those seeking further information on CMR modelling. The computer program CAPTURE (Rexstad and Burnham, 1991) includes modifications of the Lincoln-Petersen model that provide population estimates with CMR data which account for unequal capture probabilities. The Jolly-Seber model is the basic CMR model for population estimates of open populations.

Programs which provide Jolly-Seber population estimates from CMR data include POPAN (Arnason and Schwartz, 1999), JOLLY (Pollock *et al*. 1990) and MARK (White and Burnham, 1999).

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Chapter 7
Radio telemetry and bird movements

RADIO TELEMETRY

Understanding the role that wildlife play in the ecology of AI viruses requires knowledge of the detailed movements of wild birds over varying spatial scales. On the one hand, concurrence between the migratory patterns of some Palearctic breeding waterbirds and the spread of the H5N1 HPAI virus across Asia and Europe in the northern fall and winter of 2005/06 illustrates the importance of studies designed to identify specific migratory routes, stopover points and non-breeding areas that may span entire continents. On the other hand, studies documenting the local movements of wild birds between poultry farms and nearby wetlands may be invaluable to establish viable pathways of H5N1 HPAI transmission from poultry to wildlife (or vice versa).

Radio telemetry is a technique for determining bird movements over areas ranging in size from the restricted breeding territories of resident bird species to the movement patterns of international migratory species (reviewed in Fuller *et al*. 2005). Radio telemetry has important applications in the investigation of infectious diseases of migratory species, including H5N1 AI virus ecology. Specific objectives for AI-related telemetry studies have already been identified during the FAO-OIE International Scientific Conference on Avian Influenza and Wild Birds in May 2006[10]. In fact, telemetry projects tracking the local movements and migration routes of wild birds identified as potential virus hosts are already under way[11].

The basic concept of a radio telemetry study sounds simple; attach a radio transmitter to an animal and track the signal to determine the animal's movements. Because radio-marked birds can be relocated more frequently and consistently than those marked by other methods, telemetry can provide a history of detailed movements that is not possible with simpler mark-recapture or mark-resight studies. However, while it may be tempting to radio-mark a sample of animals just to "see where they go", the fact is that radio telemetry is a rather expensive proposition compared to mark-recapture/resight studies, and a successful telemetry project requires careful consideration, thorough planning, and specific objectives.

Having identified achievable objectives, several issues regarding the proposed telemetry project need to be addressed, including, but not limited to: 1) the type and size of radio transmitter; 2) the least invasive attachment technique; 3) capture and marking of the radio-transmittered sample; 4) the optimal tracking technique(s); and 5) data analysis

[10] www.fao.org/avianflu/en/conferences_archive.html
[11] www.fao.org/avianflu/en/wildbirds_home.html

options. Entire books have been dedicated to the subject of planning and conducting radio telemetry studies, so a thorough discussion of all the relevant issues is obviously beyond the scope of this Manual. The reader is instead directed to excellent reviews by Kenward (2001) and Fuller *et al.* (2005) for more detailed discussion of radio telemetry techniques.

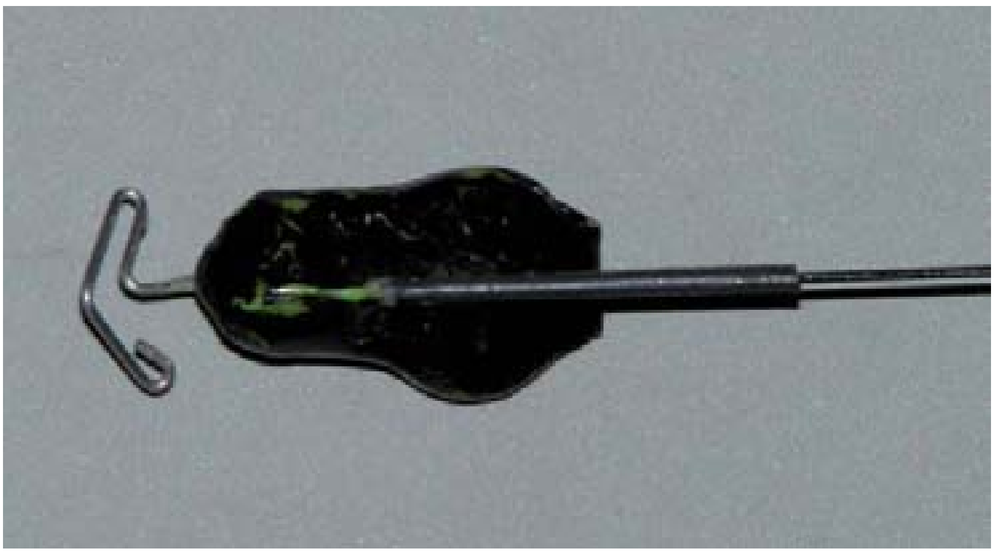
The capture, handling and marking of wild birds are activities strictly regulated in most countries. Researchers should always be aware of and comply with local and national laws regarding these activities and obtain all the required local, state, provincial and federal permits.

Radio transmitters

In the past, radio transmitters were simple very high frequency (VHF) transmitters attached externally to the bird or implanted (Figure 7.1) with an accompanying power supply, antenna and mounting material. Recent technological advances have resulted in the development of Platform Terminal Transmitter (PTT; Figure 7.2) and Global Positioning System (GPS; Figure 7.2) transmitters with capabilities far beyond those of conventional VHF radio transmitters. While modern PTT and GPS transmitters operate using the same basic principles as VHF radio transmitters (emission of an electromagnetic signal at a specified frequency which is detected by receivers tuned to the frequency), these more advanced transmitters use orbiting satellites to receive and relay transmitter signals. Thus, VHF, PTT and GPS transmitters have very different characteristics which render them suitable for very different species and studies (Table 7.1).

The size of the transmitter in relation to the bird can be a limiting factor when considering PTT and GPS transmitters. The general rule of thumb is that a radio transmitter should not exceed 2-3 % of the bird weight, although this may increase to 3-4 % for smaller birds (<50 g). Using this measure, VHF radio transmitters are available for all but the smallest bird species, with the smallest transmitters weighing slightly less than 1 g. In contrast, the

FIGURE 7.1
Implanted VHF radio transmitter



CREDIT: DARRELL WHITWORTH

FIGURE 7.2
Platform terminal transmitters (PTT; three on the left); Global Positioning System (GPS) transmitter (one on the right)

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TABLE 7.1
Characteristics of radio transmitters used in avian telemetry studies

	Radio transmitter type		
	VHF	**Satellite (PTT)**	**Satellite (GPS)**
Transmitter wt.	< 1 g to 12	12-18 g	30-60 g
Species	> 20 g	> 500 g	> 1 kg
Minimum cost	US$ 100/ea	US$ 3,200/ea	US $3,800/ea
Attachment	Anchor, feather, implant	Collar, backpack, implant	Collar, backpack, implant
Power source	Battery	Battery or solar	Battery or solar
Duration	Days to months*	Months to years	Months to years
Range	0.1 to 100+ km*	Unlimited	Unlimited
Tracking	Manual	Satellite	Satellite
Tracking interval	Continuous*	4 hours	Continuous
Accuracy	± 5 m to 1 km*	±100 to 200 m	±10 to 20 m
Frequency	VHF	UHF	UHF

* Depends on the size of the transmitter and the tracking method employed.

smallest PTTs weigh 12-18 g, limiting their use to species weighing 500 g (e.g. small ducks and gulls) or more. GPS transmitters weigh 30-60 g, thus can only be used with large species weighing 1 kg or more (e.g. geese and swans).

PTT location accuracy is generally good (within 100–200 m) for most uses, although the larger and more expensive GPS transmitters greatly increase location accuracy (10-20 m). Location accuracy for VHF transmitters will depend mostly on the tracking method used and the effort expended. If radio-marked birds are tracked and visually sighted, locations can be pinpointed to within 5 m, but in many cases visual sightings are not possible and locations are estimated with varying degrees of accuracy using techniques specific to the tracking method (see *VHF Telemetry Tracking*).

A variety of useful options can be incorporated into VHF and satellite-based radio transmitters, although these options invariably increase the weight, power consumption and cost of the transmitter. Activity, temperature, pressure, and mortality sensors convey data by changing the transmitter pulse rate. Timers programmed to switch the radio transmitter on and off at specified times are a particularly useful energy-saving option. Timers can switch transmitters on to coincide with pre-scheduled tracking periods or times when predictable orbiting satellites are scheduled to pass overhead.

There are considerable differences in cost between satellite-based and VHF transmitters (Table 7.1) that may prevent the use of the PTT and GPS transmitters in projects with limited funds. However, PTT and GPS transmitters eliminate the need for expensive tracking equipment and personnel.

PTT and GPS transmitters

Despite their size and cost, if PTT or GPS transmitters are suitable for the species to be radio-marked, the logistic advantages compared to conventional VHF telemetry are considerable. PTT and GPS tracking is automated and conducted by satellite systems. Because PTT and GPS signals are received by orbiting polar satellites, there are no spatial tracking biases because the signals can be received from anywhere in the world, including remote or inaccessible areas where marked birds may be otherwise undetectable.

Fortunately, PTTs are suitable for radio-marking waterbirds and several other large species (>500 g) vulnerable to the H5N1 HPAI virus, thus satellite telemetry has broad applications in AI-related wildlife studies. Satellite telemetry provides opportunities to follow the movements and migratory routes of waterbirds that are not possible with any other technique. PTT transmitters provide a nearly continuous history of a bird's movements, revealing detailed information concerning the migration route, rate of travel and stopover duration during flights that may cross entire continents.

The longevity of solar-powered satellite-based transmitters allows long-term studies that can determine annual fidelity to specific migration routes and stopover points, data which may help identify high-risk disease outbreak zones. The accuracy of PTT and GPS transmitter locations also permits analysis of spatial and temporal habitat use, including possible overlap with poultry production and disease outbreak sites.

Strategies in which a few individuals are marked with PTTs and larger samples with conventional VHF radio transmitter or other marks (see *Mark-Recapture (Resight) Studies*) can increase sample sizes and mitigate the high costs of PTT transmitters.

VHF radio transmitter

Many smaller species susceptible to the H5N1 HPAI virus, including shorebirds, cormorants, rails, coots, grebes, corvids and sparrows, as well as the smaller ducks, gulls, raptors, herons and egrets, are not suitable candidates for satellite telemetry studies, either because they are diving birds or are too small (<500 g) to accommodate PTTs. The current limitations of satellite-based telemetry technology leave VHF transmitters the main option for these species.

Studies examining the long-range migratory movements of these species with VHF telemetry have been conducted, but are logistically difficult because they require the mobilisation of telemetry tracking teams across vast areas, many of which may be inaccessible to observers on the ground. Therefore, practical applications of VHF telemetry to AI-related studies are more likely to address issues such as the local movement of birds to assess their utilisation of areas where risks of exposure to AI viruses may be increased, such as on farms.

VHF telemetry studies require considerably more logistical planning than satellite-based studies, mainly due to the manual tracking efforts that are required. The need for manual tracking makes VHF radio transmitter features such as transmitting power (range) and operating life important considerations. Radio transmitter batteries are finite power sources, thus there is a direct trade-off between the range and operating life; increasing range decreases life and vice versa. The optimal compromise between the two depends on study objectives.

The transmitting range of the radio transmitter greatly affects the search effort required to locate the signal, thus range should be increased (at the expense of transmitter life) if the species is expected to move over a large area. Conversely, if the species is expected to remain in a relatively confined area, search effort is reduced and the range can be decreased, extending the transmitter life. Because the range and operating life are directly related to the size of the radio transmitter, the geographic scope and duration of telemetry studies on smaller species are limited compared to larger species.

VHF radio transmitters are available from a number of reputable vendors. The best advice we can offer is to read the telemetry literature and speak to knowledgeable and experienced researchers to determine which type is preferred for the species of interest. It should be noted that radio transmitter features (e.g. frequency, pulse rate, power and duration) are specified when the transmitters are ordered and are difficult if not impossible to modify once the transmitter is constructed.

CAPTURE AND RADIO-MARKING

It is generally assumed that radio-marking will have some effects on the animal, but efforts can be made to minimise marking effects so that they do not disrupt the normal movements and behaviour of the marked individual. This is good for the marked animal, and it is also good for the study. Detrimental effects of radio-marking can be reduced by: 1) minimising capture and handling time; 2) using the smallest possible radio transmitter suitable for the objectives of the study; and 3) using the most inconspicuous and best fitting attachment method available.

Capture techniques have already been discussed (*see Chapter 3*) and it is assumed that a reliable technique has been identified and preferably field-tested before actual radio-marking is conducted. Well-planned efforts will help minimise the time in captivity and the stress associated with capture and radio-marking. Having said this, a brief observation period in a quiet secluded holding area is advisable after radio-marking to allow the bird to recover from the procedure (especially if anaesthetics are used) and to detect any problems before release.

To minimise time in captivity, marking procedures should be conducted at or as near as possible to the capture site. If possible, schedule captures to avoid periods when birds may already be physiologically stressed, such as breeding or migration. If bird movements during these sensitive periods are of interest, attempt to capture and mark individuals a few weeks before the event, when handling is less likely to disrupt breeding or migratory behaviour. This also gives the bird time to recover from capture stress and become accustomed to the transmitter before nesting or migration begins.

The long-term effects of radio-marking on an animal depend largely on the radio transmitter itself and method used to attach it. Intuitively, larger and more cumbersome transmitter/attachment packages are more likely to have greater negative effects. There is always a tendency to use the largest radio transmitter suitable for the species of interest, regardless of the study objectives; however, the use of smaller transmitters is strongly advised if they meet the objectives of the study, because they are less disruptive and less costly.

External transmitters undoubtedly increase aerodynamic drag during flight (and hydrodynamic drag for diving species) and several studies have documented decreased survival, reduced reproductive success, lower chick feeding rates and other detrimental effects. Ideally, the transmitter would remain attached for the duration of study, then fall off soon

FIGURE 7.3
Telemetry transmitter attachment using a neck collar

CREDIT: USGS WESTERN ECOLOGICAL RESEARCH CENTER afterwards, but this is seldom the case. Transmitter retention for the duration of the study is never guaranteed, regardless of the method used.

External attachment techniques have been developed that indirectly attach transmitters to birds via a neck collar (Figure 7.3), backpack harness (Figure 7.4) or leg band (Figure 7.5). Neck collars and backpacks generally have excellent transmitter retention (often for the life of the bird) and are currently the only attachment methods available for PTT and GPS transmitters. Various harness designs are available that provide better fits to particular species because poorly fitting harnesses may cause abrasions or impede wing movement. VHF transmitters attached with leg bands also have excellent retention, but problems with transmission range have been noted, possibly due to the shorter antennae and proximity to the ground.

Other external radio-marking techniques use adhesives (e.g. glue, tape, epoxy, resins, etc.), sutures and stainless steel prongs (Figure 7.6), either alone or in combination, to attach VHF radio transmitters directly to the bird. Transmitter retention is generally good for a few weeks to a few months (rarely longer); although a certain amount of early transmitter loss should be expected when using these techniques. Care should be exercised when applying adhesives because some are known tissue irritants. The use of sutures and prongs requires relatively simple medical procedures, but they are still invasive techniques and the assistance of qualified veterinarians is highly recommended until the researcher gains some experience with these methods.

External radio marks may disrupt behaviour for short periods as the bird adjusts to the transmitter, and some species will not tolerate the transmitter at all. For species intolerant of external radio transmitters, abdominal or subcutaneous implants are an option. Radio

FIGURE 7.4
Telemetry transmitter attachment using a backpack harness

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FIGURE 7.5
Telemetry transmitter attachment using a leg band

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FIGURE 7.6
Telemetry transmitter attachment using a subcutaneous prong and suture

CREDIT: SCOTT NEWMAN

transmitter implants involve highly invasive surgical procedures and are best left to qualified veterinarians or wildlife biologists specifically trained for the technique.

Again, the best advice is to read the telemetry literature and speak to knowledgeable and experienced researchers to determine which attachment technique has proven most effective for the species of interest. Field trials marking small numbers of birds are always helpful to identify detrimental radio-marking effects and potential transmitter retention problems before costly projects begin.

VHF TELEMETRY TRACKING

It is sometimes assumed that the difficult part of a telemetry project is finished once the radio-marked animals are roaming freely, waiting to be located. While this may be true for satellite telemetry studies, VHF telemetry studies require considerable search effort devoted to finding and determining location coordinates for the transmitter-marked samples. All the expense and effort that goes into radio-marking a sample of birds is wasted if effective telemetry tracking techniques are not employed.

Telemetry tracking employs a VHF receiver (Figure 7.7) connected by coaxial cables to a receiving antenna(e) to search for signals emitted by the radio transmitters. The most practical receivers allow the user to programme the desired frequencies into the unit, scan for signals at pre-set intervals, and stop the scan when a signal is detected. Adjustable volume and gain (power to receive the signal) controls are also useful. Some models have jacks to attach headsets, a particularly important option to block extraneous noise when conducting aerial surveys. Advice from experienced researchers is invaluable when considering the different receiver models available.

The most important features to consider with telemetry antennae are portability and directional capability. Directional capability is a result of the reception pattern of the antenna in which noticeable peak and null signals are heard depending on the orientation of the antenna in respect to the signal source. The most common antennae used in avian telemetry studies are the Adcock H and Yagi antennae (Figures 7.8, 7.9 and 7.10).

The H antenna has less directionality compared to the Yagi, but has only two elements, thus is smaller and easier to use when tracking on foot. The Yagi has the best directional capability of all the common telemetry antennae, but with a number of long cross ele-

FIGURE 7.7
VHF receiver and switch box used for aerial tracking in conventional telemetry studies

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ments, is also the most cumbersome. Yagi antennae are used primarily on masts mounted onto vehicles, at fixed receiving stations, or attached to the wing struts of aircraft.

Telemetry tracking surveys are most often conducted from ground-based or aerial platforms, but the methods for determining location coordinates differs between the two platforms. Aerial surveys are conducted with a single receiver attached to two directional antennae mounted on either side of the aircraft. The receiver is set to scan frequencies

FIGURE 7.8
Four-element Yagi antenna mounted on aircraft strut

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FIGURE 7.9
Yagi antennae mounted on a tower at a remote tracking station attached to a data logger

CREDIT: SCOTT NEWMAN

FIGURE 7.10
Hand-held Adcock H antenna

CREDIT: SCOTT NEWMAN

through both antennae as the observer(s) listen through a headset. When a signal is detected, the observer switches back and forth between antennae with a switch box to determine on which side of the aircraft the signal is located and directs the pilot to manoeuvre the aircraft accordingly. As the signal is "boxed" in with a series of turns, the signal becomes progressively stronger until it is the same on either side of the aircraft, at which point the location coordinates are recorded.

Ground-based surveys, conducted on foot or in a vehicle, utilise a technique called "triangulation" to accurately locate signals. Scanning from a fixed location with known position coordinates, the signal is detected with a directional antenna and the bearing to the point of the strongest signal is recorded. Shortly thereafter, the procedure is repeated at another nearby location. When the bearings from the fixed listening stations are plotted, two intersecting lines are produced that indicate the approximate signal location. Some vehicle-based systems increase directional capability by using two precisely-configured Yagi antennae mounted on a mast.

A combination of aerial and ground-based (or shipboard) surveys is often the most effective and cost-efficient tracking strategy. Aerial surveys offer more extensive spatial coverage and greater signal reception range, but locations are less accurate and surveys are more expensive. By contrast, ground-based surveys provide more accurate locations, often allow observations of marked individuals and are less costly. Utilising the strengths of both techniques, aerial surveys can be used to approximately locate signals over a large area and direct ground-based surveys for more accurate locations. Although ground reception range is limited compared to aerial surveys, scanning from hills, towers and other elevated points will greatly increase the range.

Programmable data loggers are data storage devices that are attached to or incorporated into receivers and allow remote tracking from fixed receiving stations. Data loggers are most useful for recording the presence/absence of marked birds within a restricted area and could have important applications for AI-related studies, such as continuous monitoring for the presence of marked birds in poultry farms or near disease outbreak sites.

Like receivers, data loggers have an internal battery, but external power sources (e.g. solar panels or 12-V batteries) can greatly extend the time between maintenance visits. Data loggers can be programmed to conduct continuous scans or scan at pre-set intervals to save battery power. Data can be downloaded directly into laptop computers in the field.

With the availability of reliable, accurate and affordable GPS units, the days of marking telemetry locations on topographic maps are just about over. Hand-held GPS units are particularly useful for marking the location coordinates of radio transmitter-tagged animals or monitoring stations, and delineating areas covered during telemetry tracking surveys. Their ease of use, portability and compatibility with most spatial analysis software makes GPS units required equipment for any radio telemetry study.

DATA ANALYSIS

Since its inception as a wildlife tracking technique in the early 1960s, radio telemetry has been used to study local movements, dispersal and migration routes, estimate home ranges, habitat use and selection, estimate population abundance, examine intra- and inter-specific relationships and estimate survival. Analysis of animal movement and distribution has become a sophisticated science in itself, and the details of specific analysis techniques are best found in reviews by White and Garrott (1990) and Fuller *et al.* (2005).

Fortunately, from the perspective of AI ecology, the primary use of radio telemetry data is relatively straightforward; examining movement and habitat preferences of potential host species that may acquire and transmit the viruses, determining the potential overlap between wild bird and poultry habitat use, and determining if wild bird movements temporally coincide with new outbreaks in wild birds or poultry. For example, telemetry data can establish current migration routes of waterbirds to reveal possible temporal and spatial relationships between their movements and patterns of AI outbreaks in wildlife and poultry. This could be accomplished simply by plotting telemetry locations together with disease outbreak data and visually inspecting the resulting map. However, care should be taken when designing telemetry studies to make sure that the observed movements are representative of the population, because different portions of a population (sexes or age cohorts) may exhibit different movement patterns.

Over smaller scales, using telemetry data to establish the local movements and habitat preferences of wild birds might involve home range analyses to examine direct overlap with poultry farm operations, as well as indirect exposure to infectious materials such as poultry farm waste runoff into wetlands. Home range analysis uses telemetry locations to describe an animal's spatial distribution over a specified time. Home range analysis could be as simple as connecting locations to form a minimum convex polygon that theoretically encompasses the animal's total area of use. Or it may involve complicated probabilistic models reflecting differential use patterns over an area (e.g. adaptive kernel home range) that require sophisticated geographic information system (GIS) programs.

Familiarity with GIS is by now an indispensable skill for those working with animal movement and spatial data. The program Arcview GIS, among others, offers a vast array of options which allow the user to plot locations, quickly calculate distances and movement rates, and perform movement, home range, habitat use and a variety of other spatial analyses. GIS programs also have sophisticated mapping capabilities which permit visual and statistical analyses of relationships between marked birds and habitat or climatic variables.

The availability of high quality satellite imagery of the earth's surface through computer programs such as Google Earth[12] with add-on capabilities will allow the user to plot GPS locations and visualise movements of birds in relation to their environment.

MARK-RECAPTURE (RESIGHT) STUDIES

Prior to the advent of radio telemetry technology, studies of animal movements were conducted using mark-recapture or resighting techniques. Mark-recapture (or resight) studies are conceptually simple and straightforward. Basically, animals are captured, marked for later identification, and released. Subsequent recaptures or resightings, depending on the marking technique, provide information concerning the movements of the marked individuals. Mark-recapture studies are applicable to any bird species which can be safely captured and marked, and depending on the range of the species, can extend over extensive geographic areas limited only by the efforts of the research team.

The marking of wild birds is widely used to investigate the location-specific aspects of avian biology where large numbers of birds may be marked with a combination of colours and/or numbers to provide individual bird recognition. Individual marking provides a valuable tool to study movements of migratory waterbirds and has been increasingly used in conjunction with avian influenza surveillance. It is important that any planned marking project is approved through the responsible country or regional agencies to ensure that the proposed scheme will not conflict with other current or planned marking programmes.

There are well-coordinated marking schemes for different species in Eurasia through EURING[13], Africa through AFRING[14], the Asia-Pacific region[15], and a variety of schemes within the Americas.

The principal consideration when choosing a marking method is to avoid techniques that will adversely affect the health, survival, behaviour or reproductive success of the marked individual. Some techniques that are appropriate for one species may not be appropriate for another. Trial studies marking small samples may be warranted to assess

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12 Available for free download from http://www.earth.google.com.

13 http://www.cr-birding.be/

14 http://www.safring.net

15 http://wetlands.tekdi.net/colorlist.php

effects before marking large numbers of birds. As for all capture and handling activities, the marking of wild birds is strictly controlled in most countries and permits should be acquired from the proper local, state, regional, provincial and federal authorities.

Table 7.2 lists a variety of marking techniques and some important characteristics to consider when planning a mark-recapture study. Does the technique allow identification of marked individuals or are birds marked as a group? Is the technique invasive? Is recapture or resighting the most efficient means of obtaining the desired data? Answers to these questions will help determine the optimal marking technique.

Numbered metal leg rings (or "bands") are the most common and widespread method of marking birds. Rings are (or should be) placed on every bird that is captured and released into the wild. Numbered metal rings allow for individual identification of marked birds, but birds must be recaptured in order to read the numbers. A combination of metal and coloured plastic rings (Figure 7.11) has been used on a variety of long-legged bird species (e.g. shorebirds). The coloured plastic rings or flags permit individual recognition without the need for recapture. Rings and ringing techniques were discussed in more detail in *Chapter 4*.

Although birds marked with metal leg rings require recapture, rings are probably the least disruptive of the marking techniques described here. The other techniques result in conspicuous external marks that may be visible from a distance, but also may cause detrimental physical and behavioral effects. In fact, patagial and web tags require an invasive procedure in which the skin is punctured to attach the tag. Birds marked with patagial or web tags can be identified from a distance, but may need to be recaptured if the tag numbers are too small to read.

Neck collars (Figure 7.12), nasal discs (Figure 7.13), nasal saddles, and coloured leg bands or flags provide conspicuous marks that permit identification of marked individuals over long distances with the aid of binoculars or a spotting scope. With highly visible external marks, these techniques are especially valuable for local studies examining habitat

TABLE 7.2
Wildlife marking techniques commonly used in avian studies*

Marking Technique	ID	Invasive	Codes	Recapture -Resight	Duration
Leg Rings					
Metal	Ind	Non	Number	Recap	Life
Plastic, Darvic	Ind	Non	Colour	Resight	Months/Life
Neck collars	Ind	Non	Colour+number	Resight	Life
Nasal discs	Ind	Inv	Colour+shape	Resight	Life
Nasal saddles	Ind	Inv	Colour+number	Resight	Life
Streamers	Group	Non	Colour	Resight	Weeks
Flags	Ind	Non	Colour+number	Resight	Life
Plumage dyes	Group	Non	Colour	Resight	Weeks
Patagial tags	Both	Inv	Colour+number	Both	Life
Web tags	Both	Inv	Colour+number	Both	Life

* Characteristics for each technique include whether the mark permits identification of birds as individuals (Ind) or a group; invasiveness of the technique (invasive [Inv] or non-invasive [Non]); whether number, colour or shape codes are used to identify marked birds; whether data are acquired through recaptures or resightings; and duration of the mark.

FIGURE 7.11
Coloured leg rings used as a marker in mark-recapture or resight studies

CREDIT: ROB ROBINSON

overlap between domestic and wild waterbirds near open farm systems. In fact, many of these techniques are commonly used in waterbird studies. However, care should be exercised when attaching nasal discs or saddles because poorly fitting marks can easily become entangled in vegetation and their use is not recommended for diving species.

Plumage colouring agents provide conspicuous external marks that are often visible over very long distances but do not permit individual identification. Dye, paint or bleaches applied to feathers are generally most conspicuous on evenly coloured species; dark dyes for lighter birds and light dyes or bleaches for darker birds. Birds marked with dye, paint or bleaches should be visible until feathers are shed during the next body moult, thus timing of the marking relative to moult patterns is critical. Care should be exercised when applying colouring agents because they may irritate sensitive tissues.

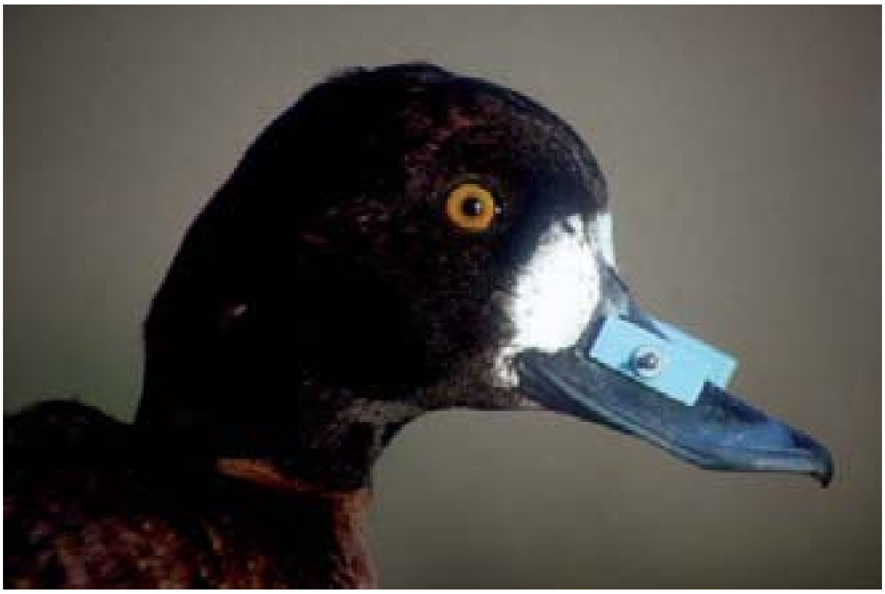
Coloured plastic streamers offer another conspicuous external mark that is visible over long distances, but does not permit individual identification. Plastic streamers and plasticised PVC tape attached to leg bands, neck collars or tail feathers provide a short-term mark that should degrade and fall off over time (weeks to months). Streamers should be cut to a length that is visible from a distance, but short enough to avoid entanglement in vegetation.

Most mark-recapture studies require the capture of sizable samples of birds, and several capture techniques were discussed in *Chapter 3*. However, creative remote marking techniques have been developed that avoid the stress that invariably accompanies capture and handling. Remote marking techniques for birds usually involve the application of non-toxic dyes or paints that colour the plumage when birds visit nest sites or water sources where the agent has been applied. These methods generally do not allow for individual identification of marked animals, but should be considered if group marking fits the objectives of the study. For example, dyes introduced into water sources in open poultry farms can be used

FIGURE 7.12
Neck collar used as a marker in mark-recapture or resight studies

CREDIT: NYAMBAYAR BATBAYAR

FIGURE 7.13
Nasal disc used as a marker in mark-recapture or resight studies



CREDIT: DARRELL WHITWORTH

to temporarily mark wild birds and determine if movements between farms and natural wetlands are occurring.

Mark-recapture studies require considerable post-release recapture or search effort, often over large geographic areas, to obtain the desired movement data. Marking should only be conducted if adequate resources are available to conduct follow-up surveys. Communication and coordination with other researchers and wildlife managers (always a good thing) to make them aware of the presence of marked individuals will maximise the recapture and resighting returns.

STABLE ISOTOPE ANALYSIS

The recent emergence of stable isotope analyses (SIA) has added a powerful tool to the study of broad avian migration patterns. The utility of stable isotopes (e.g. hydrogen, carbon, nitrogen) as indicators of avian migration patterns is based on the strong correlation between the concentration of some isotopes in the environment and the concentration of these same isotopes as they are assimilated in avian tissues, most notably feathers. Since some isotopes in the environment tend to demonstrate predictable patterns over continental scales, the concentration of isotopes in feathers can reflect the general location of the bird when moult and feather growth occurred. SIA requires sophisticated laboratory techniques that are beyond the scope of this Manual, but Hobson (1999) provides an excellent review.

The spatial resolution of SIA is probably in the order of many hundreds of kilometres on latitudinal scales and even greater longitudinally. Although SIA cannot be used to examine detailed movements or identify specific breeding sites, it can reveal broad migration patterns that do have applications in AI-related studies; for example, determining the general breeding areas of waterbirds captured on staging or non-breeding grounds, or collected at disease outbreak sites.

Despite its limitations, the SIA technique does have advantages. Birds only have to be captured once and need not be marked in any way to determine their broad scale movements. The SIA sampling procedure, removing a small number of feathers, is very simple and can be performed on any species regardless of size. SIA does not suffer the geographic biases associated with mark-recapture or VHF radio telemetry studies in which remote areas are rarely sampled. Although satellite telemetry does not suffer from similar geographic biases, it is very expensive compared to SIA.

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Annex A

Guidance on taking photographs of birds for identification purposes

(source: European Commission DG Sanco 2006)

The following simple guidance will assist non-specialists in taking photographs, especially of dead birds, that will allow subsequent identification to species. Different bird species are identified by differing characteristics, so it is difficult to provide universal guidance applicable in all situations. However, the following is a minimum standard that should be followed.

All wild birds collected for analysis for HPAI should have digital photographs taken as soon as possible after collection. The bird should fully fill the photograph[16] and wherever possible include a ruler or other scale measure. Photographs should be taken of:

- the whole bird, dorsal side, with one wing stretched out and tail spread and visible;
- the head in profile clearly showing the beak;
- close-up photos of the tips of wing feathers can often determine whether the bird is an adult or a juvenile (bird in its first year);
- ideally photographs of both dorsal and ventral views of the bird should be taken[17]; and
- any ventral photographs should show the legs and feet (since leg colour is often an important species diagnostic). If any rings (metal or plastic) are present on the legs, these should be photographed in situ as well as recording ring details.

Any conspicuous markings/patterns should be photographed.

In late summer (July - late August) many waterbirds and especially ducks and geese undergo moult and can be especially difficult to identify by non-specialists. At this time of year there is especially the need for clear photographs to aid identification of duck carcasses. The patch of colour on the open wing (called the "speculum") is often especially useful. The identification of young gulls at any time of the year is also difficult and typically they will also need to be photographed and identified by specialists.

Photographs should be retained, linked to an individual specimen, at least until laboratory tests are returned as negative for AI.

Photographs can be used immediately if identification of the species of bird is in any doubt, and for subsequent checking of the identification if necessary.

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[16] Each photograph should be taken at the highest resolution possible and if the camera has a 'date stamp' feature then this should be enabled so that the image is saved with a time reference – this may help verify the sequence of images taken at a site on a day. Images should be downloaded to a computer as soon as possible and information about location and date added to the file properties.

[17] Photographs of the upper and under surfaces of the wing and spread tail will facilitate aging and sexing of birds (*e.g.* Pintail *Anas acuta*).

FAO ANIMAL PRODUCTION AND HEALTH MANUALS

1. Small-scale poultry production, 2004 (E, F)
2. Good practices for the meat industry, 2006 (E, F)
3. Preparing for highly pathogenic avian influenza, 2006 (E)
4. Wild Bird HPAI Surveillance – A manual for sample collection from healthy, sick and dead birds (E)
5. Wild birds and Avian Influenza – An introduction to applied field research and disease sampling techniques (E)

Availability: December 2007

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4. Epidemiology, diagnosis and control of poultyr parasites, 1998
5. Recognizing peste des petits ruminant - A field manual, 1999 (E, F, A)
6. Manual on the preparation of national animal disease emergency preparedness plans, 1999 (E)
7. Manual on the preparation of rinderpest contingency plans, 1999 (E)
8. Manual on livestock disease surveillance and information systems, 1999 (E)
9. Recognizing African swine fever. A field manual, 2000 (E, F)
10. Manual on Participatory Epidemiology - Method for the Collection of Action-Oriented
    Epidemiological Intelligence, 2000 (E)
11. Manual on the preparation of african swine fever contigency plans, 2001 (E)
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13. Recognizing contagious bovine pleuropneumonia, 2001 (E, F)
14. Preparation of contagious bovine pleuropneumonia contingency plans, 2002 (E, F)
15. Preparation of Rift Valley fever contingency plans, 2002 (E, F)
16. Preparation of foot-and-mouth disease contingency plans, 2002 (E)
17. Recognizing Rift Valley fever, 2003 (E)

The highly pathogenic avian influenza H5N1 strain has spread from domestic poultry to a large number of species of free-ranging wild birds, including non-migratory birds and migratory birds that can travel thousands of kilometres each year. The regular contact and interaction between poultry and wild birds has increased the urgency of understanding wild bird diseases and the transmission mechanisms that exist between the poultry and wild bird sectors, with a particular emphasis on avian influenza. Monitoring techniques, surveillance, habitat use and migration patterns are all important aspects of wildlife and disease ecology that need to be better understood to gain insights into disease transmission between these sectors. This manual contains chapters on the basic ecology of avian influenza and wild birds, capture and marking techniques (ringing, colour marking and satellite telemetry), disease sampling procedures, and field survey and monitoring procedures.