NARRATIVE REPORT
WILDLIFE CONSERVATION SOCIETY – GLOBAL
AVIAN INFLUENZA NETWORK FOR SURVEILLANCE
(GAINS)

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GAINS Activities
Quarter 10- Months 28-30, July 1-September 30, 2008

HIGHLIGHTS FOR THIS QUARTER:

- Two studies were published on risk mapping of avian influenza. The first study provided a review of risk mapping techniques in the context of avian influenza[1] and the second provides the first quantitative assessment of high pathogenic avian influenza risk for decision makers in the southern Africa subregion, and indicates that major urban centers in South Africa would be greatest risk for future outbreaks[2]. The first article is included herein as Appendix III and both articles are available online here: http://www.ecologyandsociety.org/viewissue.php?sf=39.

- A successful surveillance mission was conducted at Kyzykol Lake in Kazakhstan in which field team members from several organizations including Wetlands International and the National Aviary placed 23 satellite PTT units on migratory water birds.

- A new version of the Wildlife Information System for Disease Observation and Monitoring (WISDOM) Map Explorer (WME 3.1) was released allowing the user a more customized experience.

- A WCS/University of Georgia collaboration demonstrated that Mongolian wild bird populations from areas of prior HPAI H5N1 outbreak have been exposed to H5 avian influenza viruses and therefore may be acquiring immunity to H5 subtypes of AI.

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[1] *Peterson, A. T., and R. A. J. Williams. 2008. Risk mapping of highly pathogenic Avian Influenza distribution and spread. Ecology and Society 13(2): 15. [online] URL: http://www.ecologyandsociety.org/vol13/iss2/art15/*

[2] *Cumming, G. S., P. A. R. Hockey, L. W. Bruinzeel, and M. A. Du Plessis 2008. Wild bird movements and avian influenza risk mapping in southern Africa. Ecology and Society 13(2): 26. [online] URL: http://www.ecologyandsociety.org/vol13/iss2/art26/*

- Wild bird surveillance continued at four locations in Southern Africa including: Strandfontein and Barberspan located in South Africa; Lake Chuali, north of the capital of Maputo in Mozambique; and Lake Chivero in Zimbabwe.

- Dr. Marcela Uhart was a keynote speaker in Panama on “Prevention and Conservation: Wildlife Health, Zoonoses and improving wildlife disease surveillance in Latin America" at the conference on “Pandemic Influenza and Other Emerging Zoonoses: A constant Threat.” Dr. William Karesh provided a keynote address to APEC Health Ministers working meeting in Lima, Peru on GAINS and emerging infectious disease surveillance and prevention.

- Wild bird surveillance missions continued, with support from USDA and local community members, in four Bolivian states despite the continued threat of civil unrest.

EXECUTIVE SUMMARY

The aim of the Wild Bird Global Avian Influenza Network for Surveillance (GAINS) project is to expand operational field capabilities, improve the understanding of viral strains and transmission of influenza viruses in wild birds, and to disseminate information to all levels of governments, international organizations, the private sector and the general public. GAINS is an initiative of the Wildlife Conservation Society (WCS) with collaborating partner institutions around the world. Please refer to Appendix I for a table summarizing recent activities and GAINS project partners listed by country. Through GAINS, WCS and its partners have collected tens of thousands of samples for highly pathogenic avian influenza (HPAI) H5N1 analysis, and are making available census data from more than 105 million bird observations via an open access database and mapping system (http://www.GAINS.org). WCS staff and GAINS project partners have also trained thousands of individuals worldwide in wild bird handling, sampling, and data collection for the purpose of understanding and controlling the spread of HPAI H5N1.

TABLE OF CONTENTS

INTRODUCTION **5**

ASIA **5**

MONGOLIA 6
Surveillance *6*
Epidemiological findings *6*
Training and Outreach *8*
CAMBODIA 8
Surveillance *8*
Collaborations *9*
VIETNAM 10
Training and Outreach *10*
Collaborations *11*
INDONESIA 12
Surveillance *12*
Training, Outreach and Collaborations *12*
KAZAKHSTAN 13
Surveillance *13*
Afghanistan *14*

AFRICA **15**

SOUTHERN AFRICA 15
Surveillance *15*
Ongoing migration studies *17*
NIGERIA 17
Surveillance *17*

LATIN AMERICA **18**

ARGENTINA 19
Surveillance *19*
Training and Outreach *19*
BRAZIL 20
BOLIVIA 20
Surveillance *20*
COLOMBIA 22
Training and Outreach *22*
PERU 22
Surveillance *22*
Training and outreach *23*
COSTA RICA 23

CROSS CUTTING ACTIVITIES **24**

INTERNATIONAL WATERBIRD CENSUS 24
Neotropical Waterbird Census (NWC) *24*
Western Palearctic and South West Asia (WPSWA) *24*
GAINS TECHNOLOGY 25
WISDOM Map Explorer (WME) *25*
Document Management System *26*
Supplementary Species Information *27*
www.GAINS.org Website Usage *27*

APPENDICES **28**

List of Appendices:

Appendix I. Summary of GAINS partners and countries

Appendix II. "Clean vs. Dirty": A protocol to prevent cross-contamination during the handling of wild bird surveillance sampling in the field.

Appendix III. Peterson, A. T., and R. A. J. Williams. 2008. "Risk mapping of highly pathogenic Avian Influenza distribution and spread." *Ecology and Society* 13(2): 15.

INTRODUCTION

WCS uses a collaborative approach to address the complexities of maintaining ecosystem health. The United States Agency for International Development (USAID) and the Centers for Disease Control and Prevention (CDC), among others described below, provided funding to WCS to administer the Wild Bird Global Avian Influenza Network for Surveillance (GAINS) project that was launched in 2006. GAINS is a smart and targeted investment in the US government's fight against highly pathogenic H5N1 avian influenza, since, for example, wild birds can serve as sentinels for early detection of the virus' presence.

Under the GAINS project, WCS unites a network of US-based and international partner institutions, including governments, NGO's and universities. These partners work together to improve our understanding of the dynamics of avian influenza, to evaluate disease risks, and to use timely information to help protect people, wildlife, and domestic animals from the threats posed by HPAI H5N1. Across the globe, field surveillance for avian influenza in wild birds is underway.

A primary purpose of GAINS is to share international disease information through an interactive, publicly accessible web-based database, which is available online at www.GAINS.org. GAINS surveillance includes systematic monitoring of wild birds along major global flyways, with information on over 105,000,000 bird observations already entered into the mapping and database systems.

Below, we are pleased to report on our activities during Quarter 10 of the GAINS project.

ASIA

WCS institutional collaborations for GAINS in Asia include the private sector (Cargill Inc., the largest distributor of meat and poultry products in the US) and US-based and international agencies such as the United Nations Food and Agriculture Organization (FAO), and the National Institutes of Health (NIH) via the University of California Los Angeles Center for Rapid Influenza Surveillance and Research (CRISAR) and the University of Minnesota Center of Excellence for Influenza Research and Surveillance (MCEIRS) programs. These funding partnerships have made possible WCS contracts and collaborations for GAINS with top research institutions and laboratories in the US and abroad, including the University of California Davis, the Center for Tropical Disease in Los Angeles, the University of Minnesota, and a host of foreign laboratories that contribute in-kind services and materials. Over $1.5 million has been leveraged through the five largest funding partnerships for GAINS in Asia, for work in Indonesia, Laos PDR, Cambodia and Mongolia.

All data collected through these partnerships are incorporated into the growing GAINS database (please see the Cross-Cutting Activities section of this report for more details). Specifics of our many regional and local partnerships with researchers, universities and NGOs are also mentioned in individual country sections.

Mongolia

Surveillance

The WCS field team identified five dead whooper swans (*Cygnus cygnus*) in early August at Erhel Nuur, where two of the three prior Mongolian outbreaks of highly pathogenic avian influenza (H5N1) occurred at this same time of year. The team used a Synbiotic rapid test kit onsite (a screening test suitable for field conditions) and found one bird that tested positive for influenza A virus. The team then alerted the State Central Veterinary Laboratory of Mongolia (SCVL) and the USAID mission in Ulaanbaatar, and sent additional samples from the site to SCVL's Ulaanbaatar laboratory for confirmation testing. The samples tested negative for AI using real time PCR tests, indicating the screening test may have been a false positive result. This incident represents the successful execution of timely, organized response among partners at WCS, USAID, Mongolian national authorities, and laboratories.

Epidemiological findings

Results from wild Mongolian bird serology samples from WCS surveys in 2006-2007 indicate that a surprisingly high percentage of the 454 birds sampled had been previously exposed to AI subtype H5 (6.2%; 28 birds). One individual had been previously exposed to the H7 subtype. Certain species exhibited more prevalence of H5 antibodies than others: 15% of all whooper swans and 5% of all Bar-headed geese sampled had been exposed.

The results are interesting for several reasons. All but one of the samples with antibodies to H5 came from previous HPAI H5N1 outbreak zones in north/central Mongolia. If the birds that were subsequently sampled for the present follow up study had also been exposed to HPAI H5N1, that would mean some birds are surviving the infection and developing immunity, which would reduce the capacity of H5N1 to persist in the wild population. If antibodies to H5 are cross-reactive to HPAI H5N1, a likely scenario, then 15% of the whooper swans in the study area may now be immune from future infection with H5 strains.

Caution is required in interpreting the findings, because we do not know whether these birds were exposed to a high or low pathogenicity strain of H5, nor what the corresponding N-subtype or viral strain was; we cannot conclude that the birds were necessarily exposed to H5N1 (for example, they may have been exposed to multiple viruses including H5N3 and H3N1). Further testing is being conducted to determine the

N subtype and help narrow the range of possible H5 subtypes to which the birds could have been exposed.

A complete report of the findings is being prepared for publication.

In its most productive sampling season to date, the Mongolian field team continued regular surveillance efforts in Hovsgol, Bulgan and Arkhangai provinces (locations of previous outbreaks of HPAI). Highlights of the work include:

- 1,377 birds from 33 species were sampled. The Mongolia team is now adapting its sampling strategy by reducing the breadth of species sampled and increasing the number of samples taken from individual species (~ 100 individuals per sampling effort). This shift will facilitate robust conclusions regarding whether certain species may be more or less likely to carry or form a reservoir for AI, as mentioned in the collaborative UGA study above. Six focal species this quarter included whooper swan (*Cygnus cygnus*), bar-headed goose (*Anser indicus*), swan goose (*Anser cygnoides*), ruddy shelduck (*Tadorna ferruginea),* Mongolian gull (*Larus vagae mongolicus*) and great cormorant (*Phalacrocorax carbo).* Samples are being analyzed at University of California, Davis to detect the presence of general avian influenza gene segments as well as genes specific to H5 and H7 subtypes. A duplicate of any sample found positive for a virus by UC Davis will be submitted for viral isolation at Hong Kong University (to attempt to grow and further characterize any virus present).

- Ongoing studies are being conducted to better understand migratory patterns of birds and what they may mean for the risk of spread of AI.

- The team continues to collect feather and claw samples for future stable isotope studies to help determine specifically where birds had traveled prior to capture. (Please see the Quarter 9 GAINS report for a summary of a prior isotope study supported by GAINS, or go to www.GAINS.org to download the entire report.)

- 554 migratory Mongolian swans and geese were fitted with color banded neck collars which can be identified by observers internationally, and provide clues to wintering and migration areas. Four of the 50 bar-headed geese (*Anser indicus*) marked by the team in 2007 banding were recaptured and sampled in 2008. With greater numbers of birds marked this year we can expect to build a picture of the health and disease exposure of individual birds throughout their lifetimes.

- The Mongolian national bird marking scheme was initiated with numbered metal bird rings in collaboration with the Institute of Biology at the Mongolian Academy of Sciences. Mongolian ornithologists will benefit from this program for years to come.

Training and Outreach

One of the biggest challenges to national AI surveillance activities is the critical shortage of highly trained ornithologists and wildlife veterinarians in Mongolia. The WCS field team is expanding its training activities to address this. The two new courses were led during Quarter 10 in Ulaanbaatar by WCS epidemiologists and pathologists. Attendees focused on principles of pathology and its application in diagnosing key wildlife diseases.

Cambodia

Surveillance

Highlights for field surveillance this quarter included:

- 352 individual birds from 19 species were sampled
- 570 samples were collected during market and merit bird surveillance in the provinces of Battambambang, Siem Reap, Kratie, Stung Treng, Kandal and Phnom Penh. Species sampled in markets and merit bird trade included plain-back sparrow (*Passer flaveolus*), common myna (*Acridotheres tristis*), Eurasian tree sparrows (*Passer montanus*), barred buttonquail (*Turnix suscitator*), spotted dove (*Streptopelia chinensis*), peaceful dove (*Geopelia placida*), house sparrow (*Passer domesticus*), streaked weaver (*Ploceus manyar*), baya weaver (*Ploceus philippinus*), Asian golden weaver (*Ploceus hypoxanthus*), and scaly-breasted munia (*Lonchura punctulata*). Results of testing at University California, Davis, are pending.

Wild birds at the Battambang markets. (Top) Green sandpiper and lesser whistling duck (Bottom) Purple swamphen. (Photo: WCS Cambodia)

The Quarter 9 GAINS report detailed the case of a wild greater painted snipe (*Rostratula benghalensis*) sample collected in Ang Trapaeng Thmor Sarus Crane Reserve in the Banteay Meanchey province which tested positive for avian influenza subtype H5. Complete viral isolation is underway at the World Organization for Animal Health/Food and Agriculture Organization of the United Nations reference laboratory in Padova, Italy.

Collaborations

During the wet season, when wild bird flocks are dispersed and unpredictable weather conditions make bird captures very difficult, the team in Cambodia focused on market sampling and strengthening collaborations for building national capacity for surveillance of wild birds. This quarter, the field team visited the Angkor Center for Conservation of Biodiversity (ACCB) and Sam Veasna Center for Wildlife Conservation (SVC) to discuss and organize synergistic training opportunities, such as bird identification, capture and handling.

The United Nations Food and Agriculture Organization (FAO) Cambodia is interested in funding a second season of wild bird surveillance by WCS to complement its own activities in the region.

Cambodia field team members Dav Sophea and Chhin Kunthea collect a tracheal swab from a common myna. (Photo: WCS Cambodia)

Vietnam

Training and Outreach

WCS has been a leader in seeking to improve the quality of data that feeds into global disease surveillance databases through the GAINS project. A fundamental but often overlooked aspect is the physical processing of samples in the field, which has profound implications for the scientific validity of large-scale surveillance endeavors.

Avoiding cross-contamination of samples under the normal conditions of disease surveillance field work can be challenging. Cross-contamination occurs when diagnostic samples become contaminated with virus that does not originate from the animal sampled, but rather from contact with other people or other samples. Opportunities for such errors increase when multi-national field teams are learning to communicate across language, cultural and educational barriers while working with little sleep, in makeshift laboratories in tents, and must hurry to prevent samples from spoiling. To make it easier to prevent the risk of cross-contamination under such field conditions, WCS veterinarian Joost Philippa and field teams in Vietnam and Indonesia have developed a new protocol to institutionalize safe practices for handling and processing AI surveillance samples.

The protocol employs a simple conceptual model in which participants visualize their roles in the teamwork spatially, and separate into a “Clean” team which handles record keeping and supplies prior to use, and a “Dirty” team which handles and samples birds. All staff train to be competent members of both “teams” and the protocol has proved highly effective (attached as Appendix II).

As detailed in the “Clean vs. Dirty” protocol manual. Red (“dirty”) and green (“clean”) team members never handle the same objects, even when working in close quarters as pictured here, to avoid cross-contamination. (Photos: J. Philippa)

Collaborations

Collaborative relationships are being pursued with Vietnam-based organizations including:

- The Oxford University Clinical Research Unit (OUCRU) at the Hospital for Tropical Diseases in Ho Chi Minh City, for targeted surveillance of zoonotic/economically important pathogens (bacterial and viral) in birds and other species at markets.

- Vietnam National University of Natural Sciences for partnerships with the Zoology Faculty.

- Partners and local communities to help develop a new focus on wildlife farms for AI surveillance in non-avian species such as monitor lizards, macaques, and civets and high profile diseases in other species sold for human consumption.

- Department of Animal Health (DAH), Hanoi, for field work facilitation and a joint wild bird AI surveillance proposal and budget to be submitted to FAO

- Google.org for human-wildlife health studies in South East Asia as part of the "Predict and Prevent" initiative (collaboration between WCS, OUCRU and Google.org).

Indonesia

Surveillance

- 214 birds were sampled during surveillance of hunter-caught birds this quarter in Amuntai, South Kalimantan and Kota Bangun, East Kalimantan. Species were mostly wandering whistling duck (*Dendrocygna arcuata*) but also a small number of purple swamphens (*Porphyrio porphyrio*) and a Sunda teal (*Anas gibberifrons*). Due to high water levels, ducks were dispersed on the lakes and there were thus fewer hunters active in the area, which was reflected by the team's lower catch numbers in comparison with prior sampling for GAINS at this time of year. However, low numbers may also be the result of intensive hunting of wandering whistling ducks in the area for years. Samples are being tested at the Disease Investigation Center (DIC) in Banjarbaru; results are pending.

- 200 birds of 39 species were sampled during surveillance in Wonorejo, Surabaya, Java. The team opportunistically sampled of range of non-shorebird species due to low shorebird numbers at this time of year. Sampled species included small blue kingfishers (*Alcedo coerulescens*), spotted doves (*Streptopelia chinensis*), brown prinias (*Prinia familiaris*), sacred kingfishers (*Todirhampus sanctus*), scarlet-headed flowerpeckers (*Dicaeum trochileum*), striated herons (*Butorides striatus*) and clamorous reed-warbler (*Acrocephalus stentoreus*). Samples are being tested by real-time PCR at the DIC in Wates, Jakarta, for presence of the matrix gene for avian influenza virus; results are pending.

- The Australasian Wader Studies Group (AWSG), a GAINS collaborator which monitors shorebirds in the East Asian Australasian flyways, notified GAINS of sightings of birds flagged by the field team in Indonesia. Significant sightings included a marsh sandpiper (*Tringa stagnatilis*) originally flagged in Java and resighted in Jingqu, Dagang, China. This is an uncommon species and is the first re-sighting of a flagged wader from Java.

Training, Outreach and Collaborations

- 10 volunteers from the veterinary program at Indonesia's Airlangga University joined the field team during surveillance in the village of Wonorejo to learn surveillance techniques such as mist netting, bird banding and sampling.

- In the same village, 44 children (kindergarten to junior high) participated in educational enrichment activities including learning about shorebirds and work by WCS and partners in Wonorejo, and playing games that teach about highly pathogenic avian influenza.

- Field team members attended a two-day training by the Indonesian Institute of Sciences-Lembaga Ilmu Pengetahuan Indonesia (LIPI). Techniques were taught to collect additional biological samples from wild caught birds during AI surveillance, to contribute to ongoing studies at LIPI.

- WCS Field Veterinarian Zulfi Arsan, a member of the Indonesia National Committee for Avian Influenza and Influenza Preparedness (KOMNAS), attended "The National Strategy on Wild Birds Surveillance Draft Finalization Meeting" in Bogor to help finalize national strategies and guidelines for AI surveillance of wild birds.

- Staff of the Viral Disease Program of the United States Naval Medical Research Unit of Indonesia (NAMRU-2) discussed NAMRU protocols and possibilities for future collaborations with WCS.

- The forestry Faculty at Bogor Agricultural University (IBP) have been conducting wildlife trade surveys for 3 years and are interested in collaboration with WCS including sample collection from wildlife traded at markets in Bogor, Jakarta and Cibinong.

Kazakhstan

Surveillance

Thirty-four (34) influenza A positive birds were found among 751 sampled birds (2,644 samples) that were collected by the field team during 2007. All the positive samples will be analyzed in a reference laboratory in Germany for further typing, as well as an equal number of negative samples.

In Quarter 10, the field team in Kazakhstan continued its surveillance work at multiple sites, including Kyzykol Lake (also known as Red Lake) which hosts hundreds of thousands migratory waterfowl each autumn on their migratory routes. A highlight this quarter was a collaboration to use satellite telemetry Platform Transmitter Terminals (PTT), funded by United States Geological Survey (USGS) and United Nations Food and Agricultural Organization (FAO), to elucidate the migratory routes of wild birds of the

region and shed light on potential transmission routes for AI along flyways. In September, partners from WCS, FAO, USGS, Wetlands International and United States' National Aviary attached satellite transmitters equipped with PTT units to 23 migratory ducks including 8 common teal (*Anas crecca*), 6 mallard (*Anas platyrhynchos*), 4 northern shovelor (*Anas clypeata*), 3 red-crested pochard (*Netta rufina*) and 2 gadwall (*Anas strepera*).

Red-crested pochard affixed with FAO/USGS funded PTT unit at Kyzykol Lake, Kazakhstan. (Photo: T. Katzner, National Aviary)

The team simultaneously collected samples for AI testing from over 150 birds from nearly 30 species including ruff (*Philomachus pugnax*), little stint (*Calidris minuta*), pin-tailed snipe (*Gallinago stenura*), Eurasian sparrowhawk (*Accipiter nisus*), long-eared owl (*Asio otus*) and Eurasian eagle owl (*Bubo bubo*). Samples are being tested at the Institute of Microbiology and Virology in Almaty, Kazakhstan, and results are pending.

Sam Iverson, USGS, releases common teal after sample collection in Kyzykol Lake, Kazakhstan. (Photo: T. Katzner, National Aviary)

Afghanistan

Field surveillance was postponed during Quarter 10 due to security issues related to civil unrest in the country.

AFRICA

Southern Africa

(South Africa, Mozambique, Zimbabwe)

Dr. Graeme Cummings, of Percy FitzPatrick Institute, University of Cape Town, and leader of the field team in Southern Africa, spearheaded a special feature on "Risk mapping for avian influenza: a social-ecological problem" in the peer-reviewed journal *Ecology and Society*. Two of the studies in the issue were supported by GAINS:

- A. Townsend Peterson and his colleague coauthored an overview of the problem of risk mapping, providing an overview of the transmission cycle of avian influenza viruses, how risk-mapping can be most fruitfully applied, and an outline of how risk mapping and larger scale influenza monitoring should be linked to provide a more responsive information base for decisions and initiatives related to H5N1 and other emerging bird-borne diseases. (The full reference is: Peterson, A. T., and R. A. J. Williams. 2008. "Risk mapping of highly pathogenic Avian Influenza distribution and spread." *Ecology and Society* 13(2): 15. Download at http://www.ecologyandsociety.org/vol13/iss2/art15/)

- Dr. Graeme Cumming and colleagues at the Percy FitzPatrick Institute coauthored a study assessing spatial aspects of potential risk of AI outbreaks in South Africa. The study represents a simple, quantitative description of potential risks, and shows that urban centers of Johannesburg and Cape Town present the highest risk. This work provides the first quantitative assessment of AI risk for decision makers in the subregion, and presents a model for similar assessments in areas where relatively little field data is available. (The full reference is: Graeme S. Cumming, Philip A. R. Hockey, Leo W. Bruinzeel and Morne A. Du Plessis. 2008. "Wild Bird Movements and Avian Influenza Risk Mapping in Southern Africa." *Ecology and Society* 13(2). Download at http://www.ecologyandsociety.org/vol13/iss2/art26/)

Surveillance

To date, 143 point counts, representing 2,538 birds, have been recorded and uploaded to the GAINS database from observations in the Southern Africa region.

A total of 1132 samples have been collected thus far from three prime target species for AI surveillance in the region: Egyptian goose (*Alopochen aegyptiacus),* red-billed teal (*Anas erythrorhyncha*) and yellow-billed duck (*Anas undulata*).

The WCS team in Southern Africa continues monitoring the presence of highly pathogenic AI in wild bird populations at four key sites: Strandfontein (at the mouth of the Olifants River) and Barberspan (in the Northwest province) in South Africa; Lake Chuali, north of the capital of Maputo in Mozambique; and Lake Chivero, southwest of Harare in Zimbabwe. Highlights for Quarter 10 included:

- In South Africa at the Barberspan site, 140 wild birds were captured and sampled including black-crowned night heron (*Nycticorax nycticorax*), little bittern (*Ixobrychus minutus*), as well as two rarities for the area: the rufous-bellied heron (*Ardeola rufiventris*) and terek sandpiper (*Xenus cinereus*). Final sample results are pending. A total of 50 birds were captured and sampled at the Standfontein site (due to inclement weather in July).

- In Mozambique, the team captured and sampled over 60 species, with African jacana (*Actophilornis africana*) being the most numerous.

- In Zimbabwe, 150 birds were captured and sampled including red-billed teal (*Anas erythrorhyncha*), curlew sandpiper (*Calidris ferruginea*), greenshank (*Tringa nebularia*) and African snipe (*Gallinago nigripennis*).

Southern Africa field team members Andrew Nkosi, Ngoni Chiweshe and Mduduzi Ndlovu set up a mist net at Lake Chuali, Mozambique. (Photo: M. Kock, WCS)

Ongoing migration studies

Satellite GPS transmitters affixed to wild birds in previous missions continue to yield useful data about the movements of ducks from Strandfontein, Barberspan and Lake Chivero. The first long-distance movement of an Egyptian goose occurred in August, with a single individual flying 850km Southeast in two days (from Barberspan south to Stutterheim, near East London, in the Eastern Cape). Warmer, drier weather may have contributed to the long range of movement.

The team attempted to attach 3 satellite transmitters to Egyptian geese during the mission in Zimbabwe mentioned above in Quarter 10, but capturing the birds proved impossible; the transmitters will be attached at the next opportunity.

Nigeria

Surveillance

Laboratory results are still pending from the Institute of Immunology in Luxembourg on nearly 1,100 individual birds sampled for AI surveillance at the Dagona Waterfowl Sanctuary in Shugum wetland area in northern Nigeria's Hadejia-Nguru Wetlands. The samples include over 650 wild birds and nearly 420 domestic birds. Wild species include cattle egret (*Bubulcus ibis*), spur-winged goose (*Plectropterus gambensis*), red bishop (*Euplectes franciscanus*), Heuglin's masked weaver (*Ploceus heuglini*) and African jacana (*Actophilornis africana*).

Field team members of Nigeria, aided by local villagers, collect samples from domestic birds. (Photo: Wetlands International)

Seasonal occupation of the wetlands by Palearctic and intra-African migratory birds and the proximity of the wetlands to local settlements where local poultry and domestic ducks are reared could serve as a possible source for transmission of avian influenza into or out of the country. In 2007, surveillance data showed that 2 waterfowl out of 255 sampled in the Dagona wetland were positive for HPAI[3]. The field team has concentrated on that area to monitor whether cases of HPAI are still present.

The field team, led by Dr. Tim Dodman with participation by AP Leventis Ornithological Research Institute (APLORI), also sampled local domestic poultry, ducks and feral doves in villages and markets around the Dagona wetlands. They gained permission from village leadership and captured the birds with the help of children and other local residents.

LATIN AMERICA

Field veterinarian Dr. Marcela Uhart and her colleagues in the region have reached out to diverse audiences in recent months to promote awareness of the need for surveillance of avian influenza and other zoonotic diseases, provide training on how to design and interpret results of surveillance, and diffuse scientific information obtained through GAINS project activities. Examples include:

- Dr. Uhart was a keynote speaker in Panama on “Prevention and Conservation: Wildlife Health, Zoonoses and improving wildlife disease surveillance in Latin America" at the conference on “Pandemic Influenza and Other Emerging Zoonoses: A constant Threat”, organized by the Instituto Conmemorativo Gorgas de Estudios de la Salud.

- Dr. Pablo Beldomenico and Dr. Uhart co-authored “The ecoepidemiology of avian influenza virus” to be published in *Revista FAVE - Ciencias Veterinarias.*

- Dr. Beldomenico was the lead author of a paper focusing on the links between general health and well-being of animal populations and the spread of infection, an issue with critical implications in AI epidemiology. (Beldomenico, PM; Telfer S; Gebert S; Lukomski L; Bennett M; Begon M. Poor condition and infection: a vicious circle in natural populations. *Proceedings of the Royal Society B: Biological Sciences* 275: 1753-1759. 2008).

Other highlights of activities in the region are detailed below by country.

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[3] *Gaidet, N. et al. 2008. PLoS Pathog 4(8): e1000127. URL: http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1000127*

Argentina

Surveillance

Samples were collected in Santa Fe province wetlands by Drs. Hebe Ferreyra, Virginia Rago and Marcelo Romano from hunter-killed wild ducks in July. Species sampled included rosy-billed pochard *(Netta peposaca*) fulvous whistling duck (*Dendrocygna bicolor)*, white-faced whistling duck (*Dendrocygna viduata)*, Brazilian Duck *(Amazonetta brasiliensis*), silver teal (*Anas versicolor*), ringed teal *(Callonetta leucophrys*), cinnamon teal (*Anas cyanoptera*), red shoveler *(Anas platalea*),and black-headed duck *(Heteronetta atricapilla*). Samples are being analyzed by RT-PCR at INTA (National Institute of Agriculture), the reference laboratory in Argentina for AI surveillance.

Dr. Hebe Ferreyra (front right) works with field team on hunter-killed wild duck necropsies in Santa Fe, Argentina. (Photo: H. Ferreyra, WCS)

Training and Outreach

Drs. Hebe Ferreyra and Marcelo Romano continue training veterinary students and young veterinarians while conducting field work for GAINS. During the last quarter, trainees included: Darío Manzoli, Nicolas Acosta, Agustín Quaglia, Carolina Magni and Ana Correa, veterinary students from Esperanza University and Buenos Aires University.

Little is known about AI viruses in wild ducks in South America. About 500,000 wild ducks are hunted annually in Argentina for sport and control of agriculture damage to rice crops. Hunter-killed ducks provide an opportunity for sample collection and training, and offer a detailed picture on AI virus prevalence in natural hosts.

Dr. Hebe Ferreyra weighs a hunter-killed duck during sample collection for AI surveillance. (Photo: H. Ferreyra, WCS)

Brazil

Dr. Flavia Miranda is in contact with USAID representatives in Latin America, whose principal office is being transferred from Brazil to Lima, Peru. Dr. Miranda continues to encourage collaboration with the Interministerial Committee for Avian Influenza in Brazil. Next quarter she will also participate in migratory bird captures for AI surveillance to be coordinated by the Ministry of Health and Ministry of Agriculture in Rio Grande do Sul.

Bolivia

Surveillance

With support from the Unites States Department of Agriculture (USDA), wild bird AI surveillance was conducted this quarter in four Bolivian states. Work continued despite significant civil unrest in Bolivia in recent months (road blockades, civilian confrontations, etc). However, there have been delays in sample analysis by the LIDIVECO Laboratory in Cochabamba, where all samples for AI surveillance are sent, related to the unrest. Initial laboratory results will be reported in the Quarter 11 report.

- Sampling in the state of Oruro was led by veterinarian Fabian Beltran with support from a veterinary student and participation of the governmental institution PRONESA (National Avian Health Program), the Institute of Ecology of the

Major University of San Andrés (UMSA) and the national NGO BIOTA. Activities included wild bird sampling, a necropsy, and fecal swabs obtained from the shores of the Uru Uru lake.

- Sampling in the state of Santa Cruz (Kaa Iya National Park) was led by Dr. Sixto Angulo with support from 3 volunteers and a biologist.

- Sampling in Cochabamba state was led by Dr. Angulo with participation of three veterinarians from the governmental institution PRONESA (National Avian Health Program). Extremely windy conditions in the former made captures impossible, but several fecal swabs were obtained indirectly via pools collected from lake shores.

- Sampling in the state of La Paz was conducted by Dr. Angulo with veterinarians Fabian Beltran and Erika Alandia, and two veterinary students, included captive birds kept at the La Paz zoo, birds sold at the live market in El Alto, and wild birds at Achocalla and Titicaca lakes.

Dr. Erika Alandia (right) and Natural Resource Management Coordinator from CIPTA (Takana Communities Government) prepare a blood smear during disease surveillance activities in the state of La Paz, Bolivia. (Photo: E. Alandia, WCS)

Colombia

Training and Outreach

The team strengthened and consolidated inter-institutional collaborations for capacity building and AI field surveillance. A formal agreement with the International Center for Training and Medical Research (CIDEIM) was established to provide laboratory assistance for activities conducted under agreements with the Cauca Valley parastatal organization (CVC) and the Ministry of Environment, Housing and Territorial Development (MAVDT). Agreements with various other NGOs are in their final stages of being signed (see the Quarter 9 GAINS report for details on these organizations).

The first team training for GAINS activities with the Regional Autonomous Corporation of the Cauca Valley – CVC was carried out in August. Twelve birds were sampled from five different species. Samples are stored in the International Center for Training and Medical Research (CIDEIM) and will be sent to the Colombian Agricultural Institute (CEISA Reference laboratory) for analysis.

A second team training was conducted with eight biologists on methodologies for avian influenza surveillance in the Cauca Valley. Three of them will work in Cauca Valley wetlands, and the remaining 5 (experts from the NGO Calidris specializing in Colombian waterbirds) will conduct workshops and surveillance throughout Colombia under the agreement with the MAVDT.

Many more field surveys and training activities are planned for the next quarter. With the CVC partner these include 7 surveys in Sonso Lagoon, Robles wetland, and Bocas de Tulua wetland from October to November, and 2 workshops in the Sonso and Robles sites.

Eleven training workshops are planned under the agreement with the MAVDT in sites including: Bogotá, Villavicencio, Pasto, Complejo del río Atrato-Golfo de Urabá, San Andrés Isla, Complejo de Humedales de la Cienaga de Zapatosa y humedales del río Magdalena, Estero-Mani (Humedales de Puerto Carreño – Casanare), Riohacha (Humedales costeros de la Guajira), Santamarta (Magdalena - Parque Isla de Salamanca y complejo de la Ciénaga Grande de Santa Marta).

Peru

Surveillance

The collaborative team in Peru continues monitoring AI in wild bird markets. The team's surveys to date have produced more than 3,100 records of domestic and wild birds housed together at markets – conditions conducive to the mutation and spread of avian influenza viruses. Markets in Lima, Iquitos, and Pucallpa were regularly monitored this quarter with the help of three new field assistants who joined the team (the team now

includes 8 people monitoring trade in 8 markets). Traders are increasingly comfortable with the presence of the monitors, which facilitates more accurate bird censuses. To date, about half of all birds sampled at Lima, Pucallpa and Iquitos have been analyzed and tested negative for avian influenza.

This study is one of the first of its kind in the region. The team is on the alert for indications of the presence of low pathogenic AI strains in asymptomatic (apparently healthy) wild birds in markets. Such birds are likely to come into contact with domestic fowl, allowing viruses the opportunity to reach new species hosts and to mutate into highly pathogenic forms. Results of remaining samples will be reported in the next GAINS report.

The team confirmed that there has been decreased trade as compared to previous years. They are seeking to establish whether fluctuation in trade volume is a normal seasonal pattern, an effect of increased vigilance by Peruvian authorities, or an artifact of survey methodology.

Training and outreach

- Four Peruvian veterinarians and one biologist were trained in bird identification, market surveys and informational communication with traders. Training for new assistants included bird handling and restraint, sample collection and information management.

- An informational brochure about diseases in apparently healthy birds and project activities was designed with input from a broad spectrum of experts and stakeholders and is awaiting printing.

- Dr. Donald Brightsmith presented on “Infectious Diseases in the live bird trade in Peru: Monitoring global threats” at the annual meeting of the Association of Avian Veterinarians in Atlanta Georgia.

- Dr. Mendoza and a project assistant spoke at the Faculty of Veterinary Medicine of Universidad Nacional Mayor de San Marcos on advances in bird trade surveys at Peruvian markets and identification of common birds in markets.

Costa Rica

This quarter WCS entered into a new GAINS subaward agreement with Universidad Nacional de Costa Rica and the National Animal Health Service. The partners seek to determine the presence of avian influenza virus in migratory and resident wild birds belonging to the orders Anseriformes and Charadriiformes in at least four wetland areas of Costa Rica: Palo Verde National Park, in the northern part of Costa Rica; Caño Negro National Park, located in the North Pacific region; “Mata-Redondo” lagoon,

located in the North Pacific; and Chomes y Punta Morales, located on the north central Pacific Coast.

CROSS CUTTING ACTIVITIES

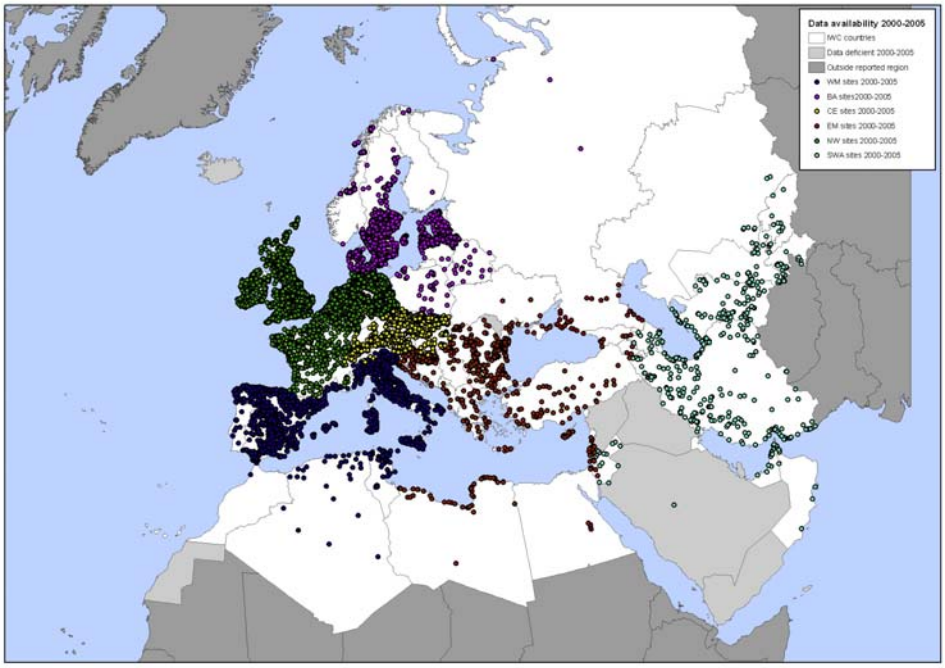
International Waterbird Census

The IWC is a global program for counting water birds at “wintering” sites, coordinated by Wetlands International. Over 30 million birds are counted annually in over 12,000 sites globally, covering Europe, Africa, most of Asia, and large parts of Latin America. Counting largely occurs through a volunteer network. Water bird data are stored in the IWC database, a decentralized database managed in four regions. The main database with the longest running data set is for Western Palearctic and South West Asia (WPSWA). Other regional databases exist for Africa, Asia, and the Neotropics. All these databases use the same data format, but require a uniform data management system. Until now, lack of capacity and resources for effective coordination have resulted in highly uneven data quality. GAINS is supporting development of a uniform data management system to improve management and distribution of these data.

Neotropical Waterbird Census (NWC)

WCS partner Wetlands International, South America Program, in close cooperation with Wetlands International Headquarters in The Netherlands, continues to improve the database for the Neotropical Waterbird Census (NWC) for the International Waterbird Census (IWC). As discussed last quarter, the team continued analyzing entries in NWC from 1990-2006 on 25 selected bird species that could be possible reservoirs for avian influenza. Also this quarter, the team worked on georeferencing the coordinates for the NWC sites where testing and sample collection occurred.

Western Palearctic and South West Asia (WPSWA)



Data availability in the Western Palearctic and South West Asia (WPSWA) region of the International Wetlands Census (IWC) from 2000 to 2005.

WCS partner Wetlands International continues working on the web-based International Waterbird Census (IWC) database application for GAINS. This quarter the technology team for IWC worked on allowing the web based IWC database application to function as a central web-based system for storage of data from the International Waterbird Census. To enable this, a revised data exchange policy has been written that will facilitate the development of an XML-based exchange of data between the IWC database and the GAINS portal. This XML standard will allow the GAINS.org to obtain a copy of the latest updates from the IWC database, but present aggregated data in order to respect the intellectual property the data providers. In addition, the technology team worked on updating and correcting existing data in the IWC Western Palearctic and South West Asia (WPSWA) from 2000-2005. New data was inserted and data corrections were carried out for Qatar, Algeria, Croatia, Jordan, Tunisia, Turkey, United Arab Emirates, Estonia, Turkmenistan, Azerbaijan, Uzbekistan, Cyprus and the Czech Republic.

GAINS Technology

WISDOM Map Explorer (WME)

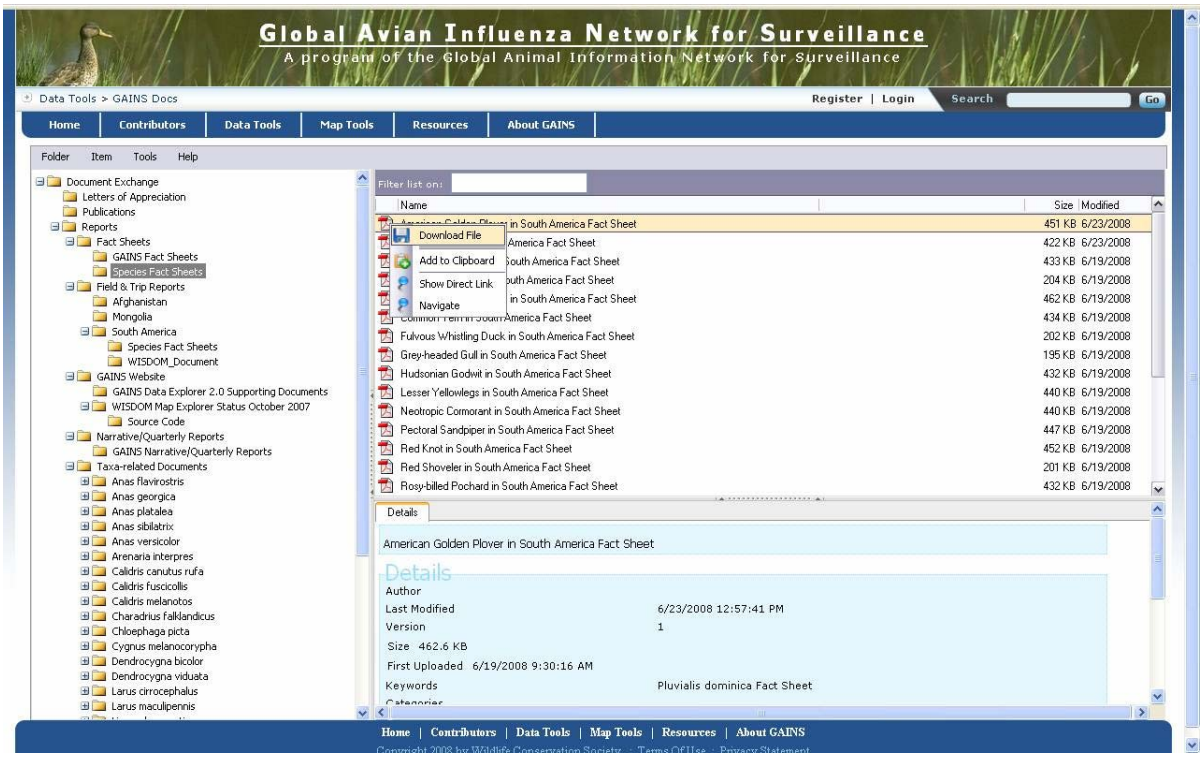
A new version of the Wildlife Information System for Disease Observation and Monitoring (WISDOM) Map Explorer (WME 3.1) was released allowing users to: overlay data on the map using KML and Shape files, and WMS services; annotate the map with lines, shapes, colors and text; export the map into KML and Shape files, image format; and as a comprehensive report, save and revisit map sessions, as well as share

with other users; customize the filtering criteria for data; change the base map layer; and zoom directly to a specified location or coordinate.

The WISDOM Map Explorer showing H5N1 highly pathogenic positive samples at Hunt Lake in Mongolia, with an overlaid poultry density layer.

Document Management System

The document management system was upgraded, providing users a richer navigation experience through the use of AJAX technology, allowing asynchronous actions. The new version also enables greater control through email, allowing users who are not logged into the system to interact with greater flexibility (e.g., submitting and receiving files using email commands).



A screenshot of the document management system showing the option to download one of many supplementary species documents.

Supplementary Species Information

Supplementary species information has been made available through the GAINS reporting system to support the data accessible in the WISDOM database. These materials include abundance maps and fact sheets, and are accessible through the point location report associated with the WISDOM Map Explorer.

Usage of the www.GAINS.org Website

The GAINS web site was visited 6,912 times by individuals from 114 countries and territories during Quarter 10; users spent an average of 9 minutes on the site during each visit.

APPENDICES

APPENDIX I – SUMMARY OF GAINS PARTNERS AND ACTIVITIES BY COUNTRY

Partial list of contributing WCS partners and subawardees in the GAINS project

1. United States Agency for International Development (USAID)
2. United States Department of Health and Human Services, Centers for Disease Control and Prevention (CDC)
3. United States Department of Defense
4. United States Department of Agriculture, South East Regional Poultry Laboratory (SEPRL)
5. United States Department of the Interior, United States Geological Survey (USGS)
6. Texas A&M University
7. Freidrich Loeffler Institute, Germany
8. Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD)
9. United Nations Food and Agriculture Organization (FAO)
10. Centro de Investigación de Enfermedades Tropicales (CIDEIM), Peru
11. Centro de Investigación de Enfermedades Tropicales (CIDEIM), Colombia
12. Naval Medical Research Unit - Cairo, Egypt
13. Naval Medical Research Center Detachment, Peru
14. Woodland Park Zoo
15. Instituto Nacional de Tecnología Agropecuaria (INTA Castelar), Argentina
16. Instituto Colombiano Agropecuario (ICA), Colombia
17. Cargill, Inc.
18. National Institutes of Health (NIH)
19. Australasian Wader Study Group (AWSG)
20. Tanzania Bird Atlas
21. IDEXX via University of Georgia and SERPL
22. Environmental Protection in the Caribbean (EPIC)
23. National Aviary, United States
24. National Ecological Center of Ukraine
25. University of Cape Town through Percy FitzPatrick Institute of African Ornithology
26. The Regents of the University of California on behalf of The Center for Tropical Research at UCLA
27. Wetlands International
28. The University of Kansas Center for Research, Inc. on behalf of the National History Museum and Biodiversity Research Center, University of Kansas
29. Universidad Nacional de Costa Rica and the National Animal Health Service

Summary of Selected Activities by Country

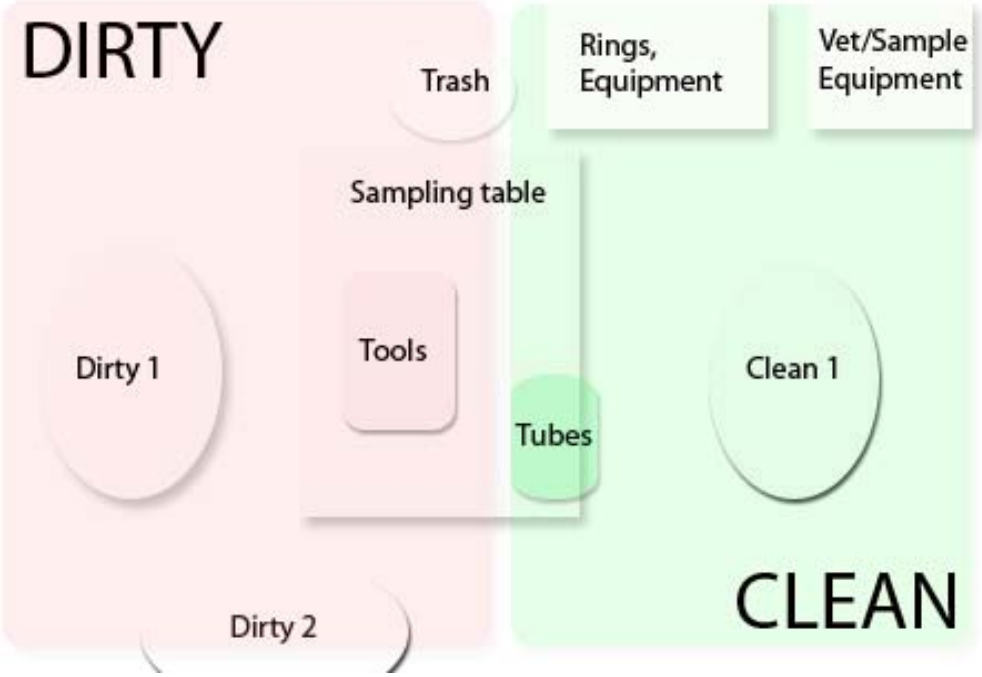
Country or State	Collaborator(s)	Activities in 2007 - 2008	Planned Activities
Afghanistan	WCS/DoD-NAMRU/FAO	Ongoing wild bird surveillance and training of local biologists and veterinarians	Sampling for AI in waterbirds, market wild birds, bridge species
Argentina	WCS/Wetlands Int. Argentina	Ongoing wild bird surveillance and training; courses organized by GAINS with USDA support	Wild waterbird sampling, training in AI surveillance and preparedness
Bolivia	WCS	Monitoring for AI in flamingo populations in Laguna Colorada	Wild waterbird/market sampling, training
Botswana	Percy FitzPatrick Ornithological Institute	Wild bird surveillance including sampling at Lake Ngami	Surveillance/migratory satellite telemetry
Brazil	Cargill, Inc./ FAO/GAINS/CIRAD	New projects to monitor wild birds for AI in the Amazon region and to examine interactions between migratory wild and domestic birds with a focus on AI prevention	Surveys and sampling for AI, training activities
Cambodia	WCS/UC Davis/FAO/AWSG/ USAID	Surveys and sampling of waterbirds, market wild birds, bridge species	Surveys/sampling of waterbirds, market wild birds, bridge species; education in AI preparedness
Cameroon	WCS/UCLA/Birdlife	Sampling domestic and wild birds of 65 species for AI testing	Fieldwork complete
Caribbean region (including Anguilla, Antigua, St. Kitts, St. Martin and St. Kitts)	EPIC (Environmental Protection in the Caribbean)	Sampling over 500 waterbirds from 5 countries, Training local biologists	Fieldwork complete
Chile	USDA-APHIS/WCS/SAG	Training in AI surveillance methods to personnel from the Chilean Agriculture and Livestock Service (SAG)	Surveys and sampling; adding existing AI surveillance data from Chile to GAINS
Colombia	WCS	Creating a national level AI monitoring program for wild waterbirds with local NGOs and government	AI surveillance in the Valle del Cauca area
Costa Rica	Universidad Nacional de Costa Rica and the National Animal Health Service	Wild and domestic bird surveillance of wetlands	AI surveillance in four wetland areas
Ecuador	Charles Darwin Research Station	Sampling of waterfowl for AI; training of biologists	Fieldwork complete

Country or State	Collaborator(s)	Activities in 2007 - 2008	Planned Activities
Egypt	Wetlands International/NCE	Waterbird surveys and training courses in collaboration with Nature Conservation Egypt	Surveys and sampling of waterbirds and bridge species
Falkland Islands	Falklands Conservation/WCS	Sampling waterbirds, training vets and technicians, mortality surveillance	Mortality surveillance and live wild bird sampling
Gabon	WCS	Census and mortality surveillance in waterbirds	Census and mortality surveillance in waterbirds
Ghana	University of Kansas (UK)	Analysis of samples previously collected by UK	Global risk map analysis with UNH
India	Wetlands International India	Census and AI sampling of waterbirds, bridge species; 4 training courses in 2007-08	Fieldwork and training complete
Indonesia	WCS/Wetlands Int. Indonesia/Cargill/AWSG	Census and AI sampling of market wild birds, waterbirds, bridge species, training gov't and NGO staff; local education	Census and AI sampling of market wild birds, waterbirds, bridge species
Kazakhstan	National Aviary/ Wetlands International/ FAO	Census and AI sampling of waterbirds, bridge species; placing satellite transmitters to monitor migratory movements	Census and AI sampling of waterbirds, bridge species; additional transmitters
Laos PDR	NIH/UMN	Funding from NIH via UMN supporting sampling plans	AI surveillance
Mongolia	WCS/FAO/USGS/NIH/UMN/AWSG	Census and AI sampling of waterbirds, bridge species; bird marking migration studies; training courses; stable isotope migration study	Census and AI sampling of waterbirds, bridge species, migration studies, training
Mozambique	Percy FitzPatrick Ornithological Institute	Census and AI sampling of waterbirds, bridge species	Census and AI sampling of waterbirds, bridge species; satellite - telemetry of waterbirds
Nigeria	WCS/CIRAD/Wetlands International	AI sampling of waterbirds, bridge species	AI sampling of waterbirds, bridge species
Peru	Cayetano Heredia University/DoD-NMRCD/Texas A&M	Wild bird and market surveillance	Waterbird sampling in new wetland sites; monitor market trade in wild birds
Philippines	University of Kansas (UK)	Analysis of samples previously collected by UK	Global risk map analysis with UNH
Republic of Congo	WCS	Census and mortality surveillance of wild birds	Census and mortality surveillance
Russia	Wetlands International/USGS	Waterbirds sampling for AI	Transmitters for migration studies
South Africa	Percy FitzPatrick Ornithological Institute	Census and AI sampling of waterbirds, environmental sampling, risk analysis and mapping	Census and AI sampling of waterbirds, bridge species; transmitters
South Korea	WCS, FAO, National Agriculture Department of South Korea	AI outbreak investigation	Fieldwork complete

Country or State	Collaborator(s)	Activities in 2007 - 2008	Planned Activities
Sudan	WCS, Wetlands International	Building collaborative relationships with government agencies and NGOs	Waterfowl sampling and censuses
Tanzania	Tanzania Bird Atlas	Census and AI sampling of waterbirds, bridge species, scavenger species	Census/sampling of waterbirds, bridge spp., transmitters for birds that fly the Nile-Rift Valley pathway
Ukraine	Wetlands International Black Sea Program; Friedrich-Loffler Institute, Germany	AI sampling of waterbirds	Fieldwork complete
Zimbabwe	Percy FitzPatrick Ornithological Institute	AI sampling of waterbirds, bridge species	AI sampling of wild and domestic birds
CONTRIBUTING WILD BIRD DATA TO www.GAINS.org	**Organization**	**Species groups**	**Supporting Agencies**
Burkina Faso	CIRAD	Anatidae, Waders	FAO
Chad	CIRAD/Wetlands Int.	Anatidae, Doves	FAO
Egypt	CIRAD/Wetlands Int.	Waders, Cormorants, Cattle Egrets	FAO
Ethiopia	CIRAD	Anatidae, Waders	FAO
Iran	Wetlands Int.	Anatidae	FAO
Jordan	CIRAD	Waders	FAO
Kazakhstan	CIRAD/Wetlands Int.	Waterbirds	FAO
Kenya	CIRAD/Wetlands Int.	Anatidae, Waders, Flamingo	FAO
Malawi	CIRAD/Wetlands Int.	Anatidae, Waders, Rails	FAO
Mali	CIRAD	Anatidae, Waders, Rails	FAO
Mauritania	CIRAD	Anatidae, Waders, Gulls	FAO
Morocco	CIRAD	Anatidae, Waders	FAO
Niger	CIRAD	Waterbirds	FAO
Nigeria	CIRAD/Wetlands Int	Waterbirds	FAO/WCS
Romania	CIRAD	Anatidae, Waders, Sparrows	FAO
Senegal	CIRAD	Anatidae, Waders	FAO
Sudan	CIRAD/Wetlands Int.	Anatidae, Waders	FAO
Tunisia	CIRAD/Wetlands Int.	Anatidae, Waders	FAO
Turkey	Wetlands Int.	Anatidae	FAO/WCS
Zambia	CIRAD	Anatidae, Waders	FAO

STANDARD OPERATING PROCEDURES AVIAN INFLUENZA VIRUS SAMPLE COLLECTION

Wildlife Conservation Society
Global Health Program
Indonesia / Vietnam



CLEAN 1*:*

<u>NO</u> GLOVES; (MASK)

Handles:

- writing materials
- packets (swabs, syringe, alcohol pad) to present contents to Dirty 1
- VTM tubes and swabcutter
- rings / flags in bags
- glue and applicator
- ANYTHING/EVERYTHING ELSE - EXCEPT THE BIRD AND "DIRTY" MATERIAL

DIRTY 1 &2*:*

GLOVES; MASK

Handles:

- the bird
- scale
- caliper
- rulers
- ringing pliers
- pliers for cable tie removal

- **Weighing**

Clean:

- records data

Dirty 1:

- weighs bird + bag

- **Swabbing (Oropharyngeal and Cloacal)**

Clean:

- open swab packet, present swab
- open tube
- cut swab
- close tube
- place tube in ice
- place tubes in liquid Nitrogen
  (after 30 minutes or 3 birds)

Dirty 1:

- take swab
- throw away swab handle

Dirty 2:

- assist with holding bird (where necessary)

- **Blood collection**

Clean:

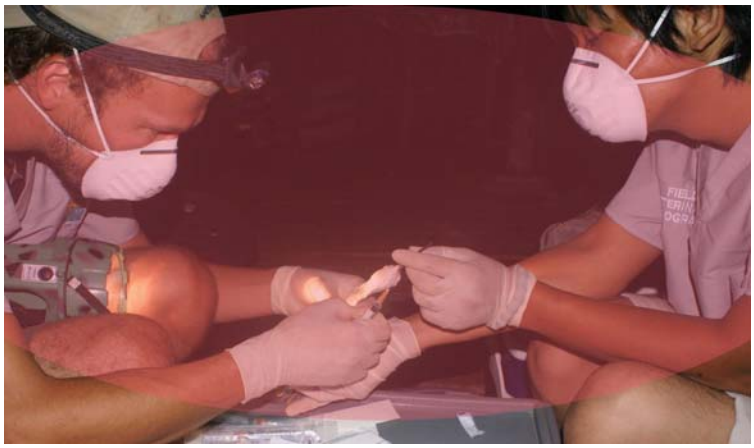
- present syringe and alcohol swab
- place empty bloodcollection tube in rack on table

Dirty 1 (<u>vet only</u>):

- take blood (when bodyweight >50g)
- insert needle in bloodcollection tube
- discard needle (sharp safe) and syringe (ziplock bag)

Dirty 2:

- assist with holding bird (where necessary)



- **Ringing / Flagging**

Clean:

- take ring / flag from case and place on table
- apply glue to flag

Dirty 1:

- apply ring / flag

Dirty 2:

- assist with holding bird (where necessary)

- **Morphometrics**

Clean:

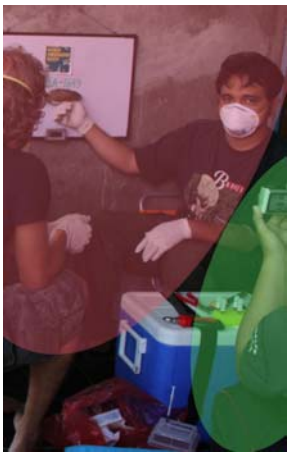
- record data

Dirty 1:

- take measurements

Dirty 2:

- assist with holding bird (where necessary)



- **Photo**

Clean:

- write bird ID number on board
- take photo

Dirty 1-2:

- hold bird

- **Cleaning/Tidying up**

Clean:

- ringing case
- vet case

Dirty 1&2:

- Disinfection - alcohol
- ringing equipment
- scale
- table

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Peterson, A. T., and R. A. J. Williams. 2008. Risk mapping of highly pathogenic Avian Influenza distribution and spread. *Ecology and Society* **13**(2): 15. [online] URL: http://www.ecologyandsociety.org/vol13/iss2/art15/

Research, part of a Special Feature on Risk mapping for avian influenza: a social-ecological problem

Risk Mapping of Highly Pathogenic Avian Influenza Distribution and Spread

A. Townsend Peterson and *Richard A. J. Williams*

ABSTRACT. The rapid emergence and spread of highly pathogenic H5N1 avian influenza begs effective and accurate mapping of current knowledge and future risk of infection. Methods for such mapping, however, are rudimentary, and few good examples exist for use as templates for risk-mapping efforts. We review the transmission cycle of avian influenza viruses, and identify points on which risk-mapping can focus. We provide examples from the literature and from our work that illustrate mapping risk based on (1) avian influenza case occurrences, (2) poultry distributions and movements, and (3) migratory bird movements.

Key Words: *avian influenza; land birds; poultry; risk mapping; wild birds.*

INTRODUCTION

The 1997 emergence and rapid spread of the highly pathogenic avian influenza strain H5N1, hereafter HP-H5N1, raised concerns regarding the potential for a global influenza pandemic (Cox and Subbarao 2000, Webby and Webster 2001, Capua and Alexander 2002). The impressive virulence of this strain, both for humans (Katz et al. 2000, Parry 2006) and for some birds (Sturm-Ramirez et al. 2004, Chen et al. 2005, Olsen et al. 2006), combined with its rapid spread across Asia (Chen et al. 2005), Africa (Ducatez et al. 2006), and Europe (Alexander 2007), indeed is cause for concern. To optimize response, forecasting and tracking the global spread of HP-H5N1 becomes of key importance, particularly in light of the diversity and contrasts of opinions that have been expressed regarding its transmission (ABC 2005, Chen et al. 2005, BirdLife International 2006, Gilbert et al. 2006, Rappole and Hubalek 2006).

In spite of the importance of forecasting and anticipating the global spread of such an important pathogen, few efforts have been made to map and forecast HP-H5N1 risk across broad regions or continents. Several publications have reviewed the HP-H5N1 situation (Alexander 2000, Feare and Yasue 2006, Olsen et al. 2006, Rappole and Hubalek 2006), but only in broad and general terms. Only a few have made more quantitative predictions or risk maps: poultry-based spread in the Netherlands (Boender et al. 2007), connections with rice cultivation in Thailand (Gilbert et al. 2007), mapping and monitoring of bird migration in various regions (Gilbert et al. 2006, Peterson et al. 2007, Winker et al. 2007), niche-based predictions of HP-H5N1 transmission risk across West Africa (Williams et al. 2008), and some more limited analyses that will not be treated in detail herein (Guo et al. 2006, Mulatti et al. 2007). Only one analysis (Kilpatrick et al. 2006) has addressed HP-H5N1 spread globally.

In this overview, we briefly review HP-H5N1 transmission routes and offer an overview of risk-mapping approaches that may be applicable to the situation. We provide examples of several such approaches, and explore how HP-H5N1 risk mapping may be improved in the future. Perhaps most importantly, given the paucity of detailed and quantitative information available, we outline how risk mapping and broad influenza monitoring can and should be linked in coming years to provide a more dynamic, responsive information base for decisions and initiatives related to HP-H5N1 and other emerging bird-borne diseases.

HP-H5N1 Transmission

The basic elements of the HP-H5N1 transmission cycle are fairly well understood (Fig. 1): in general, the virus is transmitted from bird to bird without intermediate vectors, but it can be held in bodies of water for some period of time, and mammals, including humans, are occasionally infected. Mammal-to-mammal, including human-to-human, transmission appears rare, as essentially all known human cases have some more or less direct contact with birds, usually poultry, and thus a clear connection to the bird-driven transmission cycle. Some indications of mammal-to-mammal transmission do exist (Thanawongnuwech et al. 2005), but these transmission chains are not self-sustaining.

Although the elements of the transmission cycle are not in much doubt, their relative roles are the topic of considerable debate. Some of those who opine about the topic believe fervently that the entire system is driven by movements of poultry and poultry products, and that wild birds are incidental hosts with little importance in overall HP-H5N1 transmission dynamics (BirdLife International 2006, Feare and Yasue 2006, Rappole and Hubalek 2006, Gauthier-Clerc et al. 2007) – we can term this opinion that of "wild birds as victims." A key point revealed by inoculation studies is that at least some wild birds can be infected without significant external symptoms or death (Brown et al. 2006, Boon et al. 2007), suggesting that they can serve as carriers for avian influenza viruses once infected, potentially even on migration. On the other side of the debate, several researchers have recognized significant roles for wild birds, e.g., "wild birds as vectors," in HP-H5N1 transmission and spread (Chen et al. 2005, Gilbert et al. 2006, Kilpatrick et al. 2006, Olsen et al. 2006, Winker et al. 2007), although poultry clearly play a role in this process as well. A recent commentary pointed out the relative costs of choosing the wrong null hypothesis in this debate, suggesting that the most appropriate presumption would be that migratory birds are involved (Flint 2007). The most balanced and the only global analysis to date (Kilpatrick et al. 2006) concluded that both domestic and wild birds have played roles in the global spread of HP-H5N1.

Still more shrouded in shadows, however, is the avian taxonomic distribution of host relationships of HP-H5N1 transmission and spread. The standard review of influenza ecology and host distribution (Webster et al. 1992) states clearly that waterbirds are the reservoir of avian influenza viruses, and papers in the field almost invariably cite this paper and assume this point to be well established. Although influenza prevalences are clearly highest in waterbirds (Webster et al. 1992, Olsen et al. 2006, Munster et al. 2007), the occurrence of this virus in landbirds should not be neglected in surveillance efforts (Alexander 2000, Kou et al. 2005, Kwon et al. 2005, OIE 2005). Recent inoculation experiments have indicated that landbirds can be infected and can become viremic to the point of being infective to other individuals, albeit not commonly (Boon et al. 2007). Particularly of concern is the fact that major monitoring initiatives are focusing only or chiefly on waterbirds (Gaidet et al. 2007, Winker et al. 2007): appreciating a significant role for landbirds becomes difficult or impossible when they are not even being sampled.

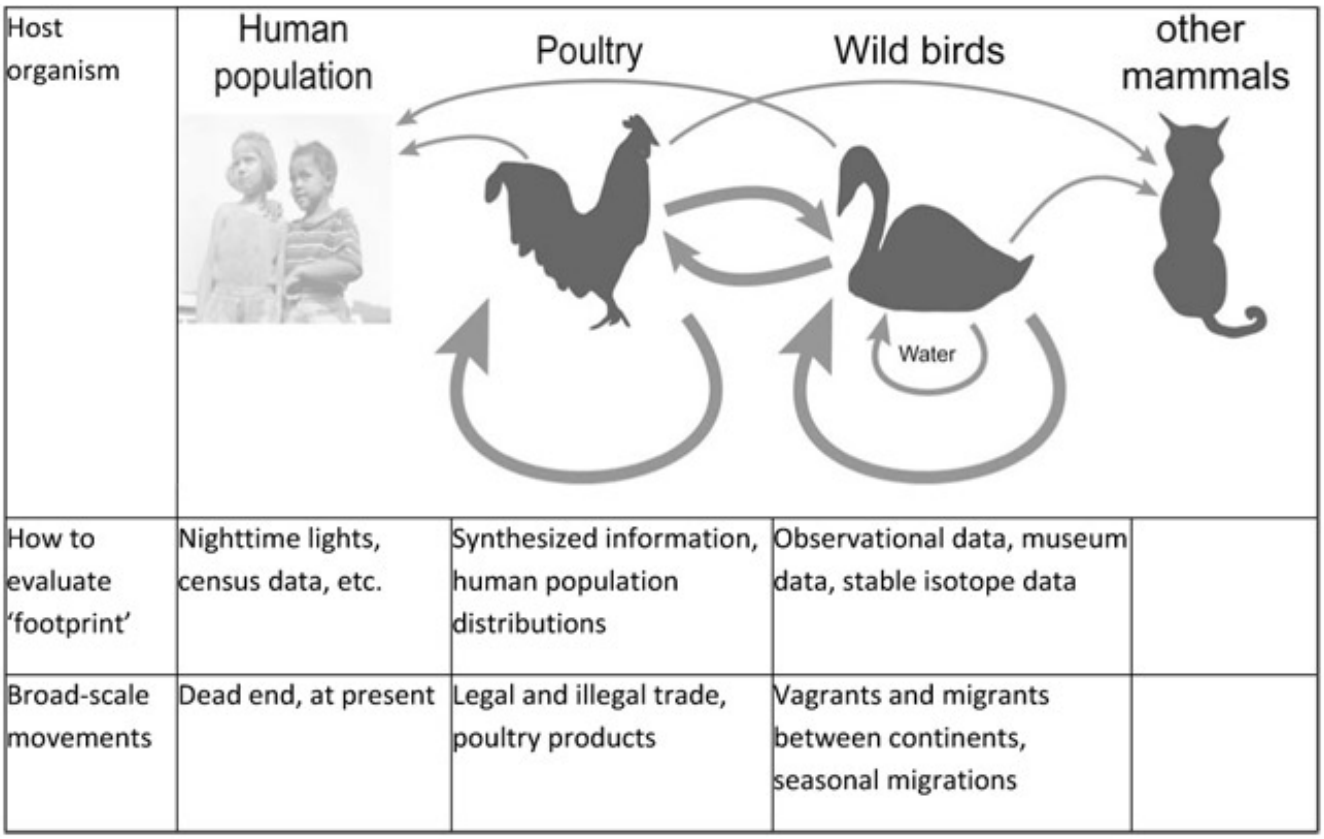
Mapping HP-H5N1 risk of transmission and spread

Three sectors of the HP-H5N1 transmission cycle may be tractable to risk mapping (Fig. 1): distributions and movements of human populations, poultry, and wild birds. In each case, broadly available data sets offer means of summarizing the distribution and movements in those elements (Fig. 1): nighttime lights and census data track human populations; specifically synthesized data or human population surrogate information can key to poultry distributions; and observational data, natural history museum data, and other ornithological data sets can inform regarding bird distributions and movements. As such, ample information resources exist on which to base risk-mapping efforts.

Three points in the transmission cycle of HP-H5N1 offer opportunities for risk mapping: (1) mapping actual cases of HP-H5N1 and extrapolating to identify areas that are similar environmentally; (2) mapping movements and commerce in poultry and poultry products; (3) mapping wild bird migration patterns, potentially educated by information from ongoing monitoring efforts. We review each of these opportunities in the paragraphs that follow, illustrating each with examples, and identifying paths toward further improvement.

An alternative approach, not explored or discussed in detail herein, is the risk analysis approach, in which hazard identification, risk assessment, management, and communication are outlined. This

Fig. 1. Diagrammatic summary of transmission routes of avian influenza viruses, showing major transmission routes (thick arrows) and minor transmission routes (slender arrows).



suite of ideas has been applied to avian influenza transmission in Ethiopia (Goutard et al. 2007) and Vietnam (Pfeiffer et al. 2007), and might be useful in refining and improving the more elemental, process-based mapping attempts outlined herein. That is to say, while we do not discount the utility of this approach, we feel that it is not sufficiently connected to HP-H5N1 transmission biology to form the basis for full risk assessments.

Finally, we note that low-pathogenic influenzas can be more numerous than the high-pathogenic viruses, and can thus provide much more abundant information regarding their ecology and distribution. HP-H5N1 is still relatively rare, and is restricted to the Old World, and detections are consequently quite rare, limiting opportunities for detailed study. As low-pathogenic strains can evolve into high-pathogenic forms (Chen et al. 2004), their detailed study is more than warranted, although differences in transmission should be borne in mind. Research attention should thus not focus only on HP-H5N1, but should extend to avian influenza viruses in general, so as to maximize the information gained and its utility in understanding a complex situation.

Mapping HP-H5N1 cases

The simplest approach to risk mapping is that of treating the entire transmission cycle effectively as a black box, and simply focusing on the spatial position and environmental characteristics of sites where humans or poultry contract the disease (Peterson 2006). This approach has the advantage of accessing a maximum of information, i.e., any

site where the disease has been detected. However, it should be borne in mind that it integrates across all of the diverse processes that make for such an occurrence being detected, that is, biases at any point from the pathogen itself through to diagnosis and reporting can create a nonrandom distribution that appears predictive (Peterson 2006, 2008). As such, independent testing and repeated challenging of models to be predictive and general are central to this application of risk mapping.

Given the simple, bird-to-bird transmission cycle of HP-H5N1, and given the broad spectrum of birds apparently involved, i.e., both poultry and wild birds, it might be expected that no clear ecological signature would be detectable. Curiously, though, the two detailed analyses that have been developed along these lines (Gilbert et al. 2007, Williams et al. 2008) have both found significant, predictive environmental signals indicative of higher risk of HP-H5N1 transmission. Clearly, this area of risk mapping for HP-H5N1 demands more attention to determine whether it is simply tracking inherent, but subtle, biases in occurrence data, or whether the environmental signals are genuinely associated with HP-H5N1 transmission.

The first "black box" study focused on free-ranging domestic duck populations in Thailand (Gilbert et al. 2007). The tie between HP-H5N1 transmission and free-ranging domestic duck populations was already well established (Hulse-Post et al. 2005), so the novelty of this study hinges on identification of coarse-scale environmental signals of domestic duck populations that are detectable repeatably and quantitatively in remotely sensed imagery (Gilbert et al. 2007). The study resulted in clear "signatures" of domestic duck populations related to the length of the growing season for second rice plantings and harvest in Thailand, suggesting that HP-H5N1 transmission risk can be mapped successfully, at least in Southeast Asia, where duck production is so key.

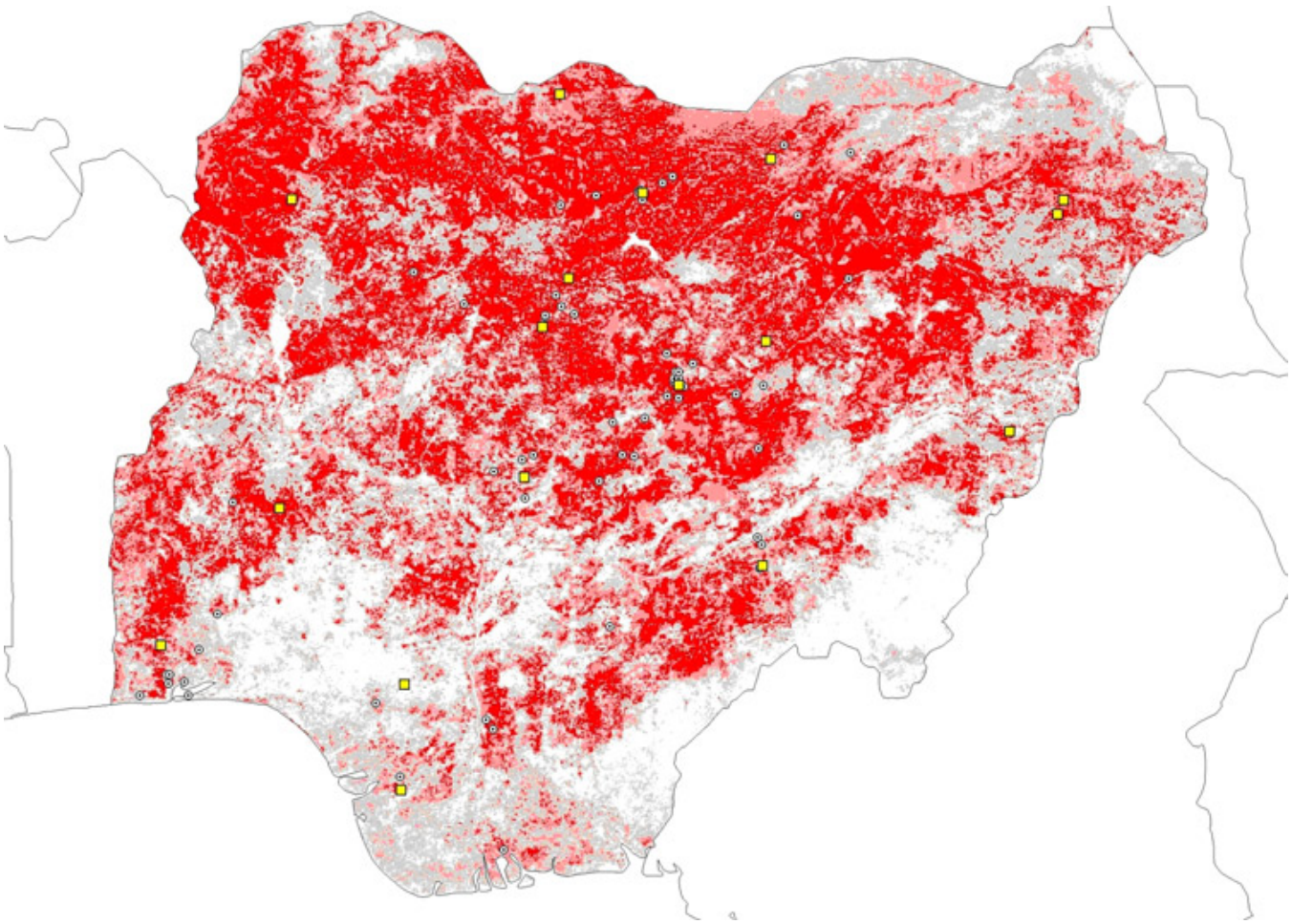
The second such study (Williams et al. 2008) is much less clear mechanistically as to why risk mapping should be possible. Here, the authors focused on HP-H5N1 occurrences in Nigeria, where poultry flocks are not clearly and consistently associated with such a prominent landscape feature as rice cultivation. Using tools for ecological niche modeling (Peterson 2006), they related sites of known occurrence to a set of remotely sensed images summarizing month-to-month variation in "greenness" (normalized difference vegetation index); models were challenged to predict broad sectors of the country and of West Africa from which occurrence data were withheld in the modeling process. The models, perhaps somewhat surprisingly given the lack of easily detectable environmental signals, e.g., rice paddies, were able to predict the great majority of independent occurrences in numerous tests (see Fig. 2 for an example); nonetheless, considerable additional testing is necessary before such analyses can be considered to be genuinely predictive of HP-H5N1 transmission potential.

This sort of black-box risk mapping works only if consistent environmental signals are associated with HP-H5N1 transmission, and, of course, it can be applied in a region only when some number of occurrences has already occurred. In the case of individual species' distributions, the niche-modeling approach functions admirably thanks to species' ecological niches (Pulliam 2000, Soberón and Peterson 2005, Soberón 2007). For HP-H5N1, however, the case is less clear, as the black box subsumes many and very diverse species and processes, except in the clearest cases, such as the rice-paddy duck production in Southeast Asia. The environmental signal, in essence the "ecological niche" of the transmission cycle, could come from some environmentally-constrained step in the cycle, or it could come from some consistent source of bias in sampling or reporting. Clearly, this area of HP-H5N1 risk mapping will require further testing and experimentation before it can be considered as a reliable source of information.

Mapping poultry movements

Moving away from simple, black-box approaches, an important element in HP-H5N1 transmission is movements of poultry and poultry products. As has been noted frequently (Kilpatrick et al. 2006), this element in the transmission cycle is difficult to quantify and map in detail owing to the combination of local, unregulated movements and trade in poultry by peasant farmers, and the broader-scale, illicit bird trade (Van Borm et al. 2005). Consequently, detailed considerations of this factor are complex, and only two previous HP-H5N1 risk-mapping efforts have included dimensions of poultry movements (Kilpatrick et al. 2006, Boender et al. 2007).

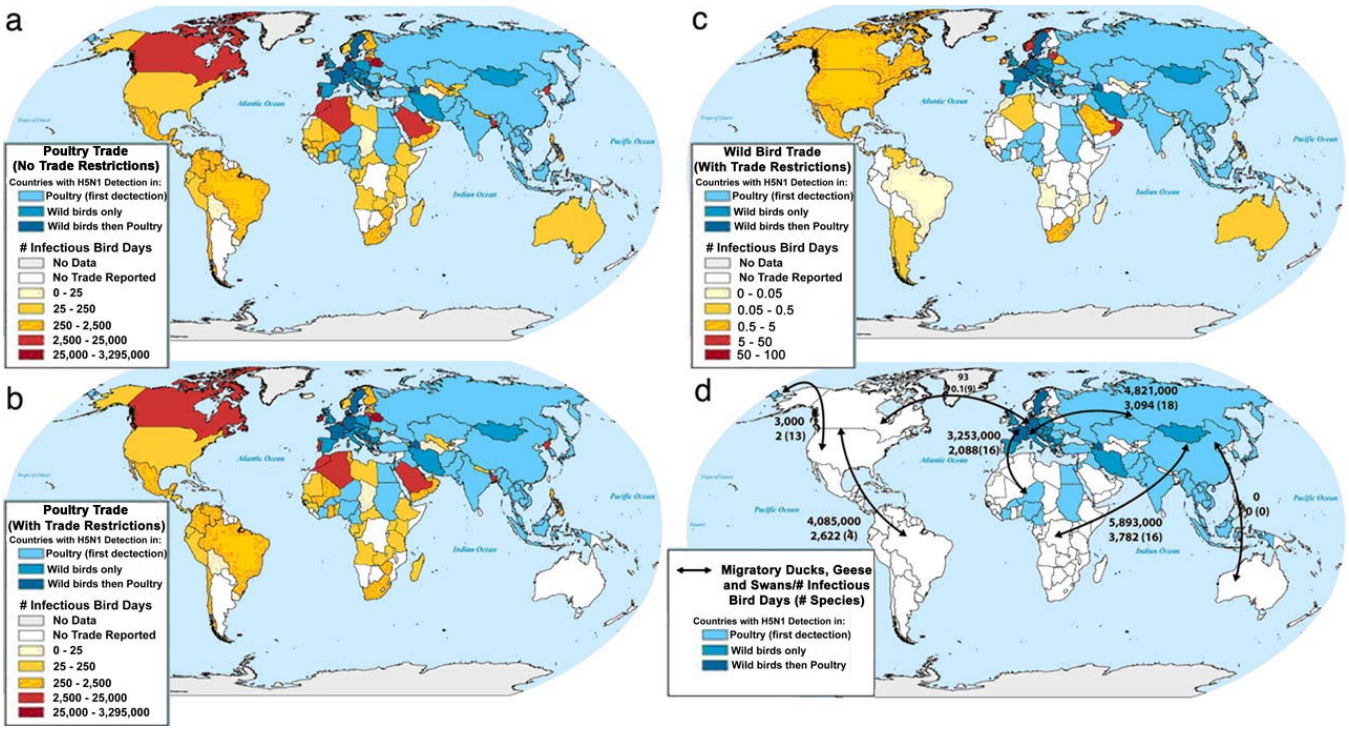
Fig. 2. Example prediction of HP-H5N1 occurrences in poultry in Nigeria (Williams et al. 2008). Dotted circles indicate 72 occurrences in January-November 2006 that were used to train the model. Model predictions are summarized as a ramp of colors, ranging from white (all replicate models agreed in predicting absence) to red (all replicate models agreed in predicting potential for presence). Yellow squares indicate independent occurrences from November 2006-January 2007 that were used to test the model predictions. Note the close correspondence between the spatial position of these occurrences and the red areas, which was statistically significantly better than random.



Boender et al. (2007) developed scenarios of spread of highly pathogenic avian influenza based on the spatial distribution of infected and uninfected poultry farms in the Netherlands, estimating farm-to-farm transmission probabilities based on inter-farm distances. The resulting risk maps are sensitive to parameter estimates, which become less precise when based on less-complete data. However, the principal limitation of this approach is that of obtaining detailed locations of all relevant poultry farms in a region, particularly when study regions are broad and cross multiple states or countries.

The second poultry-based risk mapping effort is the global HP-H5N1 risk evaluation of Kilpatrick et al. (2006). Combining information on legal poultry trade with information on bird migration (see separate discussion below), this analysis produced country-level maps of the chances of HP-H5N1 introduction (Fig. 3), and offered several novel

Fig. 3. Predicted risk of HP-H5N1 introduction from countries that have had H5N1 outbreaks (in blue). (a–c) Risk was estimated as number of infectious bird days caused by trade in: live poultry with no trade restrictions (a), live poultry with no exports from countries reporting H5N1 in poultry (b), and captive wild birds with no exports from countries reporting H5N1 in poultry (c) as in b. (d) Estimated number of ducks, geese, and swans migrating between mainland continents, number of infectious bird days, and number of species (in parentheses). Reproduced from Kilpatrick et al. (2006).

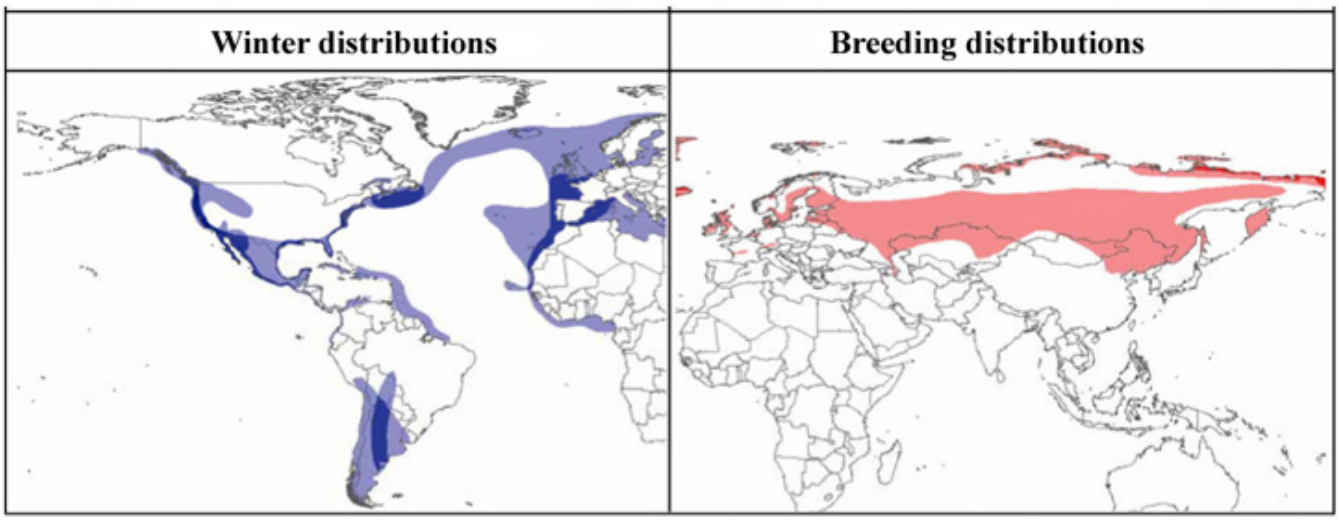


insights into introduction potential, e.g., risk of introduction into North America via South America. This study was limited in several dimensions, particularly in its focus on legal poultry trade, neglecting the much-less-regulated but not negligible illicit trade, and in its spatial resolution at the level of countries only. It is, however, worth mentioning that this analysis, i.e., the only one to date that includes dimensions of both domestic and wild birds, concluded that both sets of bird populations, i.e., poultry and wild birds, have contributed to the spread of HP-H5N1.

Mapping bird migration

The dimension of HP-H5N1 transmission that is perhaps most amenable to detailed mapping is that of bird distributions and movements. Given several centuries of study by ornithologists, as well as intense popular interest, birds rank among the best known of all major taxa (del Hoyo et al. 1992-2007), with detailed distributional information available for most species. Several studies have taken advantage of such information to develop insights into how wild bird movements could serve to disperse HP-H5N1 broadly across regions (Gilbert et al. 2006, Kilpatrick et al. 2006, Peterson et al. 2007, Winker et al. 2007), although a genuinely detailed synthesis has yet to be developed.

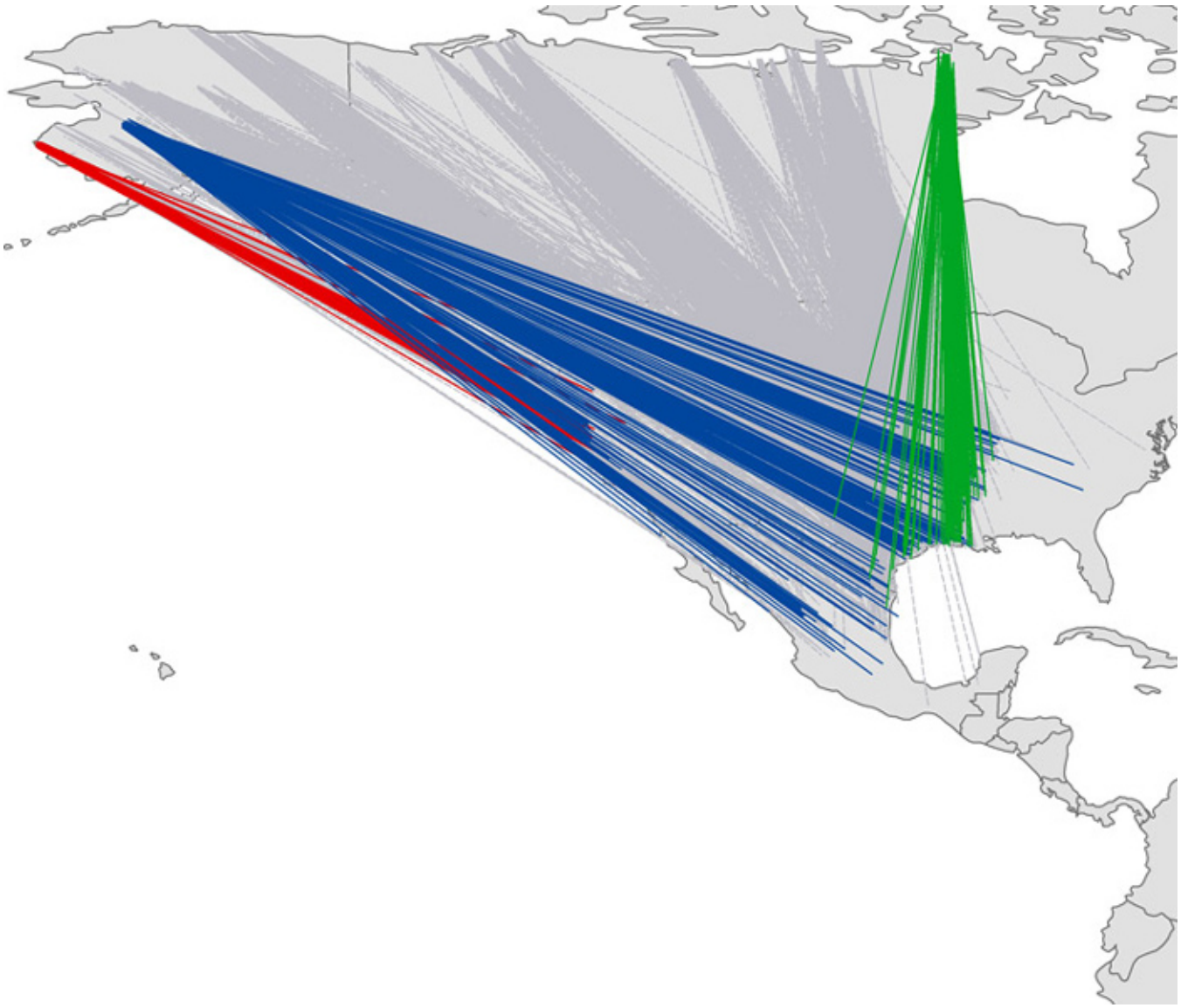
Fig. 4. Summary of wintering and breeding distributions of species that breed across both hemispheres in the Holarctic, but winter at least in part in the Americas; one species (*Larus ridibundus*) ranges considerably farther south in Russia, extending to areas that have indeed seen HP-H5N1 outbreaks. Color ramp indicates numbers of species (white = 0, darkest shade = 5 species). From Peterson et al. (2007).



The global evaluation cited above (Kilpatrick et al. 2006) relied generally on information regarding bird movements along migratory pathways, which (as will be seen below) can be deceptively simple, and can miss important details. Similarly, the regional analysis of Gilbert et al. (2006) was also based on coarse-resolution flyway information on migratory bird movements. Reliance on such summary information can limit the spatial resolution of resulting information products. As such, in the following paragraphs, we review two recent, and more detailed, studies that deal with migration summaries on a species-by-species basis not constrained by the coarse detail and resolution of flyway information.

At coarse scales, an important suite of questions revolves around bird-mediated dispersal between continents. Although these questions have seen considerable speculation in recent commentaries (Rappole et al. 2000, Rappole and Hubalek 2006), real data and quantitative approaches can be more revealing. These questions were addressed recently in an HP-H5N1 context by Peterson et al. (2007). The question was how HP-H5N1, if it is being spread across Europe, Asia, and Africa by migratory birds, could potentially reach the Americas. The conventional focus in the Americas has been on western Alaska as the most likely "port of entry" for the virus into the region (NBII 2006). Peterson et al. (2007), however, digitized summer and winter range maps for all of the 157 bird species that show regular seasonal movements between the two hemispheres, and illustrated that two very distinct patterns are present: birds that winter in southern Eurasia, breed across northern Eurasia, and have marginal breeding populations in northwestern or northeastern North America, i.e., the conventional focus, but also birds that winter in the Americas, but breed across northern North America and into northern Eurasia marginally (Fig. 4). The distributional patterns of these two sets of species contrast sharply, with the latter group ranging broadly southward into the Americas, suggesting that the western Alaska focus of attention may not be sufficient for effective HP-H5N1 monitoring. This study also illustrates vividly the perils of overreliance on summary data on migratory pathways, when individual species may behave in ways that do not 'fit' the general pattern comfortably.

Fig. 5. Illustration of the complexities of bird migration, when information on connectivity of areas is considered, even within a single species. Here, U.S. Bird Banding Laboratory data on banding and recovery events in breeding and winter seasons of Greater White-fronted Geese (*Anser albifrons*) are plotted on a map of North America. Records, i.e., lines connecting breeding and wintering records corresponding to banding and recovery of a single individual, not intended to reflect migratory routes, from particular areas of the breeding range are highlighted in different colors. Note that a significant portion of the blue lines also stops along the Pacific coast of North America, coincident with the winter destination of the red lines, and that none of the red lines extends farther east than the west coast.



At finer scales, the challenges of data resources become more acute. Ideally, patterns of connectivity of areas via bird migration would be mapped at fine scales, rather than just at the level of countries or flyways, but the technology for establishing such connectivity is only now being explored. Possibilities include stable isotope mapping (Farmer et al. 2004, Atkinson et al. 2005), fine-scale phylogeographic studies (Kelly et al. 2005), tracking of satellite transmitters attached to individual birds (Webster et al. 2002), and direct detections of connectivity from banding data (Belthoff and Gauthreaux 1991). Nonetheless, major challenges confront any broadscale efforts in this dimension.

To illustrate both the complexity of such applications and the need for probabilistic approaches to migratory connectivity, we show example patterns from Greater White-fronted Geese (*Anser albifrons*) in North America (Fig. 5) based on banding recoveries. Certainly, such analyses are subject to myriad caveats and qualifications, but the information provided for connectivity between breeding and wintering distributional areas for those species for which sample sizes are sufficient is almost unique. The resulting patterns are more complex than the simple flyway maps would suggest. In particular, while extreme coastal Alaska is connected via migration principally to the west coast of North America, individuals from areas just a few hundred kilometers inland, but still in western Alaska, range much more broadly across western North America. Only by combining information across many such individual species will a genuine and complete picture of migratory connectivity be possible.

CONCLUSIONS AND NEXT STEPS

The challenge of mapping risk of HP-H5N1 dispersal and transmission is clearly complex. Each element of the transmission cycle that can be mapped has significant caveats associated with it (see above): niche modeling of landscape suitability demands adequate identification of causal elements of landscape environments and how they actually affect HP-H5N1 transmission; poultry mapping requires more accurate summaries of official poultry distributions, but also resolution of the much more difficult challenges of mapping peasant-owned poultry and illicit poultry movements; and mapping of bird migration requires improved protocols for integrating diverse data streams into a single overall picture. However, the only path to a genuine synthesis of the situation would include combination of all of these elements into a single analysis, i.e., broadscale connectivity via migratory birds, local and broadscale connectivity via poultry and poultry products, and local landscape suitability from ecological niche models. Clearly, the complexity of data, analyses, and interpretation in such a system would be daunting.

Nonetheless, the examples cited in this overview should, we hope, offer some stimulus. That is, important components of HP-H5N1 dispersal and transmission are amenable to analysis and mapping, and good templates for such mapping efforts are available already. As such, workers in particular situations and particular regions can take techniques already prototyped, and adapt them to particular challenges and questions. In this way, important portions of the HP-H5N1 risk mapping challenge can indeed be taken on as feasible steps.

Responses to this article can be read online at:
http://www.ecologyandsociety.org/vol13/iss2/art15/responses/

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