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Migratory movements of waterfowl in Central Asia and avian influenza emergence: sporadic transmission of H5N1 from east to west

SAMUEL A. IVERSON,[1]* ANDREI GAVRILOV,[2] TODD E. KATZNER,[3] JOHN Y. TAKEKAWA,[1] TRICIA A. MILLER,[4] WARD HAGEMEIJER,[5] TAEJ MUNDKUR,[5] BALACHANDRAN SIVANANINTHAPERUMAL,[6] CARLOS C. DeMATTOS,[7] LU'AY S. AHMED[8] & SCOTT H. NEWMAN[9]

[1]*United States Geological Survey, Western Ecological Research Center, Vallejo, CA 94592, USA*
[2]*Institute of Zoology, Akademgorodok, Al-Farabi av. 93A, Almaty 050060, Kazakhstan*
[3]*Division of Forestry and Natural Resources, West Virgina University, Morgantown, WV 26506, USA*
[4]*Carnegie Museum of Natural History, Powdermill Nature Reserve, Rector, PA 15677, USA*
[5]*Wetlands International, NL-6700 AL Wageningen, Netherlands*
[6]*Bombay Natural History Society, Mumbai 400 001, India*
[7]*United States Naval Medical Research Unit 3 (NAMRU-3), Cairo, Egypt*
[8]*Egyptian Environmental Affairs Agency, Cairo, Egypt*
[9]*EMPRES Wildlife Unit, Animal Health Service, Animal Production and Health Division,
Food and Agriculture Organization of the United Nations, Rome 00153, Italy*

Waterfowl in the genera *Anas* and *Tadorna* are suspected as vectors in the long-distance transmission of highly pathogenic avian influenza H5N1. The former Soviet Republics of Central Asia are situated at an important migratory crossroads for these and other species of birds that bridges regions where the disease is prevalent. However, waterfowl movements through Central Asia are poorly quantified. In this study, historical data derived from over 80 years of bird ringing are combined with recent satellite tracking data to delineate migration routes, movement chronology and habitat use patterns of waterfowl in relation to H5N1 outbreak locations. Results confirm migratory linkage between breeding and moulting areas in northern Kazakhstan and southern Siberia, with non-breeding areas in the Caspian, Black and eastern Mediterranean Sea basins, as well as with South Asia. However, unlike the situation in neighbouring regions, most notably western China, H5N1 outbreaks have not been recurrent in Central Asia after they were first reported during summer 2005 and spring 2006. These findings have implications in relation to potential sampling biases, species-specific variation in migratory behaviour and continuing regional H5N1 transmission risks.

Keywords: *Anas*, bird migration, highly pathogenic avian influenza, Kazakhstan, ring recovery, risk mapping, satellite telemetry, *Tadorna*.

Waterfowl in the Family Anatidae (ducks, geese and swans) are the primary reservoir of avian influenza viruses in the wild (Webster *et al.* 1992). Considerable research has focused on understanding the role of wild waterfowl in the emergence and spread of highly pathogenic avian influenza H5N1 (hereafter H5N1; Horimoto & Kawaoka

2001, Webby & Webster 2001, Webster *et al.* 2006), including their potential involvement in the long-distance dispersal of this influenza virus sub-type across Asia and into Europe and Africa (Kilpatrick *et al.* 2006, Olsen *et al.* 2006). The importance of migratory waterfowl as H5N1 vectors compared with the movement of poultry and poultry products remains a subject of debate (Feare & Yasue 2006, Gauthier-Clerc *et al.* 2007). However, a growing body of evidence from

*Corresponding author.
Email: Samuel.Iverson@ec.gc.ca

laboratory and field studies suggests that individuals of at least some waterfowl species can act as disease carriers (Kim *et al.* 2009).

The former Soviet republics of Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan and Uzbekistan (hereafter Central Asia) sit at an important crossroads for waterfowl migration. The region encompasses an area of broad spatial overlap between the East Asian, Central Asian and Eurasian–African Flyways and globally significant numbers of waterfowl are funnelled into its wetlands each spring and autumn (Gavrilov & Gavrilov 2005, Kreuzberg-Mukhina 2006, Lanovenko 2006, Yerokhov 2006, Schielzeth *et al.* 2008). Central Asia is situated at a particularly important location for studying H5N1 transmission because it is bordered by China to the east, where the disease first emerged and continues to circulate (Sims *et al.* 2005, Sims & Brown 2008), and areas to the north, west and south, where H5N1 detection has been more recent and sporadic (Sims & Brown 2008). Phylogenetic similarity between virus isolates collected from wild birds at Qinghai Lake, China, and those from the Russia–Kazakhstan border region suggested a route of introduction to Russia and Central Asia from western China (Shestopalov *et al.* 2006). In accordance with these similarities, outbreaks were located near sites where large numbers of waterfowl congregate during the post-breeding period (Gilbert *et al.* 2006). However, the distribution of outbreaks also followed major trade routes for the movement of poultry and poultry products, emphasizing a need for ecological study and H5N1 surveillance sampling along important migration corridors to distinguish mechanisms of spread (Gauthier-Clerc *et al.* 2007).

Although natural history studies are numerous in Soviet ecological journals, waterfowl movements in Central Asia have rarely been analysed comprehensively and are not well described in English-language publications (see Veen *et al.* 2005, Dobrynina & Kharitonov 2006). In this paper, we integrate historical data from waterfowl ringing with current satellite tracking studies and H5N1 outbreak information. We focus on movements by waterfowl in the genera *Anas* (dabbling ducks) and *Tadorna* (shelducks) because species in these genera are numerous in Central Asia (Gavrilov & Gavrilov 2005) and several have been implicated as potential H5N1 carriers (Keawcharoen *et al.* 2008). Our objectives are to delineate migration routes, identify migratory connections between breeding and non-breeding areas, and compare movement behaviour and habitat use patterns among potential host species in relation to H5N1 occurrence patterns at a junction between eastern and western flyways.

METHODS

Multiple data types were used in our analyses, for which the sources and our applications are described below.

Ringing and recovery locations

Ring and recovery information was obtained from the Institute of Zoology, Ministry of Sciences and Education of the Republic of Kazakhstan. H5N1 was first reported in Kazakhstan in July 2005 at a poultry farm in the northern province of Pavlodar near the Russian border (FAO 2010). Additional cases were reported in the surrounding area in which H5N1 was suspected, but could not be confirmed. Outbreaks have not been reported in other countries in Central Asia and the last known case of H5N1 in Central Asia involved wild swans *Cygnus* spp. (species not reported) that were recovered in western Kazakhstan near the Caspian Sea in March 2006.

Ring and recovery location estimates were compiled for seven *Anas* species (Common Teal *Anas crecca*, Eurasian Wigeon *Anas penelope*, Gadwall *Anas strepera*, Garganey *Anas querquedula*, Mallard *Anas platyrhynchos*, Northern Pintail *Anas acuta* and Northern Shoveler *Anas clypeata*) and two *Tadorna* species (Common Shelduck *Tadorna tadorna* and Ruddy Shelduck *Tadorna ferruginea*) for individuals that were either ringed in Central Asia and recovered inside or outside the region, or ringed outside Central Asia and recovered therein. The database included records from 1927 to 2005, with more than three-quarters ringed from the 1960s onwards. Most records were obtained by wildlife professionals engaged in ringing activities (90%) and the remainder were from submissions by hunters (6%) or other members of the public (4%).

Location estimates were assumed to be accurate within 0.10° of latitude and longitude, which is a conservative estimate of location accuracy. Older reports and those derived from submissions by non-professionals were taken from marks on maps and as such could not be independently verified.

This level of accuracy is similar to the standard applied by wildlife agencies in North America for ring reports from the public (Gustafson *et al.* 1997) and corresponds roughly to the 10-km radius location accuracy category used by EURING for recoveries in Europe (Speek *et al.* 2001). Error at this scale is small relative to the large spatial scale of bird movements and was appropriate for our analyses.

When delineating bird movements from ring and recovery data it is important to note that location estimates do not constitute a random sample; rather, encounter and reporting probabilities are influenced by habitat and landscape features, human activities, and the cultural, political and economic situations within the areas into which birds range (see Clark *et al.* 2009 for a review). We suspected spatial variation in recovery probabilities within and among countries and therefore avoided potentially spurious statistical comparisons in situations where 'missing data' might bias results. Instead, for this component of our work, we identified locations for which the empirical evidence of migratory linkage is strong, but refrained from statistical comparisons of recovery rates among geographical areas.

Satellite tracking data

Satellite transmitters overcome issues associated with the non-random distribution of ring recoveries but they are subject to other considerations (reviewed by Millspaugh & Marzluff 2001). These include potential impacts of transmitters on tagged individuals and the frequency and quality of remotely sensed location data. For our study, 19 individuals were tracked to provide additional insights into habitat use patterns and migration chronology. The primary location for transmitter deployment was Kyzolkol (Red Lake; 43.75°N, 69.50°E) in South Kazakhstan Oblast (province), where 14 birds (two Common Teal, one Gadwall, seven Mallard, two Northern Shoveler and two Ruddy Shelduck) were marked in September 2007 and September 2008. Supplementary data were obtained for three birds marked in Egypt (two Northern Shoveler and one Common Shelduck) and two birds marked in India (one Garganey and one Northern Pintail) that subsequently migrated to Central Asia. The deployments in Egypt were made during January 2009 at Manzala Lake in the Nile Delta (31.27°N, 32.20°E) and the deploy-

ments in India were made during December 2008 at wetlands near Kanyakumari, Tamil Nadu (8.09°N, 77.50°E).

Capture, handling and marking procedures were standardized across studies following United Nations Food and Agriculture Organization procedures (FAO 2007) approved by the USGS Patuxent Wildlife Research Center Animal Care and Use Committee. Depending on their size, individual birds were fitted with 12-, 18- or 30-g solar-powered transmitters (Microwave Telemetry Inc., Columbia, MD, USA) that were attached with a Teflon harnesses in a backpack configuration (Bally Ribbon Mills, Bally, PA, USA). Location data were obtained using global positioning system (GPS) technology (30-g transmitters) and/or the Argos Data Collection and Geo-Location System (12-, 18- and 30-g transmitters; CLS America Inc., Largo, MD, USA). We restricted our analysis to location estimate classes 0, 1, 2 and 3, which correspond to 1-sigma error radiuses of 1000, 350–1000, 150–350 and ≤ 150 m, respectively.

Environmental data

We integrated our bird location estimates with digital thematic maps using ArcGIS, Version 9.2 (Environmental Systems Research Institute Inc., Redlands, CA, USA). H5N1 outbreak information was obtained from the FAO EMPRES Global Animal Disease Information System database (FAO 2010). Although potential reporting biases exist (much as for ring recoveries), the EMPRESS database constitutes the most comprehensive source of information about H5N1 occurrence at national and regional scales. We restricted our analysis to reports rated as reliable by FAO and included outbreak events occurring from 1 March 2000 to 30 June 2009. Habitat characteristics at bird recovery locations were inferred using the MODerate-resolution Imaging Spectroradiometer (MODIS)/Terra Land Cover Classification (https://lpdaac.usgs.gov), for which the MOD12C1 product with spatial resolution 0.05° was employed. The MODIS/Terra dataset provides information about dominant land cover type, which we grouped following the University of Maryland protocol and compiled into natural vegetation and cropland/urban categories. Poultry density estimates were derived from the FAO Gridded Livestock of the World (Robinson *et al.* 2007), which is available via Geonetwork (http://www.fao.org/geonetwork).

In the database, livestock census data are extrapolated to produce density estimates in head/km^2 and files are disseminated in raster format with a spatial resolution of 0.08°. These data were used to compare the potential for contact by wild birds with domestic birds on a landscape scale.

Data analysis

For our analyses, we followed recommendations by Flint (2007) and assumed that waterfowl movements have been consistent with expectations for an involvement in the long-distance dispersal of H5N1. We then used empirical data to assess the extent to which evidence supports or contradicts this assertion. We elected to employ this null hypothesis on the basis that premature dismissal of a potential carrier has the ability to allow H5N1 to expand its range undetected, whereas incorrectly concluding that migratory birds can disperse H5N1 when they cannot is merely inefficient from a research perspective (Flint 2007). We judged support for the null hypothesis on the basis of spatial and temporal overlap of *Anas* and *Tadorna* location estimates with H5N1 outbreak sites, as well as the use of habitats by wild waterfowl considered at high risk for direct contact with poultry.

Ring-recovery data were used to estimate migratory connectivity in relation to world regions and major landscape features. UN definitions were used to categorize world regions (http://www.un.org/depts/dhl/maplib/worldregions.htm) with the exceptions that South and Central Asia were separated into different components, and territory within the Russian Federation was split into Eastern Europe and Northern Asia at the Ural Mountains. To distinguish movements made during a single season from those made over the course of an individual's lifetime, recovery data were summarized by date, season and the interval of time between ringing and recovery events. Data pairings were categorized as spring movements when a bird was ringed between 15 December and 15 May and recovered < 6 months later, and as autumn movements when a bird was ringed between 1 June and 30 October and recovered < 6 months later. The purpose of this categorization was to censor individuals that were recovered several seasons or years after ringing and to distinguish movement directions toward or away from breeding and moulting areas.

Distance between ringing and recovery locations was calculated using the animal movement parameter tool in Hawth's Tools (http://www.spatialecology.com/htools). Characteristics of recovery locations were inferred using the majority and mean focal statistics tools in ArcGIS Spatial Analyst. A 10-km buffer was applied to each location estimate on the basis of bird recovery location accuracy and the resolution of the thematic base maps. Generalized linear models (GLMs; STATISTICA, Version 6, Systat Software Inc., Richmond, CA, USA) were used to assess statistical differences among species in the distance between ringing and recovery locations, the proportion of birds that were ringed in Central Asia and recovered inside and outside the region, the proportion of recoveries occurring in cropland/urban habitat types and poultry density at recovery locations. The proximity of *Anas* and *Tadorna* location estimates to H5N1 outbreak sites was also estimated. Evaluations were made for data pairings that occurred within 10 km and 10 calendar days of each other (irrespective of year), as well as within 50 km and 45 calendar days to assess broader scale proximity and seasonal correspondence.

The satellite tracking data were used to identify key areas of bird activity within Central Asia and define habitat use patterns, rates of movement and stopover duration during migration. Rates of movement during migration and potential H5N1 dispersal distances were estimated following procedures described by Gaidet *et al.* (2010). Briefly, the onset of migration was defined as the first set of sequential locations indicating a persistent latitudinal-orientated movement of > 100 km. The end of migration was defined as the first date of a series of nearby locations, generally < 50 km apart, situated within the species' breeding or wintering range. Each satellite telemetry location was assessed as a site and time of potential H5N1 infection. Distance travelled within sliding 8-day time-frames was used to assess maximum H5N1 dispersal distance. The 8-day interval represented the maximum asymptomatic infection period for species included in Gaidet *et al.*'s (2010) review of experimental infection studies of waterfowl inoculated with H5N1.

RESULTS

Our database included 2099 ring-recoveries. Among these, 1000 were for birds originally ringed

in Kazakhstan and 1099 were for birds ringed outside Central Asia and recovered therein. The largest percentage of birds ringed in Kazakhstan was recovered within Central Asia; however, there was also strong evidence for migratory linkage to Northern Asia, Eastern Europe, Southern Asia and Western Asia (Table 1). Less frequent movements were documented to other parts of Europe and Africa and no movements were documented from Central Asia to Eastern Asia. Among the birds ringed outside Central Asia and recovered therein, the vast majority were ringed in Eastern Europe and Southern Asia (Table 2). Birds ringed in Northern Asia and Northern or Western Europe were also recovered in Central Asia with regularity. Few bird movements into Central Asia from other parts of Asia, Europe or Africa were documented and no movements were documented into Central Asia for birds ringed in Eastern Asia.

Table 1. Regions in which *Anas* and *Tadorna* species ringed in Central Asia were recovered.

Region	*n*	%
Central Asia	471	47
Eastern Asia	0	0
Northern Asia	283	28
Southern Asia	59	6
Western Asia	43	4
Eastern Europe	97	10
Northern or Western Europe	20	2
Southern Europe	21	2
Africa[a]	6	1
All regions	1000	100

[a]Includes Northern, Eastern and Western Africa.

Table 2. Regions outside Central Asia in which *Anas* and *Tadorna* species recovered in Central Asia were originally ringed.

Region	*n*	%
Eastern Asia	0	0
Northern Asia	97	9
Southern Asia	391	36
Western Asia	1	< 1
Eastern Europe	553	50
Northern or Western Europe	51	5
Southern Europe	1	< 1
Africa[a]	5	< 1
All regions	1099	100

[a]Includes Northern, Eastern and Western Africa.

Seasonal categorization of ring-recovery location estimates revealed frequent movement between India and Central Asia during spring migration (Fig. 1a) and between Kazakhstan and the Caspian Sea, Black Sea and eastern Mediterranean Sea basins during autumn migration (Fig. 1b). Within Central Asia, complex multidirectional movements linking breeding, moulting and wintering areas were evident, particularly between breeding and post-breeding areas around the northwest Kazakhstan–southern Siberian border region and wintering areas in wetlands adjacent to the Caspian Sea (Fig. 1c).

Range maps detailing species-specific ring and recovery locations are provided in Supporting Information Figs S1–S9. Variation was evident among species in the distance between ring and recovery locations ($F_{8,2090} = 22.12$; $P < 0.001$) and the proportion of individuals of each species that were ringed in Kazakhstan and recovered outside the region ($F_{8,991} = 17.65$; $P < 0.001$; Fig. 2). These differences were driven primarily by wider dispersal ranges for Eurasian Wigeon and Garganey and more restricted ranges for Ruddy Shelduck, Mallard and Gadwall. Estimates for Northern Pintail, Northern Shoveler and Common Shelduck were intermediate, whereas Common Teal exhibited a seemingly mixed strategy, with a comparatively large estimate for the mean distance between ringing and recovery locations, but also a high proportion of recoveries occurring on wintering grounds within Central Asia.

Poultry density at recovery locations did not differ among species ($F_{8,2090} = 1.46$; $P = 0.16$). However, statistical differences were apparent in the proportion of recoveries occurring in habitats rated high in poultry density (> 100 head/km²; $F_{8,2090} = 3.11$; $P < 0.01$) and in landscapes dominated by cropland/urban habitat types ($F_{8,2090} = 2.15$; $P < 0.05$). These trends were driven by positive associations between Common Shelduck and Common Teal with areas of high poultry density and Common Teal, Mallard and Garganey with cropland/urban habitats (Fig. 2).

Satellite tracking data confirmed the broad-scale movement patterns indicated by ring-recovery (Fig. 3) as well as variation in habitat use, movement distance and movement rate. Complete migration from wintering areas in Egypt and India to breeding locations in Central Asia was documented for three birds (CS-46123, NS-91238, GA-89128; Table 3). For these individuals, total
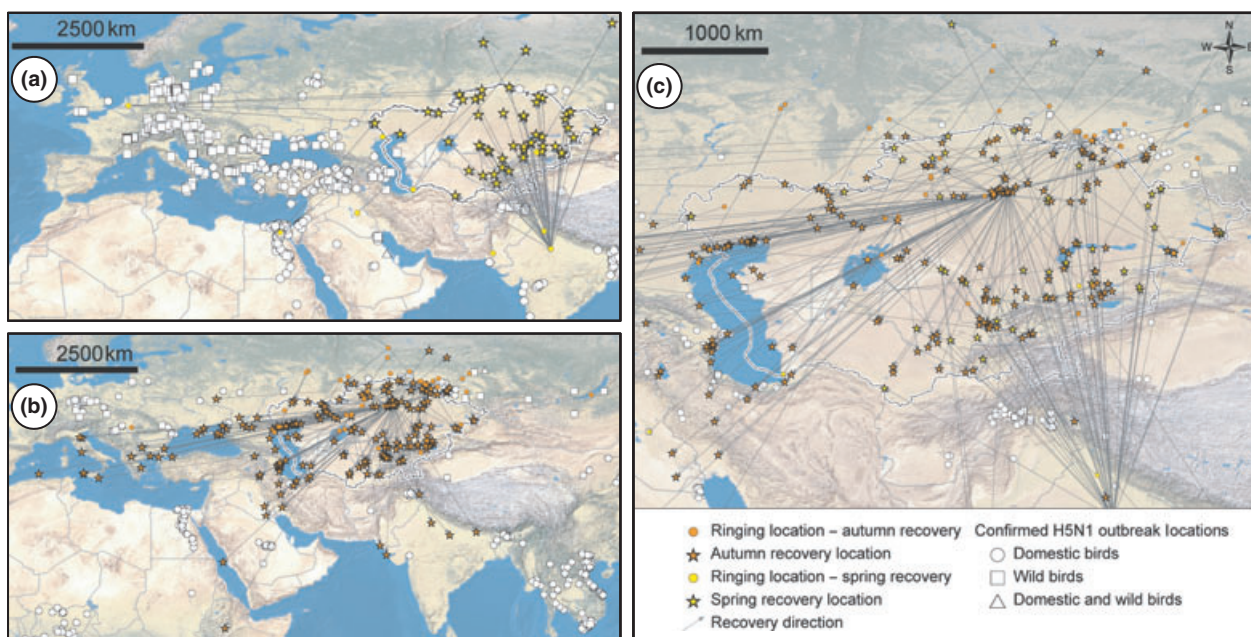
Figure 1. Location of ringing and recovery sites during the (a) spring migration period and (b) autumn migration period for *Anas* and *Tadorna* species affiliated with Central Asia. Detail of movements within Central Asia during both periods is given in panel (c). Locations where birds were ringed are marked by coloured circles and recovery locations by coloured stars. Confirmed H5N1 outbreak locations are given by the white symbols, with cases affecting domestic birds as circles, wild birds as squares, and both domestic and wild birds as triangles.

distances between wintering and breeding grounds ranged from 2450 to 3890 km. However, movements were not continuous and multiple stopover
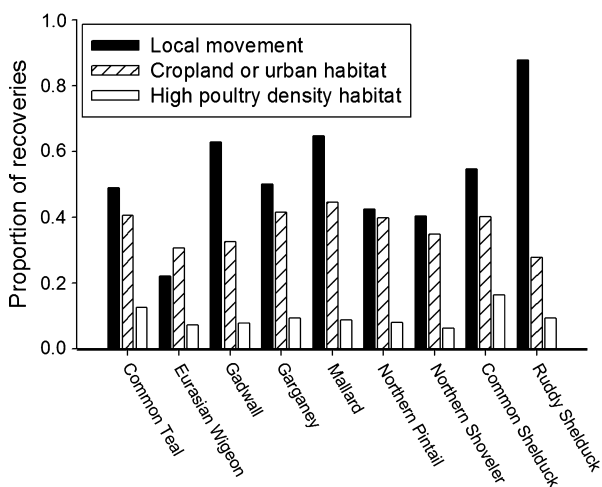
Figure 2. Ring-recovery data summary for *Anas* and *Tadorna* species affiliated with Central Asia. Bars depict local movements, which are defined as the proportion of birds ringed in Central Asia that were recovered within the region, the proportion of recoveries in cropland or urban habitats and the proportion of recoveries at locations with high poultry density (> 100 poultry/km^2).

locations were used. The maximum distances moved within any 8-day interval during migration ranged from 670 to 1560 km. Stopover duration during April and May averaged 22.8 days.

In contrast to the birds fitted with transmitters in Egypt and India, none of the 14 birds fitted with transmitters in southern Kazakhstan migrated outside the region. Satellite transmitter deployments at Kyzolkol were made in September and it is likely most birds had migrated there from more northerly areas. Total distance to wintering areas was up to 330 km, with movements from Kyzolkol to wintering areas routinely made in ≤ 8 days. Nearly half of the birds marked at Kyzolkol overwintered on the lake and its adjacent streams.

Within Central Asia, five major areas of bird activity were identified (Fig. 4). In the Caspian area, transmitter-marked birds made frequent use of wetland and agricultural habitats adjacent to the Caspian Sea and Volga River Delta (Fig. 4a). Habitats used in Northern Kazakhstan included shallow freshwater wetlands (i.e. kettle lakes), although some limited uses of upland steppe and farm fields were documented (Fig. 4b). In the vicinity of Kyzolkol, which is a semi-arid area in which very little agriculture is practised, bird locations were

Figure 3. Location estimates and migration routes for waterfowl tracked using satellite transmitters in this study. Transmitter deployment locations are symbolized by stars. Migration tracks are shown for the species listed in Table 3 and include three birds marked in Egypt, two birds marked in India and 14 birds marked in Kazakhstan.

associated exclusively with wetlands around the lake (Fig. 4c). The core of bird activity for individuals tracked to the Syr Darya area was the shallow eastern edge of the Chardara Reservoir, where the river enters along the Kazakhstan–Uzbekistan border (Fig. 4d). This area is more densely settled than most parts of Central Asia and is a location where the potential for contact between wild and domestic birds is highest. Irrigated farm fields adjacent to the river were used extensively during the non-breeding season. Similarly, the Fergana Valley area is a fertile, heavily irrigated, agricultural region. Birds tracked to the Fergana Valley predominately used agricultural fields around the cities of Quqon and Andijan, Uzbekistan (Fig. 4e).

Spatial and temporal correspondence between *Anas* and *Tadorna* ring-recovery locations and H5N1 outbreak locations was demonstrated in several instances; however, most locations were outside Central Asia. For recoveries in Central Asia, only one of 1570 occurred within 10 days

and 10 km of a known H5N1 outbreak. This occurrence involved a Mallard recovered in the coastal lowlands of the Mangyshlak Peninsula adjacent to the Caspian Sea in Mangystau Oblast. Two additional recoveries were made within 50 km of known outbreak locations, one of which was during the same season (± 45 days) and the other > 90 days apart.

Among the satellite-fitted birds tracked in Central Asia, only one was recovered < 50 km from an outbreak location during the same season in which the outbreak occurred. This involved Northern Shoveler NS-91328 as it passed into its post-breeding area in Northern Kazakhstan Oblast.

In contrast, among the 529 waterfowl ringed in Kazakhstan and recovered outside Central Asia, two were recovered within 10 days and 10 km of a known outbreak location. An additional 18 were recovered in the same season (± 45 days) and within 10 km of a known outbreak and 145 were recovered in the same season (± 45 days) and

Table 3. Summary of movements by *Anas* and *Tadorna* species affiliated with Central Asia that were tracked using satellite transmitters.

| Marking location	Common name	ID	Date marked	Date of last signal	Days signalled	General description of movement
Kyzylkol, Kazakhstan	Common Teal	CT-44754	15 Sept 2008	26 Jan 2009	134	Migrated to the Syr Darya River on the Kazakhstan–Uzbekistan border
	Common Teal	CT-82146	17 Sept 2008	12 Nov 2008	57	Migrated to the Syr Darya River on the Kazakhstan–Uzbekistan border
	Gadwall	GD-45970	16 Sept 2008	16 Sept 2008	366	Overwintered in the vicinity of Kyzolkol
	Mallard	MA-41215	21 Sept 2008	1 Nov 2008	42	Last detected at small wetland 25 km southeast of Kyzolkol
	Mallard	MA-41216	21 Sept 2008	24 Dec 2008	95	Last detected at a small wetland 25 km south of Kyzolkol
	Mallard	MA-45756	21 Sept 2008	14 Sept 2008	359	Overwintered in the vicinity of Kyzolkol
	Mallard	MA-45971	18 Sept 2007	6 June 2008	263	Overwintered in the vicinity of Kyzolkol
	Mallard	MA-46097	19 Sept 2007	23 Dec 2007	96	Last detected at a small wetland 50 km to the south of Kyzolkol
	Mallard	MA-46122	16 Sept 2007	3 Dec 2007	79	Migrated to the Syr Darya River on the Kazakhstan–Uzbekistan border
	Mallard	MA-74807	10 Sept 2007	16 Dec 2007	98	Migrated to the Assa River on the Kazakhstan–Kyrgyzstan border
	Northern Shoveler	NS-41222	20 Sept 2008	14 Dec 2008	86	Last detected on Kyzolkol
	Northern Shoveler	NS-82145	17 Sept 2008	19 Nov 2008	64	Migrated to the Syr Darya River on the Kazakhstan–Uzbekistan border
	Ruddy Shelduck	RS-44835	15 Sept 2007	9 Sept 2008	361	Overwintered in the vicinity of Kyzolkol
	Ruddy Shelduck	RS-45895	15 Sept 2007	5 Nov 2007	52	Last detected on Kyzolkol
Manzala Lake, Egypt	Common Shelduck	CS-46123	23 Jan 2009	15 May 2009	115	Migrated to the Caspian Sea; bred in Mangystau province, Kazakhstan
	Northern Shoveler	NS-91237	21 Jan 2009	17 Sept 2009	241	Last detected in Azerbaijan while migrating toward the Caspian Sea
	Northern Shoveler	NS-91238	20 Jan 2009	21 Sept 2009	275	Migrated through the Caspian Sea to Northern Kazakhstan
Kanyakumari, India	Garganey	GR-89128	21 Dec 2008	2 Sept 2009	259	Migrated to the Fergana Valley of Uzbekistan
	Northern Pintail	NP-41208	18 Dec 2008	15 May 2009	115	Migrated to northeastern Afghanistan
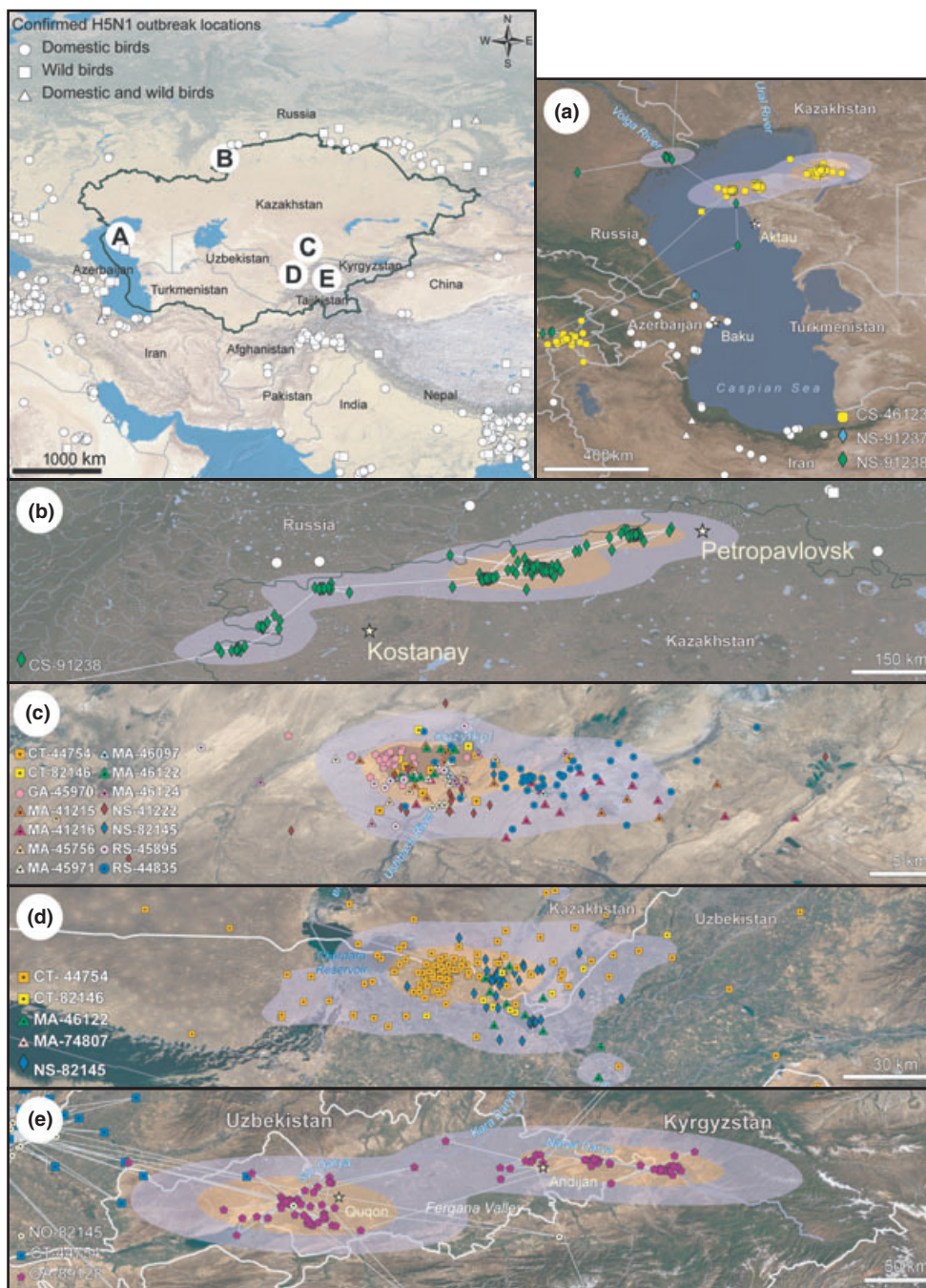
Figure 4. Movements of satellite transmitter-marked waterfowl in Central Asia within five major areas of activity: (a) Caspian, (b) Northern Kazakhstan, (c) Kyzolkol, (d) Syr Darya and (e) Fergana Valley. Confirmed H5N1 outbreak locations are marked by white symbols, with cases affecting domestic birds as circles, wild birds as squares, and both domestic and wild birds as triangles. Blue-shaded areas indicate 90% kernels and pink-shaded areas indicate 50% kernels of habitat use within each area.

within 50 km of a known outbreak. The species represented in the 20 ring-recovery instances within 10 km of an H5N1 outbreak included

Mallard, Eurasian Wigeon, Gadwall, Northern Pintail and Common Shelduck. There were nine unique H5N1 outbreak sites among these, the vast

majority of which were in cropland/urban habitats ($n = 8/9$). The H5N1 die-offs involved both wild ($n = 5/9$) and domestic ($n = 4/9$) birds and occurred in all seasons. Three were in Russia (spring, summer and autumn), two were in Egypt (winter) and single instances were documented in Iran (winter), Italy (winter), Azerbaijan (spring) and Afghanistan (spring).

DISCUSSION

The spatial epidemiology of H5N1 has received considerable attention in the scientific literature. However, many gaps remain in our ability to map transmission risks and predict virus spread. Such endeavours rely on detailed information about case occurrence, poultry distribution and movement, and migratory bird distribution, numbers and movements (Yasue *et al.* 2006, Peterson & Williams 2008). The most significant contribution of our research is delineation of migratory routes and habitat use patterns for a group of waterfowl species considered to be potential vectors of H5N1 across Asia. Such data provide a crucial context for interpreting relationships among viral lineages isolated from hosts in different locations and for evaluating hypotheses concerning long-distance transmission involving poultry and wild birds.

Gilbert *et al.* (2006) were the first to emphasize the close spatial and temporal correspondence between the H5N1 outbreak locations and water-fowl migration routes across Central Asia to the Caspian Sea and Black Sea basins. In broad terms, our findings corroborate these assertions and support the hypothesis that *Anas* and *Tadorna* movements during the autumn migration period have been spatially and temporally consistent with the spread of H5N1. However, our analyses add considerable detail with respect to species-related differences in migratory behaviours and present new challenges for interpreting continuing transmission risks.

Significant variation was evident among *Anas* and *Tadorna* species in relation to their propensity to overwinter within Central Asia and their use of agricultural and poultry-rearing habitats. Ring-recovery and satellite tracking data indicated that Eurasian Wigeon, Garganey, Northern Shoveler, Northern Pintail and Common Shelduck were the species most likely to disperse long distances, whereas Mallard, Gadwall and Ruddy Shelduck exhibited much more localized movement pat-

terns. Common Teal exhibited a seemingly mixed migratory strategy, with nearly half of all recoveries occurring on wintering grounds in Central Asia and the other half outside the region, including many of the longest distance movements recorded for any species. This observation appears to corroborate the suggestion that northern breeding populations of this species are highly migratory, whereas populations in more temperate regions are sedentary or locally dispersive (BirdLife International 2010). With respect to the potential for virus transmission between wild and domestic birds, Eurasian Wigeon, Common Teal, Mallard, Northern Pintail, Garganey and Northern Shoveler have been posited as species posing moderate to high avian influenza transmission risk in Europe (Atkinson *et al.* 2006). Our ring-recovery and satellite tracking data suggest that these species are also likely candidates in Central Asia, with Common Teal, Mallard, Garganey and Common Shelduck most frequently associated with agricultural habitats and areas with high poultry density in the region, and Eurasian Wigeon and Northern Pintail with habitats where H5N1 outbreaks occurred outside the region.

Movements during migration tended to be characterized by jumps of tens to hundreds of kilometres in length within a period of days followed by longer intervals of more localized movement. This observation has important implications for virus transmission. Experimentally inoculated captive *Anas* ducks shed measureable virus for 7–14 days after being infected with H5N1 in the laboratory (Keawcharoen *et al.* 2008). In the wild, the duration of viral shedding for low pathogenicity avian influenza has been estimated as a maximum of 8 days for Mallards (Latorre-Margalef *et al.* 2009). The extent to which infection hampers migratory performance remains unclear (see van Gils *et al.* 2007). Our results indicate that waterfowl movements across Central Asia do not typically occur within the time frames a single individual would be expected to actively shed influenza virus, implying that a large reservoir of infected individuals is a precondition for transcontinental transmission of disease and that it would have to occur in a relay fashion (Gaidet *et al.* 2010). Gaidet *et al.* (2010) also determined that a very small time frame of opportunity (5–15 days per year) occurs when migratory waterfowl movements exceed 500 km within a putative asymptomatic infection period.

Interestingly, with the exception of the eastern Caspian, H5N1 has not been detected at any of the major wetland complexes in the central or southern portions of Central Asia where bird use is heavy and agriculture is most extensive. These wetland areas bear close monitoring, as H5N1 infections in these areas would serve as early warning of virus transmission between eastern and western Asia. An obvious question arising from our research concerns the reliability of disease sampling in the region. On this topic we can only note that sampling effort in Central Asia from 2006 onward has been similar in scope to that undertaken in western China, southwestern Siberia and Western Asia, where H5N1 has frequently been documented. Analyses by Reperant *et al.* (2010) suggest that H5N1 reporting for outbreaks in wild birds has not differed in relation to population density or gross domestic product across different regions in Europe. Unfortunately, similar analyses have not been performed in Asia.

One of the most striking results of our study was the lack of evidence for direct migratory links by *Anas* or *Tadorna* species between China and Central Asia. Historically, ringing efforts have been limited in China and social and political barriers have impeded reporting of ring-recoveries (McLure 1974). Infrequent movements by migratory waterfowl between these areas is known to occur (A. Gavrilov unpubl. data); however, our data add to a growing body of evidence from ringing (Veen *et al.* 2005, Dobrynina & Kharitonov 2006, Solokha 2006) and satellite-tracking studies (Prosser *et al.* 2009, Takekawa *et al.* 2010) that suggests such movements are rare. To illustrate the importance of this point from a disease transmission perspective, consider the difference between the H5N1 situation in Central Asia and that occurring in similar bioregions at roughly the same latitude in China and Mongolia. Both areas are characterized by a mixture of desert and steppe landscapes with low population density, few commercial poultry operations and widely dispersed wetland habitats suitable for waterfowl. However, in western China and Mongolia, H5N1 outbreaks have recurred in most years since 2005 and followed predictable patterns. The first outbreaks of H5N1 involving large numbers of wild birds were documented during April 2005 at Qinghai Lake (Chen *et al.* 2005, 2006, Liu *et al.* 2005). Shortly thereafter, in August 2005, H5N1 was reported in north-central Mongolia with numerous waterfowl deaths

confirmed, most notably among Bar-headed Geese *Anser indicus* and Whooper Swans *Cygnus cygnus* (FAO 2010). The timing, location and susceptibility of the species involved suggested disease dispersal by asymptomatic carriers, which were thought to be co-mingling ducks (Newman *et al.* 2009). A similar unfolding of events occurred in China and Mongolia during spring and summer of 2006 when H5N1 re-emerged in wild birds in Qinghai and Tibet provinces, as well as Bulgan province, Mongolia. No outbreaks were documented in 2007 or 2008, but the disease re-emerged in 2009 in Qinghai, central Mongolia and adjacent areas in Tuva province, Russia and again in 2010 in Tibet and Tuva (FAO 2010).

In contrast, H5N1 outbreaks affecting wild and domestic birds along the northeast to southwest diagonal from southwestern Siberia and northern Kazakhstan to Western Asia, Eastern Europe and Northern Africa have not been recurrent. Phylogenetic evidence from H5N1 isolates collected in Africa and the Middle East suggest multiple introductions of the Qinghai-lineage strain in early 2006. However, after this initial episode of transcontinental transmission, virus populations seem to have evolved independently and without further gene flow from Eastern Asia (Cattoli *et al.* 2009). In the view of Cattoli *et al.* (2009) this was suggestive of limited virus exchange among geographically separated, susceptible host populations and disease dispersal mediated primarily by local and regional trade of poultry. In Europe, multiple sublineages sharing phylogeny with the Qinghai-strain were implicated as a source of the initial outbreaks in 2006, whereas viruses detected since probably represent lineages more local in origin (Starick *et al.* 2008).

Despite considerable research, scientific understanding of the factors underlying wild bird involvement in H5N1 transmission is poor (Yasue *et al.* 2006). Ecological study and improved information about migration are necessary to predict outbreaks and minimize their negative effects for human health, poultry production and wildlife conservation. Our findings contribute to the limited information that is available for risk assessment in Central Asia, a crucial crossroads for waterfowl migration in Eurasia, and highlight the need for results to be amalgamated from multiple species and locations to more fully understand relationships between wild birds, poultry and avian influenza.

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SUPPORTING INFORMATION

Additional supporting Information may be found in the online version of this article.

Maps depicting the location of ringing and recovery locations for *Anas* and *Tadorna* species affiliated with Central Asia. Pink-shaded areas denote core species' breeding range and purple-shaded areas denote core wintering range (BirdLife International, http://www.birdlife.org/datazone/home). Yellow circles indicate ringing locations and orange stars indicate recovery locations. For each species the sample size and mean distance between ringing and recovery sites ± sd are given.

Figure S1. Ringing and recovery locations for Common Teal *Anas crecca*.

Figure S2. Ringing and recovery locations for Eurasian Wigeon *Anas penelope*.

Figure S3. Ringing and recovery locations for Gadwall *Anas strepera*.

Figure S4. Ringing and recovery locations for Garganey *Anas querquedula*.

Figure S5. Ringing and recovery locations for Mallard *Anas platyrhynchos*.

Figure S6. Ringing and recovery locations for Northern Pintail *Anas acuta*.

Figure S7. Ringing and recovery locations for Northern Shoveler *Anas clypeata*.

Figure S8. Ringing and recovery locations for Common Shelduck *Tadorna tadorna*.

Figure S9. Ringing and recovery locations for Ruddy Shelduck *Tadorna ferruginea*.

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