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Aaron B. Shiels
USDA, APHIS, Wildlife Services' National Wildlife Research Center, Aaron.B.Shiels@usda.gov

Mele Khalsa
Island Conservation, 2100 Delaware Avenue. Santa Cruz, CA

Doreen L. Griffin
USDA, APHIS Wildlife Services, National Wildlife Research Center

Clay K. Chow
Department of Land and Natural Resources, Lihue, HI

Sheri S. Mann
Department of Land and Natural Resources, Lihue, HI

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Cattle egrets regurgitate house mouse carcasses onto a mouse-free island: implications for rodent eradications

Aaron B. Shiels[A,D], *Mele Khalsa*[B], *Doreen L. Griffin*[A], *Clay K. Chow*[C], *Patty Baiao*[B], *Sheri S. Mann*[C] *and Antoinette J. Piaggio*[A]

[A]USDA, APHIS Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA.
[B]Island Conservation, 2100 Delaware Avenue. Santa Cruz, CA 95060, USA.
[C]Department of Land and Natural Resources, Division of Forestry and Wildlife, 3060 Eiwa Street, Lihue, HI 96766, USA.
[D]Corresponding author. Email: aaron.b.shiels@usda.gov

Abstract

Context. Eradication of invasive rodents on islands typically results in positive conservation gains, and maintaining a rodent-free island requires elevated biosecurity, including prevention of assisted rodent arrival via watercraft, aircraft and animals such as birds. Cattle egrets (*Bubulcus ibis*) are widespread, and often fly several kilometres daily to roost and forage. They frequently swallow insects and vertebrates (including rodents) whole, and some regurgitate prey. Cattle egrets have been regularly observed flying between the Hawaiian Islands of Ni'ihau (where non-native mice and rats are established) and Lehua (where one species of non-native rat is established and was targeted during a recent eradication attempt).

Aims. The objectives were to identify the species of rodent that cattle egrets regurgitate following transport between Ni'ihau and Lehua islands, and to determine if any of the rodent individuals regurgitated were alive once deposited onto Lehua Island following 1.1-km oversea flights.

Methods. Eighty-five individual rodent carcasses (regurgitated by cattle egrets) were collected, preserved and identified to species using morphological characteristics and DNA sequencing.

Key results. All rodents regurgitated by cattle egrets were dead upon collection on Lehua Island. Although the Pacific rat (*Rattus exulans*) is the only rodent species on Lehua Island, and field staff suspected the regurgitated rodents were *R. exulans*, all 85 carcasses were identified as house mice (*Mus musculus*).

Conclusions. This is the first evidence (that the authors know of) showing movement of rodent carcasses, via cattle egrets, between islands.

Implications. Cattle egrets that deposit rodent carcasses onto rodent-free islands, or segments of islands, may confuse land managers and biosecurity professionals who are unaware of this phenomenon. House mice did not survive cattle egret ingestion, >1-km flight and regurgitation; therefore it is unlikely that live rodents would be introduced to rodent-free areas via cattle egrets.

Additional keywords: biosecurity; bird feeding behaviour; *Bubulcus ibis*; non-native *Mus musculus*; *Rattus exulans*.

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Introduction

Most of the world's islands contain one or more of the common non-native invasive rodents: house mouse (*Mus musculus*); Pacific rat (*Rattus exulans*); black rat (*R. rattus*); and Norway rat (*R. norvegicus*) (Atkinson 1985; Towns 2009). Rodent eradications have been conducted on many of the world's small islands to diminish the pronounced negative impacts of invasive rodents on insular native and endemic species (Howald *et al.* 2007; Jones *et al.* 2016). Maintaining a rodent-free island requires elevated biosecurity measures to prevent new rodent introductions. Boats and aircraft can easily transport stowaway rodents that may establish on the island if biosecurity prevention measures fail (Towns 2009).

In addition to human-mediated introductions of live rodents to islands, birds may represent a viable means of transporting live or dead rodents to islands. For example, raptors often fly several hundred metres from locations where they seize prey to locations where the prey is consumed (Colvin 1985; Browning *et al.* 2016). Cattle egrets (*Bubulcus ibis*) are known to consume small rodents (Funasaki *et al.* 1988), fly to distant locations and then regurgitate the boluses of consumed prey (Siegfried 1971). Young cattle egrets frequently regurgitate when they are

frightened or alarmed (Siegfried 1971; Mckilligan 1984). Both raptors and cattle egrets are known to fly long distances, including over the ocean (Arendt 1988; Garrett *et al.* 1993). Therefore, birds such as raptors and cattle egrets may represent possible alternative vectors for introducing live or dead rodents to islands. Dead rodents can also cause alarm in locations where rodents are believed to be absent, such as an island where all or some species of rodents were previously eradicated.

During observation of cattle egrets flying between the Hawaiian Islands of Ni'ihau and Lehua, we noted these birds regurgitating rodent materials while perching in trees and being alarmed by humans when on Lehua Island. We sought to determine the species of rodent the cattle egrets were transporting in their gastrointestinal tract between Ni'ihau and Lehua islands, and documented whether or not the rodents were alive once regurgitated. There was concern that the small rodents recovered on Lehua may have been Pacific rats from Lehua, which would have been interpreted as evidence that the few Pacific rats detected on the island were successfully breeding following the recent (4 months before our study) rat eradication attempt on Lehua. An alternative explanation was that the cattle egrets transported the rodents in their gastrointestinal tract from Ni'ihau Island.

Methods and results

Study site

Ni'ihau Island (21°54′N, 160°10′W; 180 km^2) is the westernmost and seventh largest inhabited island in the Hawaiian Archipelago, and is 31 km south-west of Kaua'i. Lehua Island is a small (1.1 km^2) uninhabited island just 1.1 km off the north shore of Ni'ihau. Lehua is a Hawai'i State Wildlife Sanctuary, containing large seabird nesting colonies (VanderWerf *et al.* 2007), and human access to the island is restricted above the high tide line. Both Lehua and Ni'ihau are arid and generally receive <600 mm of rainfall each year (Eijzenga 2011).

There are no native rodents in the Hawaiian Islands. The only rodent species on Lehua is the Pacific rat, and there were campaigns on Lehua to eradicate this rat in 2006 and in September 2017. There have been no formal rodent surveys on Ni'ihau, but house mice, Pacific rats and black rats have been observed on Ni'ihau (K. Robinson, pers. comm.). Cattle egrets generally forage in grassy habitats where they eat insects and some vertebrates (Mckilligan 1984; Funasaki *et al.* 1988; Gassett *et al.* 2000), and these birds often fly several kilometres daily to roost and forage. Cattle egrets were introduced into Hawai'i in 1959 to control insect pests in agricultural fields, and by 1981 the statewide population of cattle egrets was estimated to be 30 000 (Funasaki *et al.* 1988). They have been documented preying upon house mice and *Rattus* spp. in the Hawaiian Islands (Funasaki *et al.* 1988). Cattle egrets have been observed flying between islands, including daily migrations between Lehua Island (where they leave their roost in the morning and return in the evening) and Ni'ihau Island.

Field observations and sample collections

During a rat monitoring trip on Lehua Island on 18 January 2018, author MK observed dead rodents of unknown species in six locations within the vicinity of a cattle egret nesting and roosting site. This site contained 32 adult egrets, 13 juveniles and >12 eggs in four nests. On the ground at the site, there were fresh (moist and lacking decay) rodent carcasses and old dried boluses containing some rodent hair. Additionally, there was a pile of insects (katydids and grasshoppers – Orthoptera) mixed with skink (Squamata) on the ground that may have been a cattle egret bolus, and one bolus contained a fresh rodent carcass and part of a gecko's (Squamata) body. All fresh rodent carcasses were collected by placing them in labelled ziplock bags. At a second cattle egret nesting site within 500 m of the first one, a single juvenile cattle egret was observed regurgitating two dead rodents as it fled from observers. MK and additional field staff immediately collected the regurgitated rodents by placing them in uniquely labelled ziplock bags. In total, 85 rodent carcasses were collected, most of which were complete (whole), and there were no living rodents observed. The rodent carcasses were preserved by adding 70% isopropyl alcohol to each ziplock bag. Given our field observations and previous documentation in the literature (Siegfried 1971; Mckilligan 1984), we feel it is justified to assume that all 85 rodent samples were regurgitated by cattle egrets that nested, roosted or otherwise visited Lehua Island.

Rodent-monitoring devices deployed and frequently checked across Lehua Island confirmed that there were no house mice on Lehua, and that the Pacific rat was the only rodent species present on this island before, during and after our study. Additionally, after finding the regurgitated rodents, two motion-triggered cameras (Reconyx Hyperfire HC500, Holmen, WI) were placed under the cattle egret roost tree to monitor for live rodent activity. After 3 weeks of no rodent observations, the cameras were removed from the site.

Laboratory rodent identification

At the US Department of Agriculture National Wildlife Research Center's (NWRC) rodent laboratory in Colorado, all rodent specimens were removed from alcohol and individually assessed for distinguishing morphological characteristics to inform species identification. Morphological characters used are outlined in King (2005) and Shiels (2010), and included length of body, tail, ear, fur and foot. Visibility of male testes, which indicates adult status, was also noted. In addition to the experienced morphologist (ABS) having trapped, handled and identified hundreds of live and dead individuals of each *Rattus* species and the house mouse in Hawai'i, live and dead wild house mice were available at NWRC at the time of viewing all specimens; these were used as needed for additional reference during the species identification process. Although some carcasses had anatomical parts missing and two had minimal hair, suggesting they were neonatal, a subset of the carcasses with all or most anatomical parts was individually weighed and the body and tail lengths were measured (Fig. 1). Mean (s.d.) sizes for these 24 individuals were: 6.4 ± 3.4 g (total mass); 5.4 ± 0.9 cm (body length); and 5.1 ± 1.2 cm (tail length).

There were just two individuals for which morphological techniques yielded uncertain species identification. We analysed DNA sequences from these two specimens, as well as five others that were randomly chosen from those confidently identified to species morphologically. DNA was isolated from a 1-cm section of the tail of each individual using DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD). The NWRC

Fig. 1. A subset of house mouse (*Mus musculus*) carcasses collected from Lehua Island, Hawai'i, following probable regurgitation by cattle egrets (*Bubulcus ibis*). Carcasses are different sizes and show different times since regurgitation. Note that before this photograph, these specimens had been stored in alcohol and had most or all of their tails removed for DNA analysis.

genetics laboratory then performed species identification using cytochrome *b* primers MVZ05 and MVZ04 (Smith and Patton 1993) and followed the PCR and cycle sequencing parameters of Hopken *et al.* (2016). The number of base pairs for these samples ranged from 359 to 460. DNA sequences were uploaded to the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLAST) to search for matches that facilitated species identification.

All rodent carcasses regurgitated by cattle egrets and collected on Lehua, including the 78 identified solely based on morphological characteristics and the seven that were subjected to genetic and morphological analysis, were house mice (Fig. 1). The nucleotide BLAST matched each DNA sequence 100% to house mice.

Discussion

Prior to our rodent identifications, there was suspicion that the regurgitated rodent carcasses from cattle egrets were evidence that the few rats that had survived the Lehua eradication attempt in 2017 were reproducing on the island. This was not the case, however, because cattle egrets transported mouse carcasses from Ni'ihau to Lehua, and the rodent carcasses were confirmed as house mice following regurgitation, revealing that dead rodents can be dispersed onto islands by cattle egrets. All 85 regurgitated rodents recovered from cattle egrets were dead, despite being mostly whole and intact. It is unknown whether the feeding behaviours, gastrointestinal factors and regurgitation process of the cattle egrets make it possible for live rodents to survive transport between islands, but our findings suggest this is unlikely. Nevertheless, land managers and biosecurity personnel should be aware that cattle egrets can be a vector for rodent carcasses, and that this may explain how dead rodent species such as house mice may appear on rodent-free islands.

An important factor affecting prey consumption by cattle egrets is prey availability. Insects dominate the diets of cattle egrets (Arendt 1988; Funasaki *et al.* 1988). Orthopteran (comprised of grasshoppers, locusts and crickets) are consistently the most common prey items found in cattle egret stomachs and regurgitated pellets, and Lepidoptera (moths and butterflies) and Coleoptera (beetles) are also common (Siegfried 1971; Fogarty and Hetrick 1973; Gassett *et al.* 2000; Boukhemza *et al.* 2004). Vertebrates, including amphibians, reptiles, birds and rodents generally represent a very small fraction of the cattle egret diet (Mckilligan 1984; Arendt 1988; Funasaki *et al.* 1988; Gassett *et al.* 2000), but cattle egrets are highly opportunistic and will select new prey items as they become abundant (Boukhemza *et al.* 2004). Although non-native rats and mice are common in most ecosystems in Hawai'i, house mice are typically much more abundant than rats in grassland ecosystems (Shiels *et al.* 2017). House mouse outbreaks, where densities increase 4–5 times above average, are common and occur unpredictably in Hawai'i (Shiels 2010). Although mice and rats are present year-round in grassy ecosystems where cattle egrets forage, intervals of rodent outbreaks may be the most likely periods for rodent consumption and subsequent dispersal via cattle egrets. Because rodents were not being monitored on Ni'ihau, and there were only a few surviving rats on Lehua being monitored during our study, we could not determine if there were nearby rodent outbreaks at the time we collected house mouse carcasses on Lehua. It is plausible that cattle egrets were travelling from Lehua to Ni'ihau for better foraging, and that the greater abundance of rodent prey on Ni'ihau relative to Lehua was substantial during our study.

Island biosecurity plans for rodent-free islands should recognise that cattle egrets are a potential vector for dead rodent dispersal to islands. There is a growing number of small islands where invasive house mice and *Rattus* species have been eradicated, and such islands have been subsequently maintained as rodent free with strict biosecurity plans in place (Howald *et al.* 2007; Russell *et al.* 2008). For such rodent-free islands, an observation of a rodent carcass would cause alarm and may be considered a breach in biosecurity and a sign that rodents had reinvaded the island; however, although the presence of a live rodent would correctly constitute a breach, such a conclusion might be unsupported if the rodent is dead.

Rodent species identification can be confusing, particularly when small-size classes are present, observers do not have regular experience with all the rodent species in the area and DNA analysis is not an option. Body size can be a reliable characteristic for distinguishing species as long as the specimens are adults; the sizes of the four adult rodent species in Hawai'i generally average 10 g for house mice, 54 g for Pacific rats, 124 g for black rats and 230 g for Norway rats (Shiels 2010). However, juveniles of the listed rodent species can be misidentified if size is the only characteristic used for identification, and it should be noted that all *Rattus* species will be equivalent in size to juvenile and adult house mice at some point in their development. Regurgitated or decomposing rodent carcasses may also make species identification more difficult.

Conclusions

Cattle egrets can transport invasive rodent carcasses in their gastrointestinal tract to distances of >1 km, which includes passing over water and travelling between islands. All rodents regurgitated by cattle egrets in our study were house mice, dead upon regurgitation. Although house mice are swallowed whole, and regurgitation is a frequent behaviour of cattle egrets, it is unlikely that they could survive consumption, flight and regurgitation by cattle egrets. Whether rodents can survive the journey remains unsubstantiated and warrants further study. Land managers and biosecurity professionals that are actively keeping areas rodent free, such as offshore islands, should be made aware of the possibility that cattle egrets could introduce rodent carcasses to rodent-free areas.

Conflicts of interest

The authors declare no conflicts of interest.

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