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REFUGE**

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PATHOLOGY OF HOUSE MOUSE (*MUS MUSCULUS*) PREDATION ON LAYSAN ALBATROSS (*PHOEBASTRIA IMMUTABILIS*) ON MIDWAY ATOLL NATIONAL WILDLIFE REFUGE

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ABSTRACT: Invasive rodents on islands have adverse effects on native birds in island ecosystems, and rats are the most common culprits. Recently, house mice (*Mus musculus*) in the South Atlantic were found preying on three species of albatross chicks. Here, we show that house mice can also prey on nesting adult Laysan Albatross (*Phoebastria immutabilis*) on Midway Atoll National Wildlife Refuge (US). In contrast to mouse attacks on albatross in the South Atlantic, where mice targeted the rump and crown of chicks, on Midway, mice targeted nesting adults mainly on the back. For both regions, the outcome was similar with reduced nesting success. In the case of Midway, reduced nesting success was due to nest abandonment or mortality of one or both parents because of secondary bacterial infections. Mouse-induced mortality of adult albatross has the potential to have a more potent demographic effect because of their much higher natural survivorship once they reach adulthood.

Key words: Invasive mammals, islands, Laysan Albatross, mouse, *Mus musculus*, *Phoebastria immutabilis*, seabirds.

INTRODUCTION

The northwestern Hawaiian Islands, US are home to large numbers of seabird colonies comprising multiple families and species (Harrison 1990). Among these, one of the most charismatic species is the Laysan Albatross (LAAL; *Phoebastria immutabilis*), where the largest breeding colony (600,000 breeding pairs) is located on Midway Atoll National Wildlife Refuge, the penultimate atoll of the Hawaiian archipelago. The northwestern Hawaiian Islands have undergone major changes since human colonization, and significant among those are introduction of mammalian predators that threaten a variety of native seabirds (Townsend et al. 2015). Of these, rats, cats (*Felis catus*), and mice cause the most damage, in part because of their widespread distribution in island ecosystems (Townsend et al. 2015). Midway Atoll was heavily altered because of military construction and occupation during and after World War II, and in 1943, black rats (*Rattus rattus*) were introduced, leading to serious declines of burrow-

ing seabirds like Bonin Petrels (*Pterodroma hypoleuca*; Grant et al. 1981; Seto and Conant 1996). Polynesian rats (*Rattus exulans*) were documented preying on nesting LAAL on Kure Atoll (Kepler 1967), the furthest northwestern Hawaiian island, illustrating the vast prey size subject to rodent predation and the ability of rats to colonize even the most remote areas. Eradication of rats on Kure in 1993 and Midway in 1995 (Witmer et al. 2011; Vanderwerf et al. 2015) resulted in marked recovery of seabirds on both islands.

Mice are known to scavenge dead seabirds and prey on live birds, but until recently, their effect was thought to be limited to smaller burrowing seabirds (Bolton et al. 2014). However, findings in the South Atlantic have revealed mouse predation on three species of albatross chicks on Gough (Cuthbert and Hilton 2004) and Marion Islands (Jones and Ryan 2010). Here, we show that, in addition to chicks, house mice (*Mus musculus*) prey on adult nesting LAAL on Midway Atoll. Our objectives were to describe this event, with emphasis on the pathology of this condition

and consider possible reasons why this occurred.

MATERIALS AND METHODS

During the annual LAAL census in late December 2015, US Fish and Wildlife Service (USFWS) biologists observed a small and highly localized cluster of albatross (zone A; Fig. 1A) with wounds on their backs and head (Fig. 1B). Close observation of the wounds and surveys of the entire area did not reveal a cause for such as intra- or interspecific aggression. Because rats had been eradicated from Midway by 1995 and not reintroduced, they were ruled out as a cause of the trauma. Photos of wounds were sent to the US Geological Survey, National Wildlife Health Center (NWHC), Honolulu Field Station (Honolulu, Hawaii, USA), which, in turn, suggested that rodents could be responsible based on the similarity of circular to oval wounds on the back of LAAL at Kure (Kepler 1967).

We established a monitoring program that included placement of Reconyx[®] (Holmen, Wisconsin, USA) night vision trail cameras at the affected nesting sites. Camera footage revealed that mice were repeatedly entering and staying in the feathers on the backs of nesting albatrosses for prolonged periods (Fig. 1C). Albatrosses on camera showed signs of agitation (frequently standing up over their egg, shifting position on the nest, repeated preening), and all affected birds had evident wounds on the head and back (Fig. 1B). By 5 January 2016, the number of injured birds totaled 187 out of 454 nesting in the plot (41%), and on 15 January 2016, another zone (zone B) of mouse predation was discovered 400 m to the east (Fig. 1A). Details on losses of birds, nesting failures, and pilot trials to control mice in affected plots with cholecalciferol in December 2016 are outlined by Duhr et al. (2017) who recorded a sevenfold increase in adult mortality and a 10-fold increase in rate of nest abandonment in 2015–16 compared with previous years.

To obtain a better understanding of the pathology of mouse predation on LAAL, colonies were monitored daily (Duhr et al. 2017), and 27 dead adult LAAL were necropsied by the Honolulu Field Station between 5 January 2016 and 25 January 2016, during the time of mortalities. Birds were weighed (± 0.1 kg) and underwent complete external and internal exams with collection of tissues in 10% neutral-buffered formalin, which were processed for histopathology comprising paraffin embedding, sectioning at 5 μ m, and staining with H&E. Birds were sexed by visualizing gonads at necropsy because LAAL have no evident external sexual dimorphism.

Gram stains were used to visualize bacteria in affected tissues. Postmortem condition of LAAL carcasses was assessed (based on the presence of maggots) as good (no maggots), fair (small [<50] maggots and fly eggs), or poor (maggots too numerous to count). Body condition of birds was judged as good (large amounts of body fat, plump pectoral muscles), fair (moderate amounts of body fat, slight atrophy of pectorals), or poor (atrophy of pectorals, no body fat). A similar examination was done of 10 mice live-trapped and euthanized with carbon dioxide as part of mouse-control efforts implemented by USFWS during initial phases of rodent-control management. Body condition of mice was judged based on abdominal and perirenal fat stores as good (ample fat), fair (moderate amounts of fat), and poor (no fat).

Livers from three freshly dead LAAL were submitted to NWHC for routine bacterial culture on blood agar plates, and bacteria identified with the API system (bioMérieux, Inc., Durham, North Carolina, USA; Smith et al. 1972). To determine whether the bacteria recovered from LAAL might have originated from mice, oral swabs from five of 10 necropsied mice were also cultured as mentioned earlier.

RESULTS

Of 27 albatross necropsied, 18 (67%) were female and the remainder ($n=9$; 33%) were male. All birds were adults in good body condition with postmortem status ranging from 13 good (48%), 12 fair (44%), and two (7%) poor. Mean (SD) carcass weight was 2.7 (0.7) kg. The most significant gross finding in all birds was one or more holes ranging from 1 cm to 3 cm in diameter on the skin of the back associated with subcutaneous hemorrhage and trauma to the underlying skeletal muscle, ranging in depth from about 2 mm to 10 mm (Fig. 1D). Other regions were less often traumatized and included neck or axilla (three birds each; 11% each), leading edge of the skin overlying humerus, pectoralis, and left dorsal aspect of the thigh (one bird each; 4% each). Eight birds (30%) had wounds on multiple sites. Those wounds indicated ante-mortem trauma because of associated hemorrhage seen grossly, thereby differentiating those injuries from postmortem scavenging in which hemorrhage was not seen.

On histology, the skin, fat, skeletal muscle, and lungs were the most common ($>50\%$)

organs with lesions. Microscopic lesions in the skin, fat, and skeletal muscle were mainly characterized by variable amounts of necrosis associated with mixed histiocytic and lymphocytic infiltrates associated with microcolonies of Gram-negative rods (Fig. 1E). Liver had prominent periportal histiocytic infiltrates with Kupffer cells distended with bacteria. Capillaries distended with Gram-positive or Gram-negative bacteria were seen in multiple organs, including kidney, lung, and heart. Cultures of the liver in three LAAL revealed mixed growth of *Acinetobacter* sp., *Erysipelothrix rhusiopathiae*, *Proteus* sp., *Serratia marcescens*, *Staphylococcus* sp., and unidentified bacteria. In total, 19 birds (70%) had microscopic lesions caused by bacterial infections.

Of the 10 mice necropsied, six were males, three were females, and one was unknown, with all mice in good body and postmortem conditions. The mice had been euthanized and immediately frozen, so they were fresh dead. Mean (SD) weight of mice was 15.6 (2.0) g. No internal lesions or stomach contents were seen grossly. On histology, lesions were observed most often (>50%) in the esophagus and consisted of mild infestations of esophageal mucosa with unidentified nematodes eliciting no host inflammatory response. Small numbers of trematodes and nematodes were seen in the lumen of the small intestine. Cultures of the oral cavities of mice revealed *Bacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Gemella* sp., *Lactobacillus* sp., *Proteus* sp., *Staphylococcus* sp., *Streptococcus* sp., and unidentified Gram-positive bacteria.

DISCUSSION

The ultimate cause of death for mouse-traumatized LAAL was most likely septicemia in that 70% of birds had lesions compatible with bacterial infections. We had expected *Pasteurella multocida* to be cultured because this is a common bacterial infection in birds subsequent to bite trauma (Stenkat et al. 2013). However, aside from *Staphylococcus*

sp., the bacteria we found in LAAL have not been associated with rodent oral microbiota in other studies. For instance, for rat bites in human, the most common bacteria isolated were *Staphylococcus epidermidis*, *Bacillus subtilis*, α -hemolytic *Streptococcus* sp., and diphtheroids (Abrahamian and Goldstein 2011). Trudel et al. (1986), in studies of laboratory mice, found the most common bacteria were *Lactobacillus murinus* (38%), *Staphylococcus aureus* (37%), *Streptococcus faecalis* (8%), *Staphylococcus sciuri* (4%), and *Escherichia coli* (3%). How comparable this is to wild mice from Midway is debatable, given that oral microflora varies with diet (Blais and Lavoie 1990), which surely differs between wild mice on Midway and laboratory mice. Given the lack of close concordance between bacteria in mouse oral cavity and wounds in LAAL, we suspect most of the bacteria in LAAL were environmentally acquired. For instance, *Erysipelas* sp., one of the bacteria isolated in LAAL, is a common cause of cellulitis secondary to skin trauma in people (Celestin et al. 2007).

Our findings differed significantly from other instances of mouse predation in albatross in two ways. We are unaware of reports of mice acting as predators on adult nesting albatross. All other instances of mouse predation on albatross in the South Atlantic have involved smaller, downy chicks (Cuthbert and Hilton 2004; Jones and Ryan 2010) or relatively larger fledgling chicks (Dilley et al. 2016). Interestingly, our necropsy sample was heavily biased toward females. We doubt this was the result of selecting females for necropsy in the field because there is no external means to differentiate sex, so we suspect our sample is likely reflective of the sex ratio of birds on eggs during the period when the predation was first documented and specimens collected (5 January 2016 to 25 January 2016). Laysan Albatross are remarkably synchronous in their egg laying and the length of male and female incubation shifts are relatively long. The median date of egg-laying is between 29 November and 1 December, and the median of the first long-incubation shift taken by the male of the pair

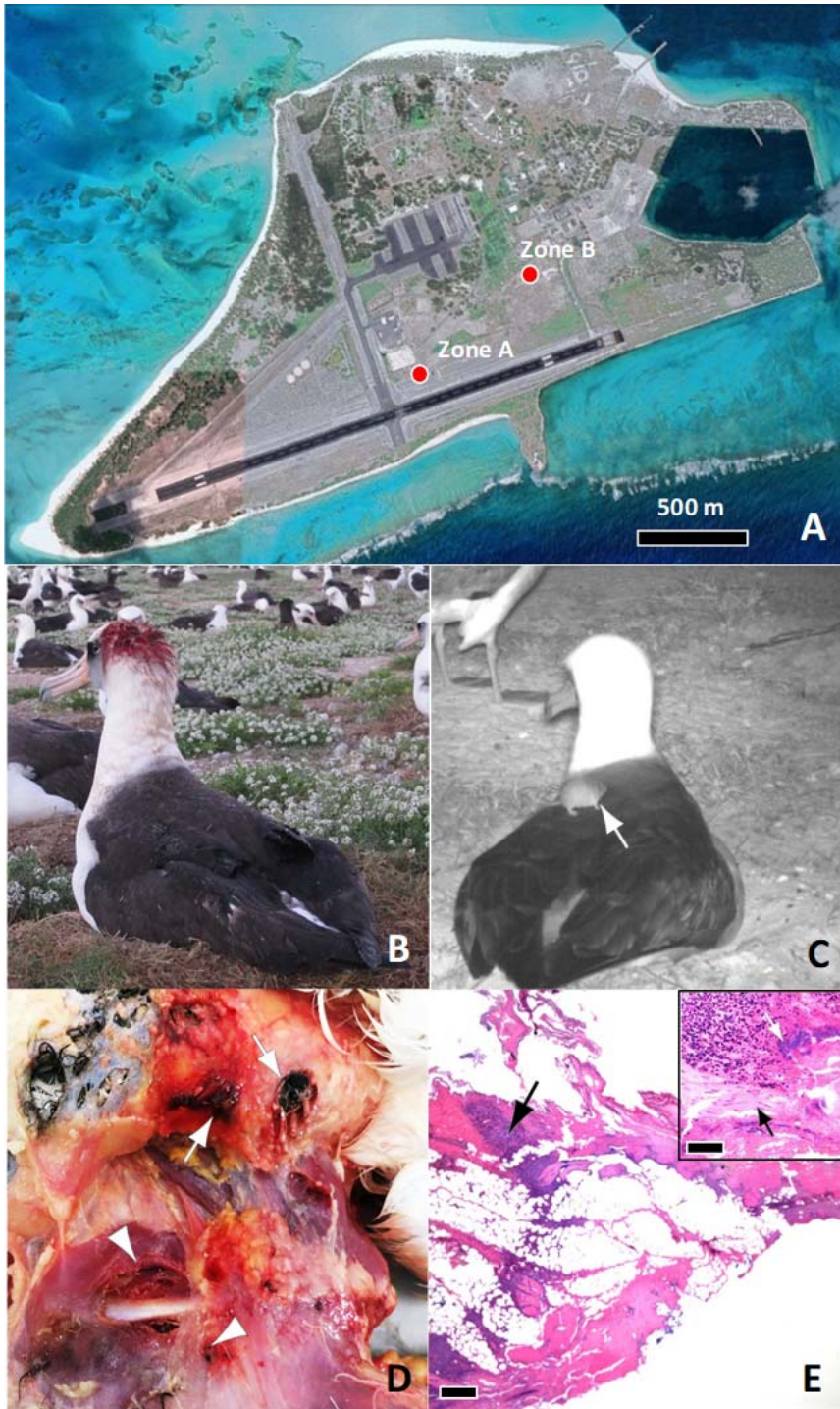


FIGURE 1. Google Earth map (Mountain View, California, USA) of Midway Atoll, USA along with photos of Laysan Albatross (LAAL; *Phoebastria immutabilis*) affected by house mouse (*Mus musculus*) predation at the gross and microscopic level. (A) Locations of initial mouse predation on adult LAAL at Midway Atoll. (B) Nesting adult LAAL with traumatic, mouse-induced head wound. (C) Night-vision camera photo of mouse

is 21 days (6–38 d, $n=451$; Fisher 1969). Therefore, it is likely that, during the period of the sample collection, most nests were being attended by the female on her first long shift (median length=24 d, range=9–58 d, $n=166$; Fisher and Fisher 1969). Such predation pressure on a demographically important component of seabirds (adult breeding females), if allowed to persist in the longer term, could have adverse effects on the population. Indeed, there was significantly lower adult survival and nesting success in plots of birds affected by mouse predation (Duhr et al. 2017).

The predominance of lesions on the back seen in LAAL at Midway seemed to differ from the pattern of lesions in other instances of mouse predation in albatross in the South Atlantic. For instance, in Tristan Albatross (*Diomedea dabbenena*) on Gough Island, mice targeted the rumps of downy chicks (Cuthbert and Hilton 2004), whereas in mouse predation of Wandering Albatross (*Diomedea exulans*) on Marion Island, >60% of lesions were on the crown of the head (Dilley et al. 2016). Although mice did attack the crowns of the head in LAAL (Fig. 1B), we did not have such cases in our necropsy samples, and in the field, those lesions were less common than lesions on the back. In both Midway and the South Atlantic, mouse predation occurred mainly at night and resulted in significant reductions in chick survival in the South Atlantic (Davies et al. 2015) and nesting success and adult survival on Midway (Duhr et al. 2017).

Mice have been on Midway since at least the time of rat introductions in 1943, so the reasons why they suddenly started to prey on LAAL in 2015 are speculative; however, there are three potential explanations for this change in behavior. First, a need for calories may have prompted mice to switch from

existing food sources to LAAL. Unfortunately, no diet studies of mice on Midway are available, so we lack evidence to support this hypothesis; however, prey switching in invasive rodents has been documented elsewhere. For instance, in the South Atlantic, mice experiencing severe food stress are more likely to prey on seabird eggs and chicks (Angel et al. 2009). In the case of Marion Island, mouse predation on albatross is suspected to be the result of secular declines in invertebrate prey upon which mice depended, leading them to switch to seabirds (Dilley 2018). Finally, the hungrier mice are, the more likely they are to switch from eating seabird eggs to seabird chicks; this was seen in sated mice preying on eggs of Marbled Murrelets (*Brachyramphus marmoratus*) but switching to eating chicks when they were hungrier (Bradley and Marzluff 2003). No evidence of seabird egg predation by mice was ever seen on Midway, so prey switching from eggs to adults is unlikely the reason for this behavior.

Second, elimination of invasive rats could have led to increases in mouse populations. It is well known that invasive predator-control efforts can have unintended consequences if the totality of community interactions in the ecosystem are not taken into account (Zavala et al. 2001), a phenomenon known as *mesopredator release* (Ritchie and Johnson 2009). For example, using before-after control impact experiments for predator control in New Zealand, Ruscoe et al. (2011) found that there was competitive release of rats after removal of possums and competitive release of mice after removal of rats. They suspected this was because rats and mice have substantial dietary overlap that fluctuates seasonally over time. Although there is some differentiation in dietary niches of rodents in Hawaii with Polynesian rats being intermediate between the mostly insectivorous house mouse

←

(arrow) on the back of nesting LAAL. (D) Gross appearance of mouse bites on dorsum; note holes in skin with hemorrhage (arrows) and traumatic ablation of underlying muscle (arrowheads). (E) Histology of skin with ulceration of epidermis (top) and underlying dermis with massive cellulitis; note mononuclear infiltrates (arrow) in subcutaneous connective tissue, fat, and muscle. Bar=200 μm . Inset is a close-up of bacteria (white arrow), inflammation to the left, and intact muscle (black arrow). Bar=50 μm .

and the mostly herbivorous black rat, there is also substantial dietary overlap (Shiels et al. 2013). Increasing mouse populations because of the elimination of rats could have precipitated dietary shifts to LAAL, perhaps because of food shortages. However, necropsies of mice revealed they were in generally good body condition, so starvation was likely not a driver of mouse predation on LAAL. In addition, there was no evident pathology, such as lesions in the brain, to explain why mice would suddenly start preying on LAAL in 2015–16, and even if such lesions had been found, correlating them to behavioral shifts absent historic pathology data on mice on Midway would have been difficult.

Finally, Duhr et al. (2017) noted that changes in seasonality of rainfall patterns observed during the 2015–16 and 2016–17 El Niño events may have shifted the timing of normal population fluctuations in the mouse population of Sand Island, in which drying conditions reduced forage and subsequently caused mass starvation. In 2015–16 and 2016–17, that crash occurred just as albatrosses began the vulnerable incubation period when the adult birds are reluctant to leave their eggs. For unknown reasons, the inability or unwillingness of albatross to kill predating rodents seems to be hard wired in these birds as evidenced by rodent predation on a variety of species; many of which are much larger than their predator (Angel et al. 2009; Dilley et al. 2016). Trophic cascades associated with precipitation leading to increased or decreased rodent populations have been documented elsewhere, for instance, with ecology of rodent-borne hantaviruses (Yates et al. 2002).

In summary, we think it most likely that a combination of increasing populations of mice and a dietary shift prompted by as-yet-unknown causes is the most parsimonious explanation for this mouse predation on LAAL. We cannot confirm this absolutely because we have no demographic data on mice on Midway. The findings herein have prompted the USFWS to begin operations to eradicate mice on Midway. Mouse eradication had not been done during rat-elimination efforts because mice were not considered a

threat to native fauna on Midway. Moreover, elimination of mice from ecosystems is far more challenging than elimination of rats, in part because of their smaller size (lower detectability) and smaller home ranges. These factors require different bait dissemination strategies and monitoring efforts than those used for larger rats (Howald et al. 2007). These actions taken by USFWS to help conserve a flagship seabird species will hopefully be successful.

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