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Rapid communication

Cats were responsible for the headless carcasses of shearwaters: evidence from genetic predator identification

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Abstract. The domestic cat *Felis silvestris catus* is known to be one of the most notorious invasive alien predators. Seabirds are typical taxonomic groups that have been impacted by free-ranging cats on islands, and their headless carcasses are frequently observed. We conducted genetic predator identification of the carcasses of streaked shearwater *Calonectris leucomelas* and described their characteristics on Mikura Island, Japan, where free-ranging cats were blamed for the recent rapid decline of the shearwater population. Eight carcasses of streaked shearwaters were found in the survey. Genetic analysis of swab samples from scarred tissues of the carcasses detected cat DNA and identified cat predation on six out of eight carcasses. All six cat-positive carcasses were headless or almost headless with the head and body faintly connected by esophagus and trachea, several of which were missing their intestines. We describe the conditions of these headless carcasses, noting the main characteristics that could lead to suspicion of cat predation. To the best of our knowledge, this is the first genetic predator identification using seabirds, and may make more stakeholders aware of the reality of cat predation worldwide. On Mikura Island, we expect that this evidence will contribute to the development of systematic cat management.

Key words: feral cats, islands, molecular forensics, molecular predator identification, saliva.

Abstract in Japanese（要旨）．オオミズナギドリの首なし死骸はネコの捕食によるものだった：遺伝学的捕食者検出によるエビデンス．イエネコ *Felis silvestris catus* は，最も有名な侵略的外来捕食者の一つとして知られている．海鳥は，島嶼においてイエネコの影響を受けてきた典型的な分類群であり，首のない死骸が頻繁に観察されることがある．本研究では，御蔵島において，イエネコによって近年個体数が減少していると言われているオオミズナギドリ *Calonectris leucomelas* の死骸を対象に，遺伝的捕食者検出を行い，さらに死骸の特徴を明らかにした．今回の調査では，オオミズナギドリの死骸を 8 個体分発見した．死骸の傷口から採取したスワブサンプルの遺伝子分析により，8 つの死骸のうち 6 つでイエネコの DNA が検出され，イエネコの捕食が確認された．これら 6 つの死骸はすべて頭がないか，頭がかろうじて食道や器官で胴体部とつながったほぼ首なしという状態で，そのうちのいくつかは消化管も消失していた．本研究では，この首なしという死骸の状態について，イエネコの捕食を疑うことができる主な特徴として提示する．我々の知る限り，本研究は海鳥を対象とした初めての遺伝的捕食者検出の事例であり，これにより世界的なイエネコの捕食の実態が，より多くの関係者に認識されるようになるであろう．さらに，この遺伝学的エビデンスが御蔵島における本格的なイエネコ対策の展開に貢献することも期待される．

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The domestic cat *Felis silvestris catus* is one of the most notorious invasive predators, responsible for the extinction and decline of native species worldwide (Medina et al. 2011; Nogales et al. 2013; Doherty et al. 2016), listed in the 100 of the World's Worst Invasive Alien Species (Lowe et al. 2000). This is particularly true for island ecosystems where native species have often evolved without native predators (Courchamp et al. 2003; Bonnaud et al. 2011; Nogales et al. 2013). However, successful cat management has been limited mainly to small and/or human-uninhabited islands (Oppel et al. 2010; Campbell et al. 2011; Nogales et al. 2013), and it is essential that any efforts be made on a wider scale to increase successful management implementations.

Seabirds are typical taxonomic groups on islands that have been impacted by free-ranging cats (Towns et al. 2011; Croxall et al. 2012). For many seabird species, islands are essential environments for breeding (Croxall et al. 2012). Many of their life history characteristics, such as slow sexual maturity, low rate of egg production, long presence as chicks in nests on the ground or in burrows, low escape ability from predators on the ground, and low vigilance, are products of their adaptation to predator-free islands, and these, in turn, make them vulnerable to cat predation (Keitt et al. 2002; Towns et al. 2011). On seabird-breeding islands where cat predation is a major concern, the carcasses of seabirds are frequently observed (Oka et al. 2002; Smith et al. 2002; Peck et al. 2008; Towns et al. 2011; Ringler et al. 2015). Because scattered carcasses on islands provide visual and intuitively persuasive evidence of cat predation to people, they can be a driver to encourage wider stakeholder consensus and better management implementation. However, it is not always possible to identify predators from the post-mortem examination of prey remains (Ratz et al. 1999; Hocken 2000; Hopken et al. 2016; Colombelli-Négrel and Tomo 2017), and sometimes failing to make associations between carcass remains and predators can lead to management inaction against the actual predator (Sundqvist et al. 2007; Caniglia et al. 2013). Therefore, reliable identification of predators is required for meaningful conservation management.

In addition, although the characteristics of carcasses attacked by free-ranging cats, of which headless carcasses are likely typical examples, have been described by some researchers and practitioners (Oka et al. 2002; Smith et al. 2002; Towns et al. 2011; Greenwell et al. 2019), these reports are not sufficiently reliable because they have not been reported with reliable predator identifications. Therefore, pairing reliable predator identification with descriptions of the characteristics of the prey remains would not only increase the credibility of the report, but would also strengthen the persuasiveness of the previous descriptions.

One useful tool for identifying predators from carcasses is the genetic approach, which detects predator-saliva-derived DNA in/around the carcass wounds (Sundqvist et al. 2007; Caniglia et al. 2013). However, to the best of our knowledge, this method has not been applied to the search for predators on the carcasses of seabirds.

The purpose of this study was to provide reliable evidence of cat predation on an island where large numbers of seabird carcasses occur, and to relate the predator identification to the characteristics of the carcasses. For this purpose, we selected Mikura Island, Japan, as a research site, where there is one of the largest breeding colonies of the streaked shearwater *Calonectris leucomelas* (Oka et al. 2002; Oka 2004), and predation of free-ranging cats on the shearwaters has been suggested and supported by fecal content analyses (Oka 2019; Azumi et al. 2021) and camera traps (Tokuyoshi et al. 2020). Since the island is inhabited and has potential predators other than free-ranging cats (e.g., rats and crows), identification of predators is important for consensus building among residents and various stakeholders to promote cat management. There, we conducted genetic predator identification of the shearwater carcasses and described their characteristics. On this island, shearwaters carcasses are frequently observed, many of which are headless, everywhere from around the residents' settlements to the depths of the forests (Oka et al. 2002; Oka 2019). Cats range freely throughout the island (Azumi et al. 2021), and researchers and conservation practitioners are almost certain that free-ranging cats are responsible for the carcasses (Oka et al. 2002; Oka 2019), although there has been no direct evidence linking the carcass remains to cat predation. On the other hand, systematic cat management has been generally unimplemented, and not many people associate the headless carcasses they frequently see with cat predation (Kusachi, personal observation). Therefore, evidence of cat predation from the carcasses is required to make more stakeholders realize the potential ecological impacts of free-ranging cats, and the potential importance of systematic cat management.

Materials and methods

Research field and target species

Mikura Island (33°52'N, 139°36'E) is located in the Izu Islands, Japan. It has steep topography, with an area of 20.54 km^2 and peak elevation of 851 m. The human population of the island is around 300, living in the only settlement in the north-northwest of the island. Its climate is temperate, with mean monthly temperature of 26.2°C in August and 9.6°C in February, and annual precipitation of 3120 mm (Kawamoto 2006). The land area from low to middle altitude is covered by broad-leaved evergreen forest consisting mainly of *Castanopsis sieboldii*, while high altitude areas are covered with shrubs and bamboo bushes (Kamijo et al. 2001).

The streaked shearwater is an endemic breeder to East Asia, and is listed as Near Threatened (NT) on the IUCN Red list (BirdLife International 2018). One of the largest breeding colonies of the shearwater is on Mikura Island, where shearwaters burrow nests on the forest floor in broad-leaved evergreen forests (Oka 2004). Shearwaters stay on the island to breed from March to the middle of November and are absent from the end of November to the end of February (Oka et al. 2002).

The breeding population of the streaked shearwater on this island has experienced a rapid decline over the last 40 years (Tokyo Metropolitan Government 1980; Biodiversity Center of Japan 2017), and cat predation is thought to be one of the causes (Azumi et al. 2021). For example, Azumi et al. (2021) estimated that even a single free-ranging cat can prey on 313 streaked shearwaters per year based on cat fecal analysis. In addition, carcasses of the shearwater, most of which were headless and thought to be killed by free-ranging cats at nighttime, were frequently observed on this island in the breeding season (Oka et al. 2002). However, no systematic cat management has been implemented. Hence, evidence from a broader perspective is needed to obtain the consensus of more stakeholders (Tokuyoshi et al. 2020; Azumi et al. 2021). Other potential predators on this island are the non-native black rat *Rattus rattus* and Norway rat *R. norvegicus* (Azumi et al. 2019); although, if anything, their main predation targets are thought to be eggs and young chicks (Oka et al. 2002; Nam et al. 2014). Diurnal avian predators, such as the jungle crow *Corvus macrorhynchos japonensis* and diurnal raptors (e.g., eastern buzzard *Buteo japonicus*), are occasionally observed either attacking shearwaters or scavenging their carcasses (Tokuyoshi, personal observation), although we can distinguish the predation of nocturnal free-ranging cats from diurnal avian predators by estimating when the shearwaters were attacked and killed. Several domestic dogs *Canis familiaris* are owned by residents, all of which are usually kept on leashes, and their involvement in predation issues on shearwater is thought to be negligible. No carnivoran species except for free-ranging cats and dogs exist on this island.

Sample collection and DNA extraction

The search for carcasses of the streaked shearwaters was conducted around the village on Mikura Island from 14 to 18 November, 2020, when most chicks fledged (Oka et al. 2002). Sunrise on the first day of the survey, 14 November, 2021, on Mikura Island occurred at 6:13. We conducted a preliminary survey throughout the study site from the day before the beginning of the main survey, and we only targeted carcasses that were not found in previous days. When new carcasses were found in the survey period, we conducted a brief visual autopsy (i.e., missing body parts, bite mark on injured area) and swabbed for scarred tissue that appeared to have been preyed upon by possible predators. We then preserved the swabs in 99.9% ethanol at room temperature until DNA extraction. We took special care to avoid contamination in sampling as follows: 1) Swabs were used only on and around the scarred tissue of the carcasses, and should not touch any other area of the skin and feathers, nor the ground, 2) after swabbing, the swabs were promptly stored in a tube with ethanol, 3) researchers never touched cats during survey period. These surveys were performed in the early morning hours to prevent carcasses killed at nighttime from losing their freshness, and to prevent scavenging by diurnal predators (i.e., avian predators).

Muscle samples were collected from two streaked shearwaters on Mikura Island in 2006 and 2017 as negative controls for the following multiplex nested PCR assessment, and stored in –20°C until DNA extraction. We also used a new cotton swab as a negative control.

DNA was extracted from the eight streaked shearwater swabbed samples (MKRaS001–008, see Results), the two streaked shearwater muscle samples, and a new cotton swab using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol.

DNA of the domestic cat and domestic dog was extracted from uterine tissue using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. These DNAs were extracted as positive controls for the order Carnivora inhabiting Mikura Island.

Multiplex nested PCR assessment

We used DNA extracted from eight swab samples of streaked shearwater carcasses for predator identification. We also used extracted DNA from two streaked shearwater, and a cotton swab as negative controls. Domestic cat and domestic dog DNA were used as positive controls.

We used primers specific for the cat and dog mitochondrial cytochrome *b* DNA regions developed by Imazato et al. (2012) for first and second multiplex nested PCR. The first and second PCRs were carried out on a ProFlex PCR System (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The first multiplex PCR was performed in a 20 µl reaction mixture containing 1 µl DNA extract (< 1 µg/reaction), 0.5 µM each first PCR primer and AmpliTaq Gold 360 Master Mix (ThermoFisher Scientific, Waltham, Massachusetts, USA). The amplification conditions for the first multiplex PCR were as follows: 95°C for 10 min; 40 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s; followed by a final extension at 72°C for 5 min. We electrophoresed the PCR products on 2% agarose gels in 1 × TAE, stained with Midori Green DNA stain (NIPPON Genetics, Tokyo, Japan) and visualized under visible LED light (500 nm). The second multiplex PCR was performed in a 20 µl reaction mixture containing 0.2 µl first multiplex PCR product, 0.5 µM each second PCR primer, and AmpliTaq Gold 360 Master Mix (ThermoFisher Scientific, Waltham, Massachusetts, USA). The amplification and electrophoresis conditions for the second multiplex PCR were the same as the first multiplex PCR.

Sequence analysis of the second multiplex PCR products

The cat specific second PCR products were purified using MinElute PCR Purification Kit (QIAGEN, Hilden, Germany), and cycle sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The two cat specific primers used for the second multiplex PCR were developed by Imazato et al. (2012). Sequences were read using an ABI3130 Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Data analyses

We performed a BLAST homology search (Altschul et al. 1990) on the National Center for Biotechnology Information (NCBI) website (https://blast.ncbi.nlm.nih.gov/Blast.cgi, Accessed 14 May 2021) to search for sequences closely related to the obtained sequences using total score, identity, and E-value.

Results

Eight streaked shearwater carcasses were found in the survey periods (MKRaS001–008, Fig. 1; Table 1). All carcasses were headless or almost headless, and several had also lost their intestines. In addition, none had conspicuous foraging marks from rats and avian predators.

The multiplex PCR of the eight swab samples from the streaked shearwater carcasses was performed to understand the possible predator species. We obtained amplified products from six swab samples (MKRaS001, MKRaS002, MKRa003, MKRa004, MKRa006, and MKRaS008) (Fig. 2). The molecular size of the products was the same as that from domestic cats (Fig. 2). As in Fig. 2 of Imazato et al. (2012), the electrophoresis photograph of the cat 2nd PCR, large size artifact bands can be seen when a high concentration of DNA sample was used. The slightly longer bands observed in lanes 1, 2, 3, 4, and 6 in our Fig. 2 may be due to the higher amount of DNA used in the 2nd PCR.

We determined DNA sequences of the cat specific second PCR products from six swab samples from streaked shearwater individuals. Four of the swab samples, MKRaS001, MKRaS002, MKRa003, and MKRa004, shared an identical sequence (242 bp). The sequences from MKRa006 and MKRa008 showed one and two nucleotide substitutions with those of MKRaS001–004, respectively. Sequences obtained in this study were registered in DNA Data Bank of Japan (DDBJ) with the following accession numbers: LC649703–LC649705. In the BLAST homology search, all the six cat specific second PCR products obtained from streaked shearwaters were 100% (242/242 bases) homologous to the *Felis silvestris catus* mitochondrial cytochrome *b* region sequences with total score = 448 and E-value = 1×10^{-121} (MKRaS001–004: MN175474, KT626623, MT499915, AB194812, AB194814, and AB004237; MKRaS006: AP023162, MN175497, MN175494, MN175480, MN175479, AB194817, and NC_001700; MKRaS008: MN175496, MN175487, MN175484, MN175478, MN175475, LC469866, KX348260, KP202275, MW267828, MW267829, FJ160761, EF689045, AY509646, AB194815, AY170102, and AB004238).

Discussion

Our genetic assessment identified cat predation on six out of eight carcasses of the streaked shearwater on

Fig. 1. The conditions of the streaked shearwater carcasses found in this study. A photo of individual MKRaS003 is missing.

Mikura Island. The method used in this study (Imazato et al. 2012) is capable of species identification from samples containing 50 pg, which corresponds to only 10 cells, and the species identification capability and accuracy are sufficiently high. Therefore, predators were reliably identified from saliva-derived DNA samples left in the wounds targeted in this study. To the best of our knowledge, this is the first genetic predator identification of seabirds. We believe that this result is widely applicable to other seabird islands, especially for human-inhabited islands where raising residents' awareness of invasive species is needed, and/or control measures on multiple invasive predators are necessary.

All six cat-positive carcasses were either headless or in a nearly headless state with the head and torso slightly connected by the esophagus or trachea. In some cases, the intestines and sternum had also been removed. In this study, we describe the conditions of these headless car-

Table 1. Streaked shearwater carcasses sampled in this study, their visual features by brief on-site autopsy, and the results of genetic predator identification

Carcass ID	Sampling date/time (2020)	Carcass condition	Extracted DNA (ng/μl)	Genetically Identified predator*
MKRaS001	14 Nov 5:09	- Head was removed. - No significant foraging marks of rodents and avian predators were found.	64.1	*F. s. catus*
MKRaS002	14 Nov 5:13	- The area from the head to the breast was consumed, and the head and body were faintly connected by esophagus and trachea. - No significant foraging marks of rodents and avian predators were found.	55.8	*F. s. catus*
MKRaS003	15 Nov 5:15	- Head was removed. - No significant foraging marks of rodents and avian predators were found.	65.3	*F. s. catus*
MKRaS004	15 Nov 6:17	- The area from the head to the breast was consumed, and the head and body were faintly connected by esophagus and trachea. - No significant foraging marks of rodents and avian predators were found.	163.2	*F. s. catus*
MKRaS005	15 Nov 6:17	- The area from the head to the breast was consumed, and the head and body were faintly connected by esophagus and trachea. - No significant foraging marks of rodents and avian predators were found.	8.3	Unidentified
MKRaS006	16 Nov 6:45	- Head, intestine, and sternum were removed. - No significant foraging marks of rodents and avian predators were found.	15.4	*F. s. catus*
MKRaS007	16 Nov 9:07	- Head and intestine were removed. - No significant foraging marks of rodents and avian predators were found.	20.1	Unidentified
MKRaS008	18 Nov 6:13	- Head and intestine were removed. - No significant foraging marks of rodents and avian predators were found.	8.4	*F. s. catus*

* *F. s. catus* represents the domestic cat *Felis silvestris catus*.

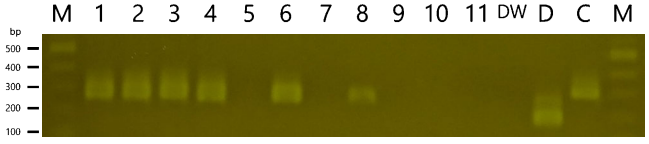

Fig. 2. Amplification products to identify predators of streaked shearwater. Swab samples from streaked shearwater carcasses (lanes 1–8), new cotton swab (lane 9), streaked shearwater (lane 10 and 11), distilled water (lane DW), dog (lane D), and cat (lane C), and 100 bp DNA ladder marker (lane M).

casses as one of the main characteristics that could suggest cat predation. Of course, we did not describe the full range of the predation behavior of free-ranging cats, as there must be other ways of killing prey, such as just biting prey animals (McDonald et al. 2015). On the contrary, headless carcasses of seabirds can also be produced by other mammalian predators, such as the common raccoon *Procyon lotor* (Hartman et al. 1997), so care should be taken to identify predators in areas where other carnivores are present. Nevertheless, linking the most obvious features (i.e., headless) of the carcass to cat predation will contribute to making more stakeholders aware of the reality of cat predation. In addition, frequent observations of headless carcasses have also been reported on various seabird islands invaded by free-ranging cats (Smith et al. 2002; Towns et al. 2011; Greenwell et al. 2019). This study provides a basis of predator estimation for such previous reports and can be more convincing about the invasion of free-ranging cats.

As mentioned above, cat DNA was not detected from two out of eight carcasses. There is a possibility that predators other than free-ranging cats killed the shearwaters, leading to a lack of cat DNA on the carcasses. However, it is not very likely that rats predated individuals of streaked shearwater older than fledging chicks (Oka et al. 2002), and there were no significant rat bite marks on wounded parts. The DNA of domestic dogs was not detected. Although cat-negative MKRaS007 was found about three hours after sunrise, leaving room for the possibility that it might have been attacked by diurnal avian predators, there were no obvious marks from avian attack (i.e., torn feathers) (Stahl et al. 2002). Therefore, it is unlikely that these other potential predators were associated with producing the two cat-negative carcasses.

The breeding population of the streaked shearwater on Mikura Island has drastically decreased from the estimated population size of 1 750 000–3 500 000 in 1978 (Tokyo Metropolitan Government 1980) to 110 000 in

2016 (Biodiversity Center of Japan 2017), which is an issue that requires close attention. Furthermore, a recent study of the feeding habits of free-ranging cats has quantified the actual predation of a large number of the shearwaters (Azumi et al. 2021). However, systematic management of cat populations has not yet been implemented. Urgent actions are needed and it is expected that our research will contribute to this through raising public awareness.

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