Functionally referential alarm calls in noisy miners communicate about predator behaviour

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Abstract Many vertebrates have alarm calls that warn conspecifics about danger, and some species even communicate about the type of predator or its behaviour, allowing for appropriate responses. However, such ‘functionally referential’ communication has been shown experimentally in only a handful of species, and requires demonstrating that individuals give acoustically distinct calls to different threats, and that the calls alone are sufficient to prompt listeners to behave as if a specific threat is present. We carried out model presentations, acoustic analyses and a playback experiment to test whether the alarm calls of noisy miners, *Manorina melanocephala*, are functionally referential. Miners gave different calls to airborne raptor models compared to terrestrial or perched raptor models, and even switched from ‘aerial’ alarm calls to ‘chur’ alarm calls when a hawk glider landed on the ground. They also behaved differently to these two types of threats, showing avoidance to aerial threats, including fleeing or freezing, but deterrent behaviour to terrestrial threats, including vigilance, approach and mobbing. The two alarm-call types were acoustically distinct, and consistent with calls to live predators. Blind scoring of video revealed that birds responded appropriately to playbacks of alarm calls alone, typically fleeing to aerial alarm calls yet becoming vigilant, approaching and calling to chur calls. Noisy miners produce alarm calls that therefore meet both criteria for functional reference, and thus become one of the few bird species in which such calls have been confirmed. Many birds appear to give different calls to airborne predators compared to during mobbing of terrestrial or stationary predators, so functionally referential alarms are likely to be common and may often categorize predators by their behaviour and not simply their taxonomic type.

Key words: acoustic communication, alarm calls, anti-predator behaviour, functional reference, mobbing calls
INTRODUCTION

Many birds and mammals give alarm calls that warn others of danger or deter predators (Caro, 2005). Alarm calls can be classified in variety of ways, including by their sound (e.g. ‘bark’, ‘croak’, ‘seet’, ‘whistle’), the context in which they are given (e.g. ‘distress’, ‘general’, ‘eagle’), or the behaviour of the caller or responders (e.g. ‘mobbing’, ‘flee’). Reflecting the diversity of labels, alarm calls vary greatly in acoustic properties and specific function (Klump & Shalter, 1984; Magrath, Haff, Fallow, & Radford, 2015; Zuberbühler, 2009). Some alarm calls are given in a wide range of circumstances, while others appear restricted to specific contexts. For example, white sifakas Propithecus v. verreauxi and red-fronted lemurs Eulemur fulvus rufus both have aerial alarm calls given specifically to raptors, as well as general alarm calls that are given to terrestrial predators and in contexts of surprise or social conflict (Fichtel & Kappeler, 2002). Alarm calls given in specific contexts can convey graded information about a predator’s proximity, size, or the degree of danger it poses (e.g. Leavesley & Magrath, 2005; Templeton, Greene, & Davis, 2005), categorical information on the type of threat (e.g. Gill & Bierema, 2013), or both graded and categorical information (Manser, 2001; Manser, Bell, & Fletcher, 2001; Sieving, Hetrick, & Avery, 2010). We focus here on communication about the type of threat rather than graded information.

The alarm calls of some species refer to very specific types of threat, allowing appropriate responses by listeners. In perhaps the best known example, vervet monkeys Chlorocebus aethiops commonly give different alarm calls to leopards, eagles and snakes, allowing listeners to respond in an appropriate way, such as looking down after ‘snake’ alarms and looking up and fleeing to cover after ‘eagle’ alarms (Seyfarth, Cheney, & Marler, 1980a, 1980b). Such alarm calls appear to refer
to specific external threats, and not simply the presence of a threat or the internal state
of the caller, such as degree of fear. These calls were initially interpreted as allowing
‘referential’ or ‘semantic’ communication about the world. Such alarm calls are now
usually considered to be ‘functionally referential’, which acknowledges that we can
observe the use and response to calls, but not the cognitive processes that are implied
in communication using symbolic signals (Evans, 1997; Macedonia & Evans, 1993).
Some authors also question the concept of functional reference, in part because they
challenge the idea that signals are designed to convey information (Rendall, Owren, &
Ryan, 2009; Wheeler & Fischer, 2014), but for others information is a central issue in
animal communication (Seyfarth et al., 2010); for a range of views, see Stegmann
(2013). Overall, many researchers find the concept of functional reference useful, and
particularly relevant in the context of alarm calls given to different predators (Gill &

Demonstrating functional reference requires considering both the production
and perception of calls. A functionally referential alarm call must be given primarily
to a specific class of threat, and the call itself must prompt responses by listeners that
are similar to those prompted by the external threat itself (Macedonia & Evans, 1993).
It follows that observational studies of natural interactions with predators cannot show
functional reference, because apparent responses to alarm calls might instead rely on
direct observation of the predator, the behaviour of the caller, or other contextual
information. As a result, playback experiments are essential in isolating the
information conveyed by the calls themselves, independent of the context (e.g.
Seyfarth et al., 1980a; review: Evans, 1997). Functionally referential calls must
therefore be both acoustically distinct, and perceived as distinct by listeners.
Surprisingly few studies have tested experimentally for functionally referential alarm calls, despite the long history of interest and observational studies suggesting they are common. Recent reviews list eight species of birds and seven species of mammals that show production and perception specificity (Gill & Bierema, 2013; Suzuki, 2016; Townsend & Manser, 2013), although smooth-bill anis *Crotophaga ani* and tamarins *Saguinus fuscicollis* and *S. mystax* should also be added (Grieves, Logue, & Quinn, 2014; Kirchhof & Hammerschmidt, 2006). All include playbacks to test response independent of context, and either observations or experiments to examine production specificity. For example, fowl *Gallus gallus* give acoustically distinct alarm calls to video images of raptors flying overhead compared to raccoons *Procyon lotor* on the ground, which are consistent with calls to natural predators, and playbacks prompt appropriate responses, such as moving to cover, crouching and looking upwards to aerial alarm calls, and standing erect and looking around after ‘terrestrial’ alarms (Evans, Evans, & Marler, 1993). Among mammals with evidence of functional reference, seven are primates (e.g. Seyfarth et al., 1980a; Zuberbühler, 2000), but both Gunnison’s prairie dogs *Cynomys gunnisoni* (Kiriazis & Slobodchikoff, 2006) and meerkats *Suricata suricatta* (Manser, 2001; Manser et al., 2001) also produce distinct calls to different predators that prompt appropriate responses.

Studies of functionally referential alarm calls have almost exclusively focused on signaling about types of predator, but signaling about predator behaviour is also relevant to escape strategy. In the only study of its type, Griesser (2008) showed that the alarm calls of Siberian jays *Perisoreus infaustus* signaled about hawk behaviour. Calls given to perched, moving and attacking *Accipiter* hawks differed acoustically, and playbacks prompted appropriate responses. For example, playback of ‘perched
hawk alarms’ prompted listeners to search for the threat, without taking cover, which is similar to the behaviour of birds mobbing a perched hawk, whereas playback of ‘attack calls’ led birds to flee immediately to cover, followed by search behaviour. Siberian jays therefore classify predators by their behaviour, not just predator type, which is relevant because appropriate responses depend on predator behaviour. In fact, avian alarm calls are often classified as ‘flee’ (or ‘warning’ or ‘aerial’) alarm calls or ‘mobbing’ alarm calls, suggesting that functionally referential alarm calls in birds—and some mammals—may often classify predators by current behaviour in addition to or instead of the type of predator.

We studied the alarm calls of noisy miners *Manorina melanocephala*, a species with putatively distinct alarm calls that refer to different threats. ‘Chur’ calls appear to signal about potential terrestrial or perched predators posing little immediate threat, while ‘aerial’ alarm calls appear to warn primarily of raptors in flight (Higgins, Peter, & Steele, 2001). However, there have been no direct experimental contrasts of the context of production and acoustic structure of these calls, and playbacks have so far been limited to chur calls (Kennedy, Evans, & McDonald, 2009). We examined experimentally both the production and perception of these calls by wild birds to test for functional reference. To do so, we used predator models of perched and flying raptors to examine the context of production, compared the acoustic properties of calls, and carried out a playback experiment in which responses were scored blind to ensure our expectations did not influence results.
METHODS

Study Species

Noisy miners are medium-sized (c. 60-70 g), highly social honeyeaters (family Meliphagidae) that breed in colonies made of loosely territorial groups (Dow, 1979). Parental care is cooperative, with the breeding female and several males provisioning individual broods (Dow & Whitmore, 1990; Pöldmaa, Montgomerie, & Boag, 1995). Miners are common in eastern Australia and typically live in habitat containing both open areas and trees, particularly eucalypts. They feed primarily on invertebrates and nectar, anywhere from the ground to the canopy (Ashley, Major, & Taylor, 2009). As a result of habitat preference, miners are common in human-altered habitats such as grazing land, urban parks and suburban gardens (Maron, 2007; Sewell & Catterall, 1998). They are aggressive to many species, including predators, and miners can exclude smaller species, particularly competitors for food, from their colonies (Dow, 1977; Piper & Catterall, 2003; Sewell & Catterall, 1998).

Noisy miners are aptly named, as they have a large repertoire of conspicuous vocalizations, including two alarm calls with a putatively distinct function (Holt, 2013). Chur alarm calls are given in the wild to potential predators that are on the ground, perched, or being harassed by miners (Higgins et al., 2001; Holt, 2013). Targets include perched raptors, mammals, snakes and large lizards (Dow, 1975). These calls have been prompted experimentally by live dogs Canis familiaris, and taxidemic models of foxes Vulpes vulpes and cats Felis cattus (Kennedy et al., 2009; Lowry, Lill, & Wong, 2012). Birds respond to playback of chur calls by approaching the speaker and often also repeating the calls, suggesting that these calls are mobbing alarm calls that alert others and incite them to harass threats (Kennedy et al., 2009). In contrast to chur calls, aerial alarm calls are given in the wild to flying raptors such as...
brown goshawks *Accipiter fasciatus* and collared sparrowhawks *A. cirrhocephalus*, which attack from the air, and have been prompted in the wild with model sparrowhawks (Magrath & Bennett, 2012) and model planes (Doohan, 2014). There has, however, been no experimental comparison of calls prompted by different threats. Furthermore, the function of aerial alarm calls has not been tested through playback experiments, except that superb fairy-wrens *Malurus cyaneus* flee to cover after playback of these calls (Magrath & Bennett, 2012), which is the same response fairy-wrens have to conspecific and other heterospecific aerial alarm calls that warn of flying predators (Magrath, Pitcher, & Gardner, 2007, 2009).

**Study Sites**

We studied noisy miners from April to September 2015 (primarily autumn and winter) in suburban parks and nature reserves in Canberra, Australia, where miners are common. Chur and aerial alarm calls are both common throughout the year, including autumn and winter (Doohan, 2014; Holt, 2013; personal observations). Experimental replication was achieved by using 15 study sites that were distributed over an area of 58 km², with a minimum distance of 750 m between sites. Individual miners have a home range size with a diameter of up to about 250 m (Dow, 1979), so the spatial separation of sites was designed to ensure that data collected at different sites were independent, despite the birds not being individually recognizable. All sites had eucalypt trees, open grassy areas, and scattered understory plants. Raptors, including *Accipiter* spp, the Australian hobby *Falco longipennis*, peregrine falcon *F. peregrinus*, and boobook owl *Ninox novaeseelandiae*, are widespread through the Canberra area (Fennell, 2009; Taylor & Group, 1992), and all commonly include birds in their diet (Higgins, Peter, & Cowling, 1999; Marchant & Higgins, 1993). One
or more of these species have been sighted at all 15 experimental sites
(http://canberranaturemap.org), and have known nest sites within 1 km of all but 4 of
the sites (Stuart Rae, pers. comm). Terrestrial predators–such as dogs, foxes, cats and
snakes–are also common in the study sites (Cunningham, pers. obs.).

Context of production

We used a matched experimental design, in which miners at each site were
presented with all treatments (two models and two controls) in order to test whether
different alarm calls are given to airborne compared to stationary threats. Airborne
models were shaped and painted to resemble an adult brown goshawk, or an adult or
juvenile collared sparrowhawk (details in Magrath, Haff, McLachlan, & Igic, 2015).
These life-sized models were made of sculpted foam; the goshawk model was similar
in appearance but larger than the adult sparrowhawk. The models were thrown by
hand, from a distance of about 10 m, and aimed to pass by the miner within 5 m of its
initial position. If thrown correctly, the models glided for 20–40 m, and remained
airborne for about 2–4 seconds. Models were covered in green cloth before being
used, so they could not be seen before being launched. Failed throwing attempts–
when a glider veered immediately into vegetation or to the ground–were repeated as
soon as possible. Stationary models were taxidermic mounts of two sparrowhawks
and a boobook owl. They were placed on the ground, on a low perch and inside a
chicken-wire cage designed to protect the mounts from attack by miners. The cage
was covered by a green cloth, which was removed by hand when a miner was within
5 m. The three models of each type were each used five times during presentations at
the 15 sites.
We used two controls to test whether our activity or the method of presentation itself affected miner calls. The control for the airborne raptor models involved an observer walking to within 10 m of a miner and miming the action of throwing a glider model. The control for the stationary taxidermic mounts involved presenting the empty chicken-wire cage in the same way as the mounts were presented. The covered cage was placed on the ground, and then the cloth was removed when a miner was nearby. These controls were designed to assess whether miner alarm calls were given to the models themselves, independently of our presence or activity during presentation. The key prediction of functional reference is that the alarm calls should differ according to the type of threat, in this case airborne versus stationary models.

All presentations were videoed and audio-recorded during model presentation to allow scoring of behaviour, and preceded by an observation period to ensure there were no alarm calls or predators seen for at least 5 min before a model or control was presented. Audio recordings were made from a mean distance of 13 m (median 15 m, range 8–26 m), using a Sennheiser ME66 directional microphone with Marantz 670 recorder, saving uncompressed wave files at 16 bits and 44.1 kHz. Birds were videoed from a similar distance by a second person using a Digital Canon XA20 video camera. There was a minimum of 1 h between different treatments at each site, during which time the observers left the site. Presentations were usually carried out over two or more days, to avoid rain and wind, and to allow scheduling of presentations at multiple sites (median 28 h between first and last presentations at a site, range 5–133 h). The order of presentation of models and controls was randomized, given that we could not include all 24 orders of four treatments, but constrained so that no type of presentation occurred more than five times in a specific order. If a random order led to
that outcome, the order was re-randomized. Later analyses revealed no affect of order
of presentation on miner behaviour or any carry-over effects (Results).

It was not possible to score the videos of model presentations blind, because
the predator models or observer were usually shown in the videos. However,
recordings and spectrograms of audio-recordings allowed unambiguous classification
by ear and eye into aerial and chur alarm calls, and detailed acoustic analyses showed
that the call types are discrete (Results). Scoring the response to hawk gliders started
when the model was thrown, and continued until 5 min after the model had landed.
Furthermore, we used the video and audio recordings to subdivide the response into
the period when the model was airborne compared with the period after it had landed.
The response to stationary mounts was scored from the time a miner first showed any
response to the model, including giving any type of alarm call, or approached within 2
m of the model. Behavioural sampling then continued for 5 min, while the model
remained visible, after which sampling stopped and the model was covered and
removed. A 5-min period was also used to score behaviour after control presentations,
timed from the action of throwing the glider or uncovering the cage.

Miners did not call to every predator presentation, especially taxidermic
models, so we gathered additional recordings of alarm calls to enable fully replicated
playback experiments (below). These supplementary presentations were used solely to
gather recordings for playback, and not to score behavioural responses to different
models or test statistical hypotheses about call structure. In these presentations, we
repeated the glider presentation to one group of miners to gather an additional call in
an aerial context. Lack of calling was more common to stationary taxidermic mounts
(Results), perhaps because their lack of movement made them less like live predators.
Therefore, to increase realism, we combined presentation of taxidermic models with 4
s playback of the Australian magpie-lark’s *Grallina cyanoleuca* general alarm call

(‘rapid’ call), which is given during aggressive encounters with conspecifics as well as to predators (Higgins, Peter, & Cowling, 2006). We used magpie-lark calls because this species is abundant in Canberra (Taylor & Group, 1992), also prefers habitat with open areas and trees, and is common at all our experimental sites. Furthermore, because rapid calls are used in a variety of social and predator contexts, we expected them to draw attention to the stationary model while giving no specific information on the type of threat. Miners always gave alarm calls in this context, so we used these calls in the playback experiment. While playback of magpie-lark alarm calls increased the probability of miners calling, they still produced the same type of calls (Results).

**Acoustic structure**

We measured the acoustic structure from spectrograms of alarm calls in Raven Pro 1.4. Spectrograms used a custom pre-set that determined both the length of time shown on the screen (1.4 s) and the spectrogram settings (Hann window function, size 10.2 ms, 3 dB filter bandwidth 141 Hz; DFT size 512; hop size 0.522 ms). Individual alarm calls were made of a sequence of discrete elements, and we measured the fundamental (first harmonic) from a random element for each individual caller (Fig. 1). We placed the selection box around the harmonic, from which we recorded: (1) duration (ms), the duration containing 90% of the energy; (2) peak frequency (Hz), the frequency at where amplitude is maximum; (3) low frequency (Hz), the frequency above which 95% of energy occurs; (4) high frequency (Hz), the frequency below which 95% of energy occurs; (5) frequency bandwidth (Hz), the frequency range containing 90% of the energy. We used these automated percentage values to minimize errors introduced by manual placement of selection boxes. In addition to
these temporal and frequency measures of the fundamental, we measured (6) the
relative amplitude of the fundamental compared with the mean of the second and third
harmonics. To do this we measured the average power of the first three harmonics,
and then we calculated the difference in dB between the fundamental and the mean of
harmonics 2 and 3. Positive values meant that the fundamental was of higher
amplitude than the mean of harmonics 2 and 3. We used this value because previous
work on miners suggested that the fundamental is loudest in aerial alarms, but that
harmonic 2 or 3 is loudest in chur calls (Holt, 2013; Jurisevic & Sanderson, 1994b;
Kennedy et al., 2009; Magrath & Bennett, 2012). We did not measure amplitudes (dB
SPL) itself because we could not control for distance and bird orientation, and were
interested specifically in the difference in structure between alarm-call types. Overall,
for the context of production experiment, we measured 14 alarm calls when a raptor
glider was airborne, 14 after a raptor glider had landed on the ground, and 7 when
there was a stationary taxonomic raptor (Results). These calls were recorded from 15
sites, following the matched sampling procedure (above), with only one sample from
a given context at each site. There was a minimum of 15 individuals, from 15
different social groups, included in the sample, but because birds could not be
identified individually (above), there could have been more individuals sampled
across the three contexts within the 15 social groups. To place these recordings in a
broader context, we also measured the acoustic properties of alarm calls produced to
natural predators (n = 9), and to calls produced during the supplemental presentation
of taxidermic raptors accompanied by magpie-lark alarm calls (n = 15). Again, each
recording in a given context was taken from a different location.
We carried out a playback experiment to determine if miners respond appropriately to the different putative alarm calls in the absence of any cues about predator type. Again, we used a matched experimental design in which unique exemplars of all four treatments were broadcast to focal individuals at each of the 15 experimental sites. Playback treatments were designed to encapsulate natural variation: (1) an aerial alarm call; (2) a short chur alarm call; (3) a long sequence of chur alarm calls; and a (4) control contact call of a harmless parrot (Supplementary Audio S1–S4). The aerial alarm, short chur call and control call were all about 2 s long, while the long chur alarm sequence was 15 s long. Aerial alarm calls are typically short, while chur calls are variable in length, and individuals can continue calling for long periods if a predator remains nearby (pers. obs.; Holt, 2013; Kennedy et al., 2009). In our sample of responses to raptor glider models, the mean aerial alarm call length was 1.7 s, with 1–2 calls being given over a period of 1–4 s. In response to taxidermic raptor models, chur calls were a mean length of 2.4 s, with calling lasting for between 6 s and 5 min (when the model was removed). Short chur call playbacks were designed to enable a direct contrast with aerial calls, while holding duration constant at 2 s, which is between the means of the two call types. We included a long sequence of chur calls to determine if the duration of calling changes response. This sequence consisted of a series of individual calls, following a natural timing. Control calls were contact piping calls of crimson rosellas, *Platycercus e. elegans*, locally abundant, harmless parrots, that occur at all study sites. *Platycercus* spp are medium-sized parrots (c. 100–130 g) that are not excluded from miner colonies (Cunningham, pers. obs; Maron, 2007; Sewell & Catterall, 1998). If miners communicate about the type of predator, then individuals should behave as if that type of predator is present,
and so flee or freeze to aerial alarm calls, but become vigilant, approach or call to chur calls. We expected that a long sequence of chur calls would prompt a longer or stronger response, as it implies a predator present for longer.

Playbacks were prepared from recordings of miner alarm calls prompted by models in the context experiment, or in supplementary presentations of the models (above). All 15 experimental sites received unique examples of aerial, chur and control calls, so the experiment was fully replicated. Aerial and chur calls were recorded at the experimental site at which they were used, and control calls recorded in Canberra. We chose high quality recordings with no distinct background sounds, and filtered at 0.75 kHz to remove sound below the frequency of all call types. Calls were standardized for duration by removing or repeating elements at the end of calls if necessary, and the long chur sequence was made of multiple calls with natural intervals. All calls were broadcast so that the loudest element had a mean element amplitude of 69 dB SPL at 10 m, the mean amplitude of chur elements at that distance and within the natural range of both alarm call types (mean ± SD: aerial 73.4 ± 3.3 dB, chur 69.3 ± 3.9 dB; Magrath & Bennett, 2012).

During experiments, calls were broadcast from a distance of about 10 m to the bird, using a Roland Edirol R-09 HR player, a custom amplifier and a Peerless 810921 tweeter speaker, all mounted on the observer’s waist. The focal bird’s response was videoed by a second person. Playbacks were only carried out after a 5-minute period during which there were no alarm calls heard or predators seen. During this 5-minute period, the observers followed an individual miner (the focal bird) that became the target of playback. Playbacks were then carried out when the bird was feeding (or occasionally preening) at a height of 2.5–8 m, and videos continued until the focal bird had resumed feeding for about 30 s or had left the area. Playbacks at a
given site were all carried out within 24 h, with a minimum of 15 min between
playbacks. A pilot project using only short alarm playbacks carried out in 2014 found
no obvious order effects with a minimum 5-minute interval between playbacks, so we
assumed that a 15-minute period would avoid substantial order effects, even with a
longchor sequence included. Like the model presentation experiment, which also had
four treatments, the order of treatments was randomized but within the constraint that
each playback type occurred approximately equally at all orders. We also tested for
effects of playback order on miner response, or carry-over effects, and found none
(Results).

The focal bird’s immediate response to playback was scored blind from video
recordings, while vocal responses were scored directly in the field. Examples of
videos are given in Supplementary Videos S1-S3. To enable blind scoring, Adobe
Premier Pro CC (2015) was used to remove sound from the file, and a visual marker
was added to mark the start of playback. The file was then given an arbitrary name by
someone uninvolved in the study, and then passed to the person (SC) who scored
responses by viewing footage at half-speed using VLC 2.2.1. It was possible to score
only the first 5 s from the start of playback on the video, because the birds often left
the camera frame after that period. Responses were scored as flee (fly from the
feeding site, but not towards the speaker), freeze (remain totally still), vigilance (stop
feeding and look around), approach (leave the feeding site and move towards the
speaker), or none (continue feeding or preening). The categories of response therefore
included avoidance (flee or freeze) and monitoring or harassment (vigilance and
approach, which are typical responses to mobbing calls in birds). After blind scoring
of behavioural responses, the complete video including audio track was checked to
confirm field scoring of aerial and chur calls. Aerial and chur call types are distinct 
(below), so categorization was unambiguous and always consistent with field scoring.

Statistical analyses

Analyses of experiments were based on their matched design, where each site 
received all treatments. We used McNemar tests, appropriate for paired dichotomous 
data (Agresti, 2007; Siegel & Castellan, 1988), to compare the probability of giving 
aerial versus chur calls to gliding versus stationary models. Paired t-tests were used to 
compare specific acoustic properties of calls prompted by raptor models in the 
matched ‘context of production’ experiment. Analyses of the playback experiment 
also entailed paired dichotomous response variables, so analyses were carried out 
using McNemar tests, when comparing two treatments, and Cochran Q tests, when 
comparing more than two treatments (Siegel & Castellan, 1988). We used exact rather 
than asymptotic probabilities in these tests and in contingency tests for carry-over 
effects (Fisher’s exact test and extensions; Field, 2013). Separate from statistical tests 
of hypotheses, that were comparisons within the planned experiment using a matched 
sampling design and randomized order, Principal Components Analysis (PCA) was 
carried out simply to visualize overall variation in acoustic properties of calls 
recorded in all contexts (Tabachnick & Fidell, 2007), including supplementary model 
presentations and serendipitous recordings of calls to natural predators. These PCA 
alyses including peak frequency, frequency bandwidth, duration and relative 
amplitude of the fundamental, because they measured attributes of calls that can vary 
independently. Low and high frequency were excluded because they were both 
strongly correlated with and are intrinsically related to peak frequency (which must lie 
between the two). Statistical tests and PCA were conducted in IBM SPSS Statistics
Ethical note

All work was approved by the Australian National University Ethics Committee, and was designed to minimize disturbance or stress to the birds. We used model predators to measure responses to different types of predator and to prompt birds to give alarm calls. Birds were therefore never exposed to real threat, and we minimized sample sizes by using matched experimental designs. Furthermore, experiments were distributed over 15 separate sites, and any one site received at most two model presentations in an experiment. Similarly, playbacks used a matched design and entailed only three alarm broadcasts at each of 15 sites. Overall, birds did display anti-predator behaviour to both models and playbacks, but individuals returned to apparently normal behaviour quickly, usually within a few minutes.

RESULTS

Context of production

Miners produced different alarm calls to different types of threats. Raptor gliders in flight prompted only aerial alarm calls in the 14 trials in which birds gave calls, whereas taxidermic models of perched raptors prompted only chur calls in the 7 trials in which birds gave any calls (Table 1a; McNemar test, alarm call type, n = 7 birds calling to both type of prompt, 2-tailed exact p = 0.016). The different call types were related to model behaviour, not whether the model was prepared from a taxidermic skin or artificial material, because all 13 birds that called to both the glider in flight and landed switched from giving aerial to chur calls after the model landed (Table 1a; McNemar, 2-tailed exact p = 0.00024). The calls were prompted by the
models themselves, and not by the way the models were presented: birds never called
to the action of throwing itself, and never when revealing an empty the cage (Table
1a). There was no affect of the order of model presentation on call type (McNemar, n
= 7 that called to both models; 2-tailed exact p = 0.45). Furthermore, if a bird called to
either model, it always gave an aerial call to the airborne model and a chur call to the
perched taxidermic model. There could be no carry-over effect on call type since it
was invariant according to the model, and we found no effect even on the probability
of giving a call. The probability was not affected by whether a model was preceded by
the other model or not (perched model, Fisher 2-tailed exact p = 0.31; glider model,
Fisher 2-tailed exact p = 1.0).

In addition to prompting different alarm calls, the different types of model
predator prompted different anti-predator responses. Raptor gliders in flight most
commonly caused birds to flee, whereas perched models most commonly provoked
attack, with the birds ‘dive-bombing’ the models (Table 1b; McNemar, 2-tailed exact
p = 0.0078; comparing fleeing and freezing versus attack, approach and vigilance).
Similarly, the miners changed from avoidance to attack after a gliding model hawk
landed (Table 1b; McNemar, n = 13 cases with a response at each stage, 2-tailed
exact p = 0.016). Similar to the vocal responses, neither the act of throwing nor
revealing an empty cage prompted any responses. There was also no effect of the
order of presentation of perched and gliding models on the type of response
(McNemar, avoidance versus attack, 2-tailed exact p = 1.0). Similarly we found no
carry-over effect; the response to the perched or gliding model did not differ
according to whether it was preceded by the other model or not (Fisher test, 2-tailed
exact p = 1.0 for each model).
Alarm call acoustic structure

Aerial and chur alarm calls were acoustically distinct, with no overlap in some acoustic measurements. The fundamental was shorter and of higher frequency by all measures in aerial compared to chur calls (Fig. 1; Table 2; data from experimental presentation of models). The fundamental was also much louder than the mean of second and third harmonics within aerial alarm calls, but similar or of lower amplitude in chur calls (delta amplitude means for gliding model hawk versus taxonomic raptor: 27.4 and -5.4 dB respectively; Table 2). Indeed, there was no overlap in measurements for delta amplitude or low, high or peak frequency for calls to gliding models hawks versus perched taxonomic raptors (Table 2).

Alarm calls given to different specific prompts, including from natural observations and supplemental recordings, grouped into either aerial or chur calls based on Principle Component Analysis of acoustic properties (Fig. 2). For example, chur calls given to a hawk glider after it had landed were similar to those given to stationary taxidermic mounts, and calls to natural predators grouped with aerial or chur calls depending on whether the predator was flying or stationary. The first two principal components explained over 90% of the variance: PC1 68.0% and PC2 23.2%. Aerial calls had higher PC1 values, which were associated with a higher peak frequency (component weighting 0.96), shorter duration (-0.82) and greater relative amplitude of the fundamental (0.95). Chur calls tended to have higher PC2 values, which were primarily associated with a greater bandwidth (0.87).

Response to alarm calls

The miners’ immediate responses to playback were appropriate to the type of playback, in the absence of any other cues about danger (Fig. 3). Blind scoring
revealed that each type of alarm-call playback prompted anti-predator behaviour in at least 14/15 cases (93%), yet did so in only two of 15 control playbacks (14%; Cochran Q, all treatments, response or not: Cochran’s Q, 2-tailed exact p < 0.001). We therefore restricted analyses of response to among alarm calls, for which we compared avoidance responses (flee or freeze) with mobbing responses (vigilance or approach). Miners always either fled or froze after playback of aerial alarm calls, yet all but two approached or became vigilant after playback of chur alarm calls of similar duration, with only one bird fleeing and another showing no response (McNemar, avoidance versus mobbing response: n = 14 with a response to both, 2-tailed exact p = 0.00024). The long chur playbacks prompted a very similar response to short chur calls, with 14 individuals showing a mobbing response, and one having no response (McNemar: long chur compared to aerial, 2-tailed exact p = 0.00012; long chur compared to short chur, 2-tailed exact p = 1.0). There was no effect of playback order on the type of response, as the responses to each playback type were almost invariant (Cochran Q, three playback types excluding the control, n = 14, 2-tailed exact p = 1.0). Given the almost invariant response to a given type of playback, there was also no evidence of a carry-over effect (contingency table with response to playbacks according to which alarm call type had preceded it or neither had preceded it: aerial, p = 1.0; short chur, p = 0.43; long chur, p = 0.53; all 2-tailed exact p values).

In addition to the different physical responses, miners had different vocal responses to the different types of playback. Birds never gave aerial alarm calls after any playback, yet they were more likely to give chur calls to chur call playbacks: none gave a chur call after the control playback, one after aerial playback, five after short chur call playbacks and 11 after long chur call playbacks (probability of giving a chur call among alarm call treatments: Cochran Q, exact p = 0.001). The difference was
only clear in a contrast of aerial versus long chur not short chur playbacks (McNemar 2-tailed exact p = 0.002 and 0.2 respectively). There was no affect of the order of playback on the probability of giving a chur call, either considering all four playback types (Cochran Q, n = 15, 2-tailed exact p = 0.93) or restricting to short and long chur playbacks (McNemar, n = 15, 2-tailed exact p = 0.74). Similarly, there were no carry-over effects; the probability of giving a chur call in response to playback did not depend which alarm call type, if any, had preceded it (contingency table, including chur or aerial or neither before: short chur, 2-tailed exact p = 1.0; long chur, 2-tailed exact p = 0.77; birds never called to aerial playbacks).

**DISCUSSION**

Noisy miner aerial and chur alarm calls meet both the production and perception criteria for functional reference. Acoustically distinct aerial calls were given specifically to airborne raptor models, while chur calls were given to perched raptor models and stationary glider models, with birds switching from aerial to chur calls when a gliding model raptor model landed. Furthermore, playback of these calls led to appropriate responses, in the absence of any contextual information. Noisy miners therefore join the small list of birds and mammals with experimental evidence for functionally referential communication about predators (reviews: Gill & Bierema, 2013; Suzuki, 2016; Townsend & Manser, 2013). Our results also support Greisser’s (2008) finding that these calls can categorize threats by current predator behaviour, not simply taxonomic differences, and so convey additional information relevant to predator avoidance.

The two alarm-call types were used consistently in our study regardless of the specific model or context, and consistent with previous descriptions of these calls.
Chur calls were given regardless of whether the prompt was a silent, taxidermic raptor, a taxidermic raptor accompanied by magpie-lark alarm calls, a gliding foam raptor after it had landed, or natural calls to live dogs or perched birds. Similarly, aerial alarms were given to gliding models or live, flying birds. These two call types are also consistent with previous spectrographic and quantitative descriptions of aerial and chur calls (Doohan, 2014; Holt, 2013; Jurisevic & Sanderson, 1994a, 1994b; Lowry et al., 2012; Magrath & Bennett, 2012; Wood, Sanderson, & Evans, 2000).

In addition to being acoustically distinct, aerial and chur alarm calls were given in specific experimental contexts, and accompanied by different anti-predator behaviour. Aerial alarm calls were given exclusively to the gliding model raptors, while only chur calls were given to the perched, taxidermic raptors, and to gliding raptors after landing. At the same time, airborne raptor models most commonly prompted birds to flee to cover, while stationary models most commonly provoked attack; the exceptions entailed birds become vigilant but not fleeing or attacking. The results from our experimental contexts are similar to previous reports on the context of production of aerial and chur calls. Aerial alarms have primarily followed airborne prompts, including model planes, and flying raptors and other large birds, while chur calls primarily follow stationary or terrestrial prompts including taxidermic cats, foxes and raptors, and live cats, dogs, humans, and large perched birds including raptors (Doohan, 2014; Holt, 2013; Jurisevic & Sanderson, 1994a, 1994b; Lowry et al., 2012; Wood et al., 2000). Overall, the context of alarm call production and associated anti-predator responses are related to predator behaviour and not predator type: airborne threats prompt aerial alarms and evasion, while those perched or landed prompt chur calls and attack.
Miners responded appropriately to playback of the different types of alarm call, which fulfills the second, perception criterion for functional reference (Evans, 1997; Macedonia & Evans, 1993). Playback of aerial calls prompted avoidance behaviour, while chur calls prompted birds to become vigilant or approach the speaker. These responses to playback were similar to their behaviour to the original prompts, and appropriate to the type of threat, showing that the calls themselves convey information about the type of threat. The responses were not identical to those to the original prompts, probably because there was no physical model that provided information on location of danger. For example, an even greater proportion of birds fled after playback of aerial calls than during presentation of the gliding model, which they sometimes just observed, perhaps because an unseen predator is more dangerous (Huang, Seiving, & St. Mary, 2012). Similarly, while birds became vigilant and approached the speaker during playback of chur calls, they never actually attacked, presumably because they could not see the threat. Given that viewing a predator directly will provide slightly different information from that conveyed by alarm calls, and different opportunities for anti-predator behaviour, the key issue for functional reference is that listeners should select ‘appropriate’ responses for the type of threat (Evans, 1997). Responses to playback will usually also be ‘similar’ to responses to the predator itself (Gill & Bierema, 2013), but not necessarily the same, as sometimes implied (Townsend & Manser, 2013).

Our results provide strong evidence of functional reference, by combining an experimental approach to both production and response specificity, along with acoustic analyses of calls and blind scoring of responses to playback. Observations of natural call production, including in miners, are important in establishing call context, but it can be difficult to get large sample sizes and to score the anti-predator
behaviour that accompanies calling (Grieves et al., 2014; Holt, 2013; Jurisevic & Sanderson, 1994a, 1994b; Manser et al., 2001; Zuberbühler, Cheney, & Seyfarth, 1999). Experimental prompting of alarm calls allows full control of context independent of location, comparable samples for acoustic analysis, and facilitates scoring of behavioural responses, which can then be compared to the responses to playback alone (e.g. Evans et al., 1993). Functional reference implies that individuals respond to calls as if the prompt was present (Evans, 1997; Macedonia & Evans, 1993), so it is desirable to directly compare anti-predator behaviour in the two situations. Blind scoring of response to playback is desirable, if possible, because it reduces the risk that scoring could be influenced by expectation (for another example of blind-scoring of response to referential alarm calls, see Grieves et al., 2014). In practice blind scoring is often difficult in studies of wild animals, but this may not be a problem in the literature on referential alarm calls. This is because responses to different types of predators and alarm calls are often distinct and unambiguous, such as approach or flee, and so easy to score in the field.

Our study supports Greisser’s (2008) conclusion that referential alarm calls in birds, and perhaps mammals, may often refer to predator behaviour and not just taxonomic categories. Specifically, avian ‘flee’ and ‘mobbing’ calls may often refer to ‘airborne’ versus ‘non-airborne’ predators, rather than specifically to aerial and terrestrial predators, as is often stated (e.g. Evans et al., 1993; Grieves et al., 2014). This being the case, it would be interesting to assess whether alarm calls can simultaneously convey information about both predator type and behaviour, as is the case for predator type and degree of danger in meerkats (Manser, 2001; Manser et al., 2001; Manser, Seyfarth, & Cheney, 2002) and some birds (e.g. Fallow & Magrath, 2010; Leavesley & Magrath, 2005; Sieving et al., 2010; Templeton et al., 2005).
Combining call types may prove a flexible method of encoding subtle information about different threats, as is the case in, for example, tufted titmice *Baeolophus bicolor* (Sieving et al., 2010) and Japanese great tits *Parus minor* (Suzuki, Wheatcroft, & Griesser, 2016).

Despite the long history of interest and research, there is ample scope for further studies of functional reference in animal communication, and alarm calls provide an excellent model because different threats predict different adaptive responses. First, we need further research on how contextual information could influence the response to alarm calls. Even if calls convey information about predator type, it does not mean that individuals ignore other sources of information to refine responses (Evans, 1997), and recent research on vervet monkeys implies that context is important. Although vervets produce different alarm calls to different predators, these calls are not always acoustically distinct from other calls in the repertoire, suggesting that contextual information is important in a listener’s response (Price et al., 2015). For example, ‘rraup’ calls are given to eagles but also during within-group aggression, so that monkeys might use the absence of fighting within the group to judge that a ‘rraup’ call warns of an eagle. Similarly, group size and caller distance might both affect the response to playback of different vervet alarm calls.

Ducheminsky, Henzi, & Barrett (2014) found no clear evidence that different alarm calls prompted appropriate responses. The authors suggest that the large groups at their study site may mean more false alarms, so that individuals respond by seeking more information rather than taking immediate action. Larger, dispersed groups also meant that playbacks were from greater distances, perhaps implying lower risk and again prompting individuals to seek further information. Clearly, even this classic example of referential communication will repay further study. Second, although the
term ‘functional reference’ was coined in part to avoid inference about cognition, it would nonetheless be interesting to assess cognitive processes underpinning communication about predator categories (Suzuki, 2016). For example, at one extreme alarm calls might prompt appropriate responses merely because of call acoustic properties (Rendall et al., 2009), while at the other extreme individuals might associate call types with specific predators and respond appropriately according to the predator, not the type of sound (Seyfarth et al., 2010; Zuberbühler et al., 1999).

Referential alarm calls provide an excellent model to address animal cognition because of the clear differences in response to playback of different calls.

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Supplementary Material
Supplementary material associated with this article can be found, in the online version, at ….
References


Table 1

The number of noisy miner (a) alarm call responses, and (b) non-vocal responses, according to the model presentation treatments.

<table>
<thead>
<tr>
<th>Call type</th>
<th>Gliding hawk airborne</th>
<th>Gliding hawk landed</th>
<th>Gliding control</th>
<th>Perched model</th>
<th>Perched control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerial</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chur</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>No call</td>
<td>1</td>
<td>15</td>
<td>1</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td><strong>(b) Behaviour</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flee</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Freeze</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vigilance</td>
<td>6</td>
<td>0</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Attack</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>No response</td>
<td>0</td>
<td>15</td>
<td>2</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>
Table 2
Acoustic differences between noisy miner aerial alarm calls, given to gliding hawk models in the air, and chur calls, given to landed gliding models and perched taxidermic raptor models. See the text for explanation of measurements. Values show means ± SD and range for all calls recorded; statistical analyses show paired t-tests with 2-tailed probability estimates for paired data.

<table>
<thead>
<tr>
<th>Model type</th>
<th>Low frequency 5% (Hz)</th>
<th>Low frequency 95% (Hz)</th>
<th>Peak frequency (Hz)</th>
<th>Band width 90% (Hz)</th>
<th>Duration 90% (s)</th>
<th>Delta amplitude (dB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glider airborne (n = 14)</td>
<td>2805.6 ± 199.0</td>
<td>3359.2 ± 236.5</td>
<td>3168.5 ± 229.6</td>
<td>553.7 ± 199.0</td>
<td>0.083 ± 0.021</td>
<td>27.44 ± 4.55</td>
</tr>
<tr>
<td></td>
<td>2497.9–3273.0</td>
<td>2756.2–3703.7</td>
<td>2584.0–3445.3</td>
<td>258.4–861.3</td>
<td>0.053–0.127</td>
<td>18.95–36.25</td>
</tr>
<tr>
<td>Glider landed (n = 14)</td>
<td>1381.2 ± 340.5</td>
<td>1855.9 ± 358.5</td>
<td>1756.5 ± 364.7</td>
<td>473.7 ± 240.7</td>
<td>0.156 ± 0.026</td>
<td>-3.89 ± 2.46</td>
</tr>
<tr>
<td></td>
<td>947.5–2411.7</td>
<td>1550.4–2928.5</td>
<td>1378.1–2842.4</td>
<td>172.3–1033.6</td>
<td>0.115–0.185</td>
<td>-8.50–1.00</td>
</tr>
<tr>
<td>Perched model (n = 7)</td>
<td>1328.9 ± 109.6</td>
<td>1710.4 ± 168.1</td>
<td>1624.2 ± 168.1</td>
<td>381.5 ± 130.2</td>
<td>0.131 ± 0.030</td>
<td>-5.42 ± 2.23</td>
</tr>
<tr>
<td></td>
<td>1205.9–1464.3</td>
<td>1550.4–1981.1</td>
<td>1464.3–1894.9</td>
<td>172.3–516.8</td>
<td>0.100–0.184</td>
<td>-8.10–2.25</td>
</tr>
</tbody>
</table>

Paired t-tests:
- Glider airborne versus landed:
  - Frequency 5%: t = 12.43, p < 0.001
  - Frequency 95%: t = 12.91, p < 0.001
  - Peak frequency: t = 13.42, p < 0.001
  - Band width: t = 1.51, p = 0.16
  - Duration: t = 9.75, P < 0.001
  - Delta amplitude: t = 34.74, P < 0.001

1. Paired differences followed normal distributions for all variables (Shapiro-Wilk, p > 0.05).
Figure 1. Spectrograms of noisy miner (A) aerial alarm calls, and (B) chur alarm calls. Spectrograms were prepared in Raven Pro 1.4, using a Hann window function, size 10.2 ms, with a 3dB filter bandwidth of 141 Hz. Selections boxes were drawn over the first three harmonics (1, 2 and 3) as shown. Automated measures of frequency, duration and amplitude were then made for the first harmonic, and amplitude alone from second and third harmonics. See text for further details of measurements.
Figure 2. Principal Components plot of the acoustic properties of noisy miner aerial alarm calls and chur calls given to different prompts, showing that these calls are acoustically discrete. The acoustic properties of aerial alarm calls given to the airborne model hawk gliders or to natural flying predators group together, as do the chur calls given to taxidermic perched raptors with or without accompanying playback of magpie-lark ‘rapid’ alarm calls, landed glider hawk models, and perched real predators.
Figure 3. Noisy miner responses to playback of alarm and control calls. The y-axis shows the number of individuals (out of 15) having that immediate response. Aerial alarm calls and short chur call playbacks were 2 s in duration, while long chur calls were 15 s in length. The control call was a 2 s contact call of a harmless parrot.