SHORT COMMUNICATION

Rhodamine-B-marked eggs identify individual predators of artificial nests

Chris Jones
Landcare Research, PO Box 282, Alexandra, New Zealand
Current address: Landcare Research, PO Box 40, Lincoln 7640, New Zealand
(Email: jonesc@landcareresearch.co.nz)

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Abstract: Investigations of nest predation are often limited by the researchers' inability to identify nest predators accurately. I tested a chemical bait marker, Rhodamine B (RB), as an indicator of egg predation at artificial ground nests. In a pen trial, the presence of characteristic fluorescent bands in one or more facial vibrissae from all treatment animals confirmed the suitability of RB as a bait marker in the introduced European hedgehog (*Erinaceus europaeus*). In a field trial in which artificial ground nests were baited with RB-dosed eggs, five of 21 trapped hedgehogs showed evidence of RB ingestion. One animal showed markings indicating two temporally separate predation events. This ability to identify nest predators to species, demographic class, or individual level could lead to more focused control programmes. Other potential uses of this technique include investigation of individual foraging behaviour, calibration of predation rates in artificial nest studies, estimation of the efficacy of poisoned eggs as a control method, and testing for bait or poison uptake by non-target species.

Keywords: bait marker; *Erinaceus europaeus*; hedgehog; nest predation; pest control

Introduction

Predation of eggs and chicks is one of the main causes of nest failure for many bird species (Ricklefs 1969; Martin 1992). However, researchers are often unable to identify the predator species involved or to estimate their relative contributions to rates of egg loss (Moore & Robinson 2004; Villard & Pärt 2004). Within species, some individual predators may prey disproportionately on a prey type, either because the prey happen to live within the individual's territory or home range (e.g. coyotes *Canis latrans* in Sacks et al. (1999)) or because the predator's gender or reproductive status influence prey selection (e.g. female mice *Mus musculus* in Miller & Webb (2001)). On river braids in the upper Waitaki Basin, Sanders and Maloney (2002) video-recorded repeated visits to ground nests by predators of the same species, but were unable to determine if these were multiple individuals or the same few animals returning to the same nests. Where nest predation is primarily due to introduced pest species, as is the case in Australia, New Zealand, and many island ecosystems worldwide, effective control of these predators may be hindered by this lack of information and may lead to expensive errors in conservation management (Larivière 1999; Sanders & Maloney 2002).

Nest predators have been identified from characteristic signs (Moors 1983), measurements of the size and spacing of tooth holes in shell fragments (Green et al. 1987), use of automatically triggered still cameras or time-lapse video (Major 1991; Brown et al. 1998; Sanders & Maloney 2002), and indirect methods such as the use of hair-sampling devices at nests, and plasticine eggs on which bite-marks can be identified (Pasitschniak-Arts & Messier 1995). Some methods, such as interpretation of characteristic sign, are unreliable (Brown et al. 1998; Marini & Melo 1998; Williams & Bohall-Wood 2002) and others, such as video monitoring, may be reliable but their use is constrained by expense or logistical requirements (Keedwell & Sanders 2002; Sanders & Maloney 2002; Thompson & Burhans 2004).

A potential alternative technique is the use of bait marker chemicals that, when applied to eggs and subsequently ingested by a predator, leave a characteristic physiological sign that can be detected at a later date. Such markers have been used to study animal movements, bait acceptance, and exposure of non-target species to control methods (reviewed by Savarie et al. 1992). Maier and DeGraaf (2000) used photographic evidence of visits by nest predators in combination with the time of appearance of the

Rhodamine-B-dyed contents of eggs to differentiate between disturbance and predation at artificial nests. My study aimed to build on these findings by testing the technique's ability to identify individual nest predators and to record repeated predation events by the same individuals. The study was in two stages: firstly, a pen trial of the suitability of the dye, Rhodamine B (RB), for marking European hedgehogs (*Erinaceus europaeus*), which are the only one of the local suite of introduced mammalian nest predators in which RB has not been tested (Ogilvie & Eason 1998; Fisher et al. 1999; Spurr 2002). A subsequent field trial using artificial ground nests with RB-dosed eggs showed marker consumption by hedgehogs that confirmed nest predation.

Rhodamine B is a non-toxic xanthene dye that is an effective biological marker for a range of mammal, bird, insect, and fish species (reviewed in Fisher 1998, 1999). It is incorporated systemically into actively growing keratinous tissues such as claws, hair, and feathers, forming fluorescent bands that are detectable with an ultraviolet light source or, more reliably, using fluorescence microscopy (Johns & Pan 1981; Lindsey 1983; Fisher 1995). The most suitable structures to test for RB marking may be mystacial vibrissae (whiskers) because their resting phase is relatively short and vibrissae are therefore more likely to be actively growing at any one time compared with other hairs (Fisher 1998). Rhodamine-B bands are effectively permanent for the life of the hair and 'travel' up from the base of a structure as it grows. This property suggests that RB can be used in pulsed trials where temporally spaced bait consumption may give rise to a series of fluorescent bands, each corresponding to a single ingestion of the marker. This would allow detection of repeated predation by an individual predator.

Methods

Pen trial

Twelve wild-caught adult hedgehogs (6 males, 6 females) were housed indoors in cages (660 × 250 × 290 mm) and supplied with dried cat food, water, and nest material. This sample size was predicted to be adequate to detect marking in 90% of treated animals versus <1% of untreated, with power = 0.80 and alpha = 0.05. Animals were weighed and visually assessed weekly. After 10 days' acclimatisation to captivity, all hedgehogs were supplied with a cracked egg of a domestic fowl (*Gallus gallus*) instead of their normal food. The eggs given to four males and five females had been injected with 25 mg of RB (0.045% by weight, based on guidelines in Fisher (1998)). This was repeated 11 days later. The 25-mg dose of RB represented a mean dose per individual of 43.4 (± 5.0, 95% CI) mg kg body mass^{-1} at the first dose and 44.3 (± 4.7) mg kg^{-1} at the second dose. Note was taken of when the eggs were consumed. All animals were humanely killed by a veterinary surgeon 21 days after receiving the second egg. Vibrissae were removed (mean number per animal = 12, range 10–13) and were examined under fluorescence microscopy by an experienced technician.

The numbers of vibrissae per animal that would need to be examined to be confident of detecting either any band or two bands were estimated using equation 1:

$$n = \frac{\log_e \alpha}{\log_e (1 - p)},$$

where n = number of vibrissae required to detect the stated number of bands with confidence level α when p is the proportion of vibrissae marked (Spurr 2002). Upper and lower limits were estimated using the 95% confidence intervals around the mean proportions found to be marked per dosed animal.

Field trial

I constructed a pseudo-colony of 20 artificial ground nests on the braided riverbed of the Ohau River, central South Island, New Zealand (44º20.0'S, 170º10.5'E) in October 2003. The habitat consisted of dry river gravels, small boulders, and silt, sparsely vegetated with mats of low vegetation, including *Raoulia* spp. and *Scleranthus uniflorus*. The 0.6-ha colony was sited 150 m from the margin of river gravels and scrub habitat. Nests were distributed randomly within the area at a density of 0.33 per 100 m^2, which is similar to the local natural density of black-fronted tern nests (0.4 ± 0.7 per 100 m^2) estimated by Keedwell (2005). Nests closely resembled natural ground nests of local wader species, each consisting of a shallow depression in the gravels containing three domestic hen-eggs injected with 25 mg of RB in solution. Eggs were placed in contact with each other, so that any disturbance could be detected, and one egg was cracked to provide an olfactory cue to their presence and allow access to egg contents to predators unable to penetrate intact hen-eggs. While there were clear differences between these nests and real ones, the primary objective of this trial was to investigate the potential of the technique rather than to quantify predation rates per se. There were no physical markers left to indicate nest locations and all human activity within the colony was carried out while wearing rubber gloves.

Eggs were left in place for four periods of four consecutive nights, each separated by 14 nights. Eggs were checked daily and any remaining eggs were removed at the end of each 4-night 'pulse'. One month after the eggs were finally removed, a trapping programme was used to sample the local predator

population. Kill-traps (two Mark VI Fenn traps per set under a plastic Philproof cover) targeted at introduced mustelids and hedgehogs were set for 12 nights in scrub habitat within 20 m of the boundary with the river gravels and also around the perimeter of the colony. Any mammals trapped were identified to species, weighed, sexed, and a sample of at least 13 vibrissae removed by plucking.

Results

Pen trial

All eggs were eaten by both control and treatment hedgehogs within 48 hours of presentation. No fluorescent bands were found in any vibrissae from the three control animals. At least one band was found in at least one vibrissa from all nine dosed hedgehogs. The mean percent of marked vibrissae from each treated individual was 32% (95% CIs 21–43%): 10% (3–18%) showed one band, and a further 22% (13–31%) had two bands. Using the equation above it can be estimated that eight (range 5–13) vibrissae would need to be sampled to be 95% certain of detecting a fluorescent band. To detect two bands with the same level of confidence 12 (8–22) vibrissae would be required. Mean growth rate of the 24 vibrissae showing two bands was estimated from the distance between the bands to be 0.16 ± 0.02 mm day^{-1}.

Field trial

There were 11 instances of predation at the 20 artificial nests and a further seven instances of eggs being disturbed, but not eaten. Most predation (67% of egg losses) took place in the first period of availability, during which eggs were eaten at four separate nests in the same night. In the third exposure period, one nest was raided on the first and third nights. Eggs were taken at least once from nine of the 20 nests.

Only hedgehogs were caught in the kill-traps, at a rate of 4.83 captures per 100 corrected trap-nights (Nelson & Clark 1973). Two (both male) were trapped on the river gravels near the colony and 19 (9 males, 10 females) in the scrub trap-line. Five hedgehogs (24%; 3 males, 2 females) showed fluorescent vibrissal bands indicative of RB ingestion. Vibrissae from one female had two fluorescent bands corresponding to two temporally separate predation events. Within- and between-individual variation in the distances of bands from vibrissal roots meant it was not possible to associate a fluorescent band with a particular predation event.

Discussion

The pen trial confirmed the suitability of RB as a bait marker in hedgehogs, as in other introduced mammalian predators in Australasian ecosystems (Ogilvie & Eason 1998; Fisher et al. 1999; Spurr 2002; Marks et al. 2003). The field trial showed that this technique can be used to identify individual nest predators. In the only other published account of the use of biomarkers in a study of individual predatory behaviour, Windberg et al. (1997) were able to estimate the proportion of a coyote population feeding on a flock of domestic goats (*Capra hircus*) that had been injected with the marker iophenoxic acid.

In the pen trial, the percentage of vibrissae from treated hedgehogs with bands (32%) was significantly less than Spurr's (2002) estimate (56%) for stoats (*Mustela erminea*) ($\chi^2 = 11.59$, d.f. = 1, $P < 0.001$). This could reflect a difference in the proportion of vibrissae growing at any one time or a difference in vibrissal growth rates between the two species. Spurr (2002) noted that markings were less likely to be detected in the first 2 weeks after dosing than later on. Sampling of hedgehog vibrissae at around 3 weeks after the second dose of RB may have given insufficient time for the bands to grow out and to be clearly distinguished from the basal bulbs of the vibrissae. It is unlikely this interspecific difference is related to dose rate (dosed hedgehogs received between 43 and 44 mg kg^{-1} body mass compared with the 62–108 mg kg^{-1} of Spurr's stoats) because there was no significant correlation between the proportion of vibrissae showing bands and the dose received by individual hedgehogs. Although hedgehogs received smaller doses than stoats, this dose was still greater than the 24 mg kg^{-1} that Jacob et al. (2002) concluded was sufficient to cause banding in mouse (*Mus domesticus*) vibrissae and the 15–30 mg kg^{-1} that caused band formation in coyote hairs (Johns & Pan 1981).

Knowledge of the growth rate of vibrissae allows the persistence of markings to be predicted. The mean vibrissal length in this study was 19 (0.7 SE) mm, so, at a mean growth rate of 0.16 ± 0.02 mm day^{-1} an RB band could conceivably persist for 119 days before being lost through natural degradation of the vibrissa tip. Sampling would therefore have to take place within this period. The longest potential gap between egg predation and kill-trapping in my field trial was 81 days. Markings persisted for up to 7 weeks in mouse vibrissae (Jacob et al. 2002) and in stoats were detectable up to 4–6 weeks after baiting and were found in vibrissae of kill-trapped wild individuals 27 days after the last possible consumption from bait stations (Spurr 2002; Purdey et al. 2003).

Although bands could not be assigned to individual predation events because of the marked variation in

the distance of these bands from the vibrissae bases, repeated egg predation by a single hedgehog was detected by 'pulsing' the availability of marked eggs. This ability to detect individual 'repeat offenders' or demographic groups causing disproportionately high rates of predation, could lead to targeted management programmes with greater focus than the species-based rationales currently in use. Evidence of such behaviour has been shown or inferred in a range of mammalian species including feral pigs (*Sus scrofa*), stoats, mice, and hedgehogs (Pavlov & Hone 1982; Ratz et al. 1999; Miller & Webb 2001; Jones et al. 2005).

Rhodamine-B-marked eggs could also be used to estimate the efficacy of poisoned eggs for pest predator control. This technique could refine estimated poison consumption rates in two ways: first, by testing the assumption that egg consumption is proportional to predator abundance, and second, by testing for uptake in non-target species.

Use of biomarked eggs in artificial nests and real nests, as additional or replacement eggs, could be useful in calibrating the relative rates of predation. Artificial nests often show different predation rates to real nests due to differential susceptibility to subsets of the local predator guild (Burke et al. 2004; Moore & Robinson 2004). This has led to the suggestion that artificial-nest-based studies are inherently unreliable (Zanette 2002; Burke et al. 2004), whereas other authors have argued that a priori identification of predators and their relative impacts on both real and artificial nests, i.e. calibration of study methods, can lead to robust conclusions (Moore & Robinson 2004; Villard & Pärt 2004). Studies that account for these relative impacts have shown clear correlations between impacts at real and artificial nests (Pärt & Wretenberg 2002; Roos 2002). Eggs injected with RB can potentially be more attractive to some predators by providing enhanced visual and olfactory cues to their presence because of leakage of dyed contents and accelerated decay rates respectively. In spite of this potential, Maier and DeGraaf (2000) found no difference in predation rates between eggs injected with RB and a reference sample.

Most methods of identifying nest predators have drawbacks, either in the reliability of results (e.g. sign-based or indirect methods) or in their cost, which limits sample size and, accordingly, inference (Marini & Melo 1998; Larivière 1999; Keedwell & Sanders 2002; Thompson & Burhans 2004). The main disadvantage of the method proposed here is that a potential predator must first be trapped before testing for signs of the marker. Trapping programmes are a common form of pest predator control in New Zealand and Australia and are often used to study predator behaviour worldwide. If kill-trapping is inappropriate for management objectives, vibrissae may be obtained from live-trapped animals (Fisher 1998). The technique suggested here is also limited in its applicability to real nests, although this could be overcome by integrating it with, for example, video monitoring, or by exploring the use of dosed eggs added to real clutches. The ability to identify nest predators to species, demographic class, or individual level could lead to more focused control programmes and may also allow assessment of the external validity of studies involving the use of artificial nests.

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