

SECONDARY ANTICOAGULANT RODENTICIDE EXPOSURE IN MIGRATING JUVENILE RED-TAILED HAWKS (*BUTEO JAMAICENSIS*) IN RELATIONSHIP TO BODY CONDITION

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ABSTRACT.—Secondary exposure to anticoagulant rodenticides (ARs) through consumption of contaminated prey has been documented worldwide in many non-target species, especially raptors. The Red-tailed Hawk (*Buteo jamaicensis*), a raptor that frequents agricultural areas and eats primarily rodents, is particularly susceptible. Because there is documentation of Red-tailed Hawk exposure to ARs in California, this study aims to describe the extent to which migrating juvenile Red-tailed Hawks are exposed to ARs, as well as any sublethal effects of AR ingestion. We collected blood samples and body morphometrics from 97 juvenile Red-tailed Hawks migrating through the Marin Headlands, Marin County, CA, from August to December in 2013 and 2015, and screened samples for the presence of ARs. Eight hawks (8.2%) tested positive for some amount of ARs. We detected first-generation (diphacinone, chlorophacinone) and second-generation (brodifacoum, bromadiolone) ARs. Although some juvenile Red-tailed Hawks are exposed to ARs either along their migration route or as resident birds in the Marin Headlands, we did not find any relationship between body condition and presence of ARs. Although this method of AR sampling of live birds is novel and increases our sampling capabilities, the short half-lives of ARs in blood make it difficult to estimate population-wide exposure rates. Future studies should focus on resident raptors near agricultural areas where AR exposure can be tested over time to better understand how this technique can be used to estimate exposure rates across whole populations.

KEY WORDS: *Red-tailed Hawk*; *Buteo jamaicensis*; *anticoagulant rodenticides*; *California*; *migration*; *secondary exposure*.

EXPOSICIÓN SECUNDARIA A RODENTICIDAS ANTICOAGULANTES EN INDIVIDUOS JUVENILES MIGRATORIOS DE *BUTEO JAMAICENSIS* EN RELACIÓN A LA CONDICIÓN CORPORAL

RESUMEN.—La exposición secundaria a rodenticidas anticoagulantes (RA) a través del consumo de presas contaminadas ha sido documentada en todo el mundo en muchas especies, especialmente en las rapaces. *Buteo jamaicensis*, una rapaz que frecuenta áreas agrícolas y se alimenta principalmente de roedores, es particularmente susceptible a los RA. Debido a que existe documentación de la exposición de *B. jamaicensis* a RAs en California, el objetivo de este estudio fue describir la magnitud a la que individuos juveniles migratorios de *B. jamaicensis* fueron expuestos a RA, así como cualquier efecto subletal de la ingesta de RA. Tomamos muestras de sangre y medidas morfométricas en 97 individuos juveniles de *B. jamaicensis* migrando a través de Marin Headlands, condado de Marin, California, de agosto a diciembre en 2013 y 2015, y analizamos muestras en busca de la presencia de RA. Ocho individuos (8.2%) dieron positivo para alguna cantidad de RA. Detectamos RA de primera generación (difacinona, clorofacinona) y de segunda generación (brodifacoum, bromadiolona). Aunque algunos individuos juveniles de *B. jamaicensis* estuvieron expuestos a RA, tanto a lo largo de su ruta migratoria o como para las aves residentes en Marin Headlands, no

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encontramos ninguna relación entre la condición corporal y la presencia de RA. Aunque este método de muestreo de RA en aves vivas es novedoso e incrementa nuestra capacidad de muestreo, las vidas medias cortas de los RA en la sangre, torna difícil la estimación de las tasas de exposición a nivel de población. Los estudios futuros se deberían enfocar en rapaces residentes en cercanías de áreas agrícolas donde la exposición a RA puede ser evaluada a lo largo del tiempo, para comprender mejor cómo esta técnica puede ser utilizada para estimar las tasas de exposición a través de poblaciones completas.

[Traducción del equipo editorial]

Anticoagulant rodenticides (ARs) are the most common method for controlling rodent pest populations worldwide (Hadler and Buckle 1992). Within the United States, approximately 30 million pounds of rodenticides and other conventional pesticides are used in agricultural, suburban, and urban settings each year (Grube 2011). Although ARs are widely used, their usage poses a potential threat to non-target wildlife though the consumption of prey already exposed to ARs (Stone et al. 1999). ARs act by inhibiting the normal synthesis of vitamin k-dependent clotting factors in the liver, thus delaying clotting time, which leads to hemorrhaging (Hadler and Shadbolt 1975). Secondary AR poisoning has been documented in many taxa including mammals such as bobcats (*Lynx rufus*; Riley et al. 2007) and fishers (*Martes pennanti*; Gabriel et al. 2012) as well as birds (Herring et al. 2017). As cases of secondary poisoning have become more prevalent and publicized, some regulatory steps have been taken to limit the use of ARs by general consumers, although many ARs are still available for use by licensed pesticide applicators (Bradbury 2008, California Department of Pesticide Regulation 2013).

ARs come in two forms that vary in toxicity; first generation (FGAR) and second generation (SGAR). SGARs are designed to kill after a single feeding, are more likely to bioaccumulate in the body, and are acutely toxic at lower concentrations than FGARs, so SGARs are far more likely to cause secondary poisoning in non-target organisms (Rattner et al. 2014). Predatory animals such as raptors and carnivorous mammals tend to have the highest prevalence of non-target AR exposure due to bioaccumulation from prey. This is particularly true among predators whose diet includes a large proportion of small mammals, because contaminated rodents partially incapacitated by ARs may be easily captured and consumed (Sanchez-Barbudo et al. 2012). Recent evidence has even suggested that AR use can result in ecological traps, as seen in some regions where raptors may be preferentially hunting

in AR-treated areas where prey are easier to catch (Vyas et al. 2017).

Several studies have documented the extent to which wild raptor populations have been exposed to ARs. Elliott et al. (2016) summarized results of 14 studies in North America and Europe in which various raptors were tested for the presence of ARs. In these studies, an average of 63% of all raptors tested positive for at least one type of SGAR. Similarly, Murray (2011) found that of 161 raptors sampled in Massachusetts, 86% were positive for ARs. Also, a study conducted in the Canary Islands found that 61% of sampled raptors tested positive for at least one type of AR (Ruiz-Suárez et al. 2014). In addition to effects seen in individual raptors, secondary poisoning also has the potential for population-level effects. For example, Thomas et al. (2011) estimated that a minimum of 11% of the sampled Great Horned Owl (*Bubo virginianus*) population in Canada is at risk of being directly killed by SGARs, although exposure levels were based on areas of high AR use and may not accurately represent risk across all of Canada.

In addition to acute poisoning leading directly to death, ARs may also lower survival of exposed animals through sublethal effects. For example, Rattner et al. (2015) exposed a captive colony of American Kestrels (*Falco sparverius*) to different doses of the FGAR chlorophacinone and concluded that sublethal responses to AR exposure, such as internal hemorrhaging and impaired clotting, could compromise the survival of wild raptors. Additionally, a negative correlation was found between AR concentration and body condition in stoats and weasels (*Mustela erminea*, *M. nivalis*; Elmeros et al. 2011). Other symptoms of AR poisoning such as lethargy and blood loss from superficial wounds may lead to impaired hunting ability and decreased ability to recover from non-fatal collisions, but a causative effect between these symptoms and AR exposure has yet to be found (Rattner et al. 2014).

The Red-tailed Hawk (*Buteo jamaicensis*) is a widespread, diurnal raptor that may be particularly

Table 1. Summary of the positive results of the anticoagulant rodenticide screen for Red-tailed Hawks captured in the Marin Headlands, California, during fall migration in 2013 ($n=20$) and 2015 ($n=77$). Trace indicates a level of rodenticide below quantifiable limits.

YEAR	AMOUNT	ANTICOAGULANT RODENTICIDE
2013	Trace	Chlorophacinone
	Positive	Diphacinone
	Trace	Diphacinone
	Trace	Chlorophacinone
	Trace	Chlorophacinone
2015	Trace	Diphacinone
	Positive	Diphacinone
	Trace	Diphacinone, Brodifacoum, Bromadiolone

susceptible to secondary poisoning. Red-tailed Hawks primarily feed on small mammals, and often frequent agricultural areas where AR application may occur (Preston and Beane 2009). Although resident Red-tailed Hawks have been documented with AR poisoning in wildlife rehabilitation facilities (Murray and Tseng 2008), no studies of AR exposure in migrating Red-tailed Hawks have yet been conducted. This study aimed to document the occurrence of AR exposure in juvenile Red-tailed Hawks migrating through the Marin Headlands, CA, and investigate relationships between AR exposure and body condition.

METHODS

Situated along the Pacific flyway near Sausalito, CA, USA, the Marin Headlands are home to the largest known hawk migration along the west coast of North America. Here, hawks are funneled by the San Francisco Bay and Pacific Ocean to the tip of the Marin peninsula on their way to overwintering sites. We collected blood samples from 97 migrating juvenile Red-tailed Hawks from mid-August to mid-December in 2013 ($n=20$) and 2015 ($n=77$). Only juvenile hawks were sampled because the vast majority of migrating hawks trapped in the Marin Headlands each year are juvenile. We used a combination of mist nets, dho-ghazas, and bow nets to trap hawks at four banding sites, and we fit hawks with US Geological Survey leg bands (Bloom 1987, Hull et al. 2013). Next, we collected a combination of morphometric and observational data including age, weight, and wing chord, and we determined sex for each bird following Pitzer et al. (2008). We then collected a maximum of 2 ml of whole blood from

the medial metatarsal vein of each hawk, and we separated plasma from red blood cells by spinning samples for 10 min at 10,000 rpm in a centrifuge. We stored the samples at -20°C until testing. Next, we sent the samples to the California Animal Health and Food Safety Laboratory in Davis, CA, where they were screened for ARs using high-performance liquid chromatography following methods described in Serieys et al. (2015). Specifically, we tested the samples for the presence of FGARs chlorophacinone, coumachlor, diphacinone, and warfarin, and SGARs brodifacoum, bromadiolone, difethialone and difenacoum. Reporting limits ranged from 1.0 to 7.0 ppb, with smaller samples resulting in higher reporting limits. We labeled detectable compound concentrations that were below quantifiable limits as “trace” concentrations. We used a general linear model to estimate a relative body condition index. We regressed wing chord and weight and then used the residuals as our condition index. We then used a nonparametric Wilcoxon rank sum test to examine differences in exposed and unexposed individuals.

RESULTS

Of the 97 hawks sampled, eight hawks (8.2%) tested positive for some amount of AR exposure (Table 1). Of the hawks sampled in 2013, five of 25 hawks (25%) tested positive for ARs compared to three of 77 hawks (3.9%) tested in 2015. One hawk in 2013 and one in 2015 tested positive for a measurable amount of AR, while the other six hawks tested positive for only trace amounts. ARs detected were chlorophacinone, diphacinone, brodifacoum, and bromadiolone, although brodifacoum and bromadiolone (both SGARs) were only detected in 2015. Additionally, one hawk in 2015 tested positive for trace amounts of three ARs, diphacinone, bromadiolone, and brodifacoum. There was no relationship between the relative body condition index and presence of ARs (Wilcoxon rank sum test; $P=0.30$, Fig. 1).

DISCUSSION

We found that 8.2% of hawks tested positive for the presence of ARs, initially suggesting a low rate of short-term AR exposure in Red-tailed Hawks migrating through the Marin Headlands. This population of migrating Red-tailed hawk reflects breeding populations in central California and the Intermountain West (Hull et al. 2008). An 8.2% exposure rate differs from those found in other studies testing

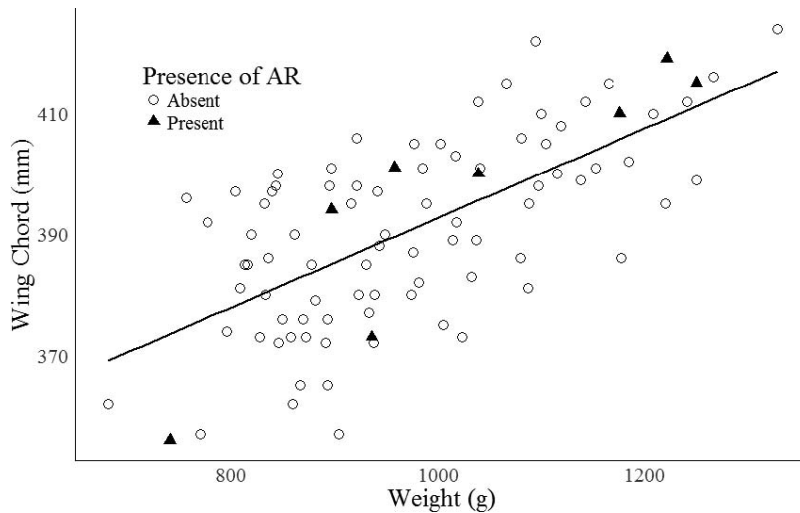


Figure 1. Relative body condition index as measured using the residuals of a regression of weight and wing chord of juvenile Red-tailed Hawks sampled on migration in the Marin Headlands, California. Black triangles indicate AR-positive individuals. Open circles indicate AR-negative individuals. The two groups did not differ in body condition ($P > 0.3$).

AR exposure in raptors from liver samples where reported exposure rates ranged from 19% to 100%, with an average exposure rate of 63% (Elliot et al. 2016). This difference in exposure rates may be due to a number of factors including both discrepancies in our sampling protocol compared to previous studies, and the behavior of migratory versus resident birds. Our results also bring into question whether or not blood sampling can give a true estimation of AR exposure rates or whether this method can only be used to infer short-term exposure.

The variation in exposure rates compared to other studies may be due to aspects of our sampling protocol causing an underestimate of the true rate of chronic and acute AR exposure in this population, specifically our use of blood serum rather than liver tissue. Tissue distribution studies have generally indicated that the highest concentrations of ARs occur in the liver and the lowest concentrations occur in the muscle, brain, blood, and fat (Eason et al. 2002). The half-life of ARs in the blood is significantly shorter than the half-life in liver tissue. For example, Eason et al. (1996) found that in common brushtail possums (*Trichosurus vulpecula*) the SGAR brodifacoum persisted in blood plasma for 35 d and in liver tissue for more than 8 mo. Although half-life data varies by compound, using blood may result in a shorter window of detection relative to previous studies only using liver samples

(e.g., Murray 2011, Ruiz-Suárez et al. 2014). Additionally, blood and liver samples may not test equally for all compounds, as a study conducted on bobcats where paired blood and liver samples were taken found that certain ARs (specifically diphacinone) were underestimated in the liver samples alone (Serieys et al. 2015). Therefore, our results are different from and should not be compared directly to results from liver samples due to differences in half-lives of ARs in blood and liver samples, and differences in the sensitivity of each type of sample to testing protocols.

Alternatively, the low short-term AR exposure rate we observed may be due to sampling migrating birds. Our study was conducted during the migration season in a migration flyway for both raptors and passerines. Raptors experiencing acute AR toxicity may not be healthy enough to continue migration and therefore may not be available for sampling at a migration station. Also, migrating birds may be eating different prey than breeding or wintering birds, as many migrant species show extreme shifts in food selection during pre-migratory periods (Bairlein and Simons 2013). It is possible that migrating birds utilize a larger variety of prey than wintering or breeding birds that may spend a longer time in high-risk areas such as agricultural fields or urban areas with high rates of household AR usage. As ARs accumulate in the blood, birds that spend a substantial amount of time foraging in these high-

risk areas may have a higher risk of exposure compared to birds that do not spend a long time in these high-risk areas. For example, adult Red-tailed Hawks with established territories had significantly higher brodifacoum levels than juveniles that were not regularly feeding in a single area (Murray 2011). As little is known about the diet and foraging range of migrating Red-tailed Hawks, more research is needed in both of these areas to investigate the validity of this hypothesis.

In addition to lethal cases of AR poisoning, there may also be sublethal effects of this exposure; there have been incidences where AR was detected in an individual, but AR exposure was not determined to be the direct cause of death. Murray (2011) sampled 161 dead raptors and found that although 86% of hawks tested positive for ARs, only 6% of hawks were known to have died directly from AR toxicosis. We investigated a connection between AR exposure and body condition, but we found no evidence of a correlation between the two. Although we did not find a relationship between AR exposure and body condition, because of the small sample size and low statistical power, we could not conclude that AR poisoning has no effect on relative body condition. It is important to note that although a raptor may test positive for only a trace amount of ARs, it is impossible to determine the exposure history of an individual. For example, it is not possible to determine whether the raptor was heavily exposed to ARs a few weeks prior to sampling and only has a trace amount of ARs left in its blood or if the raptor was only exposed to smaller amounts of ARs within a few days of being sampled. It is possible that ARs may only have a detectable effect on body condition over a longer period of exposure or that birds that were exposed to a large dose of ARs were unable to migrate, and were therefore not available for us to sample. Additionally, sublethal effects such as changes in body condition may be more detectable in different life stages, such as in nestlings (e.g., Salim et al. 2016) or in adults who have had more time for AR accumulation. Other potential sublethal effects that we did not test for include decreased clotting time, internal hemorrhaging, and lethargy leading to decreased hunting success.

As one of the first studies to use blood sampling to examine short-term AR exposure in wild raptors, our study showed that blood can effectively be used to test for both FGARs and SGARs. This method makes it possible to sample live birds, and a better understanding of the limitations of this method

can help us more accurately estimate AR exposure rates across populations. Using blood to sample for ARs across multiple time points could aid in determining underlying levels of population exposure. Regardless of the limitations of sampling live birds, this study provides a template for future long-term monitoring of short-term AR exposure in wild populations of migrating raptors. Further studies should be conducted on the effectiveness of using blood rather than liver samples with an emphasis on raptor populations near agricultural areas where there is a high likelihood of AR application.

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