A REVIEW OF BIOMARKERS USED FOR WILDLIFE DAMAGE AND DISEASE MANAGEMENT

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Abstract: Biomarkers are distinctive biological indicators used to identify, often through indirect means, when an event or physiologic process of interest has occurred in an animal. Historically, a variety of biomarkers, as well as bait-markers, have been used in wildlife management including radioactive isotopes, stable isotopes, fatty acids, systemic and physical biomarkers. The ability to successfully track, monitor, and identify animals using minimally invasive techniques is becoming increasingly important as wildlife-human interactions increase. This paper is an overview of the benefits and limitations of previously and presently used biomarkers in wildlife damage and disease management with emphasis on the use of rhodamine B as a physical biomarker as part of the USDA, Wildlife Services, Oral Rabies Vaccination Program.

Key words: bait marker, biomarker, ORV Program, rabies, raccoons, rhodamine B

INTRODUCTION

Biomarkers are distinctive biological indicators used to identify, often through indirect means, when an event or physiologic process of interest has occurred in an animal. There are many uses for biomarkers in wildlife damage and disease management; applications include vaccination, lethal control, and contraception programs as well as studies involving diet, movement, and population estimates. Programs such the USDA, APHIS, Wildlife Services, Oral Rabies Vaccination (ORV) program are extensively using tetracycline, an antibiotic biomarker, to mark animals that ingest baits filled with rabies vaccines, and thereby evaluate the success of the program. Lethal control programs often use biomarkers to identify the effects of lethal control on both non-target and target species prior to introducing toxic baits (Fisher 1999). Biomarkers have also been used to understand the movement, diet (Cerling et al. 2006) and population dynamics of many species including bears (*Ursus spp.*, Taylor and Lee 1994, Garshelis and Noyce 2006), pheasants (*Phasianus colchicus,* McCabe and LePage 1958), and small mammals (Bailey et al. 1973).

Varieties of biomarkers are used in wildlife damage and disease management to monitor animals. Each of these has benefits and limitations, but as of yet none satisfy the ideal criteria that are sought to achieve in a biomarker; characteristics that include non-invasive sampling techniques, easy to evaluate tissues of the animal for evidence of the biomarker, persistence, affordability, and preferably a tool that would only mark the animal of interest. Creation of this ideal biomarker may be in the future, but

meanwhile existing biomarkers are providing reliable estimates of exposure. Our review discusses both biomarkers used in the past and present, and is by no means a complete review of biomarkers used in wildlife damage and disease management. We will review five categories of biomarkers including radio isotopes, stable isotopes, fatty acid biomarkers, systemic markers and finally, physical markers. Each of these types of biomarkers vary in their usefulness based on the limitation and needs of the research being conducted and the species being marked.

Radioactive Isotopes

Radioactive isotopes have been used for studying wildlife since the early 1950s (Bailey et al. 1973). Radioactive isotopes have provided reliable information for a variety of research including movement and migration studies, population studies, foraging studies and studies on the metabolic pathways. Radioactive isotopes are especially useful not only because presence or absence of an event is identified but also quantitative measurements can be collected. Unfortunately, this quantitative component of radioactive isotopes is what has limited their usefulness in the field of wildlife damage, especially as broad based biomarkers. Radioactive isotopes are still useful for controlled laboratory studies, but the long-term persistence of radioactive isotopes in the environment and their detrimental effects have made using radioactive isotopes very difficult and highly regulated, and thus expensive. When using radioactive isotopes each molecule must be created, monitored, and then recovered for appropriate disposal. Even though radioactive isotopes have been very useful in the past, our expanded knowledge limits their utility in wildlife damage and disease management.

Stable Isotopes

Stable isotopes act as recorders in biotic and abiotic systems that can be identified to reconstruct ecological processes or trace activities (West et al. 2006). Stable isotopes are detected using ratios of elements such as carbon, nitrogen and hydrogen, and can be used to trace movement, in terms of migration, diet composition, physiologic processes as well as trophic interactions. These ratios can be obtained from blood samples as well as within the enamel of teeth (Cerling et al. 1997) and in hair (Cerling et al. 2006). Stable isotopes are considered medium persistence biomarkers, depending on the metabolism and habits of the animal.

Stable isotopes have been investigated for use in wildlife damage using a variety of vegetable and fish oils to define a C12/C13 ratio (J. Johnston unpublished). The utility in using stable isotopes in this manner has proven to be challenging and costly, since developing an absolute test requires considering all possible diets of the target species to identify appropriate stable isotope, their ratios and ranges. As a biomarker for wildlife damage programs stable isotopes, at this time, appear to be too costly and unpredictable.

Fatty Acid Biomarkers

Like stable isotopes, fatty acid biomarkers use deviations from an animal's traditional or ordinary diet to mark an animal. Since mammals do not have the ability to efficiently metabolize long chain fatty acids, the introduction of a novel fatty acid can be traced in the blood, hair, and adipose tissue. Fatty acids have been used to identify the diet of many marine carnivores including gray seals (Willis 2002), and research on the use of fatty acids to understand the diets of canids is underway (L. Berkley pers. comm.). One of the limitations of fatty acid biomarkers,

similar to stable isotopes, is that novel fat sources must be identified. Research into the potential of fatty acid biomarkers was conducted at the National Wildlife Research Center (J. Johnston unpublished) with the goal of developing a new biomarker option for the National ORV Program; however, finding a novel fatty acid has proven very difficult given the generalist diet of targeted mesopredators.

Systemic Biomarkers

Systemic biomarkers are chemicals and their byproducts that stain internal tissues after being eaten. Many types of systemic markers have been used in wildlife damage management including iophenoxic acid, sulfadimethoxine, mirex, all with varying success. Each has distinctive benefits and limitations that will be reviewed here. These markers are moderately invasive depending on the species being considered. Typically, restraint and anesthesia is required to collect blood samples. Many systemic biomarkers have extended persistence and are often easily applied to baits or lures. Unfortunately, systemic biomarkers can be costly, a result of the effort needed to trap and restrain targeted species as well as the costs associated with evaluation of tissues for the presence of the biomarker. Finally, some systemic biomarkers may cause long-term effects to both individuals and ecosystems.

Iophenoxic acid (IPA) has been the mostly widely used biomarker of the blood serum markers reviewed here. IPA has been used to mark a variety of mammals (Follmann et al. 1987, Knowlton et al. 1987, Eason and Batcheler 1991, Creekmore et al. 2002, Purdey et al. 2003). IPA, when ingested, results in elevated blood iodine levels. IPA is relatively simple to incorporate into baits with little taste aversion (Knowlton et al. 1987). IPA also

has some substantial limitations including affordability. Since it is necessary to capture, anesthetize and collect blood there is a large upfront cost associated with its use. In addition to these upfront costs, evaluation of blood iodine levels must be done using high performance liquid chromatography. Prior to using IPA, additional costs are incurred by the necessity of identifying the normal ranges of blood iodine levels in the animals being evaluated for the biomarker. IPA continues to be used in wildlife damage management as a biomarker and is presently being explored for immunocontraception programs.

Another systemic biomarker that has been used in wildlife damage and disease management is sulfadimethozine, a broad-spectrum antimicrobial that is used for short-term marking, lasting up to seven days. An advantage of sulfadimethozine is that it is easy to evaluate its presence in whole blood using a simple rapid card test or it can be quantified using an ELISA test (Matter et al. 1998, Youssef et al. 1998, Southey et al. 2002).

Other systemic biomarkers have been used, and continue to be explored. One of these biomarkers is Mirex, a broad pesticide. Banned in the the late 1970s due to it long term persistence in the environment as well as its carcinogenic effects, Mirex effectively marked blood serum as well as liver tissues. The case of Mirex reminds us as wildlife ecologists that it is important to understand not only the effects of a biomarker on target and non-target animals but also the long-term impacts of adding a chemical or dye into the environment.

Physical Biomarkers

There are numerous types of physical biomarkers including gut markers and calciphilic biomarkers. Examples of gut markers include, metallic flakes (glitter),

plastic bits or beads, Microtaggants®, and dyes such as rhodamine B. Each of these materials marks the feces and digestive system of animals that ingest the material. Gut markers are affordable and can be mixed or sprinkled over a food source or bait, with minimal risk of taste aversion. There are many uses for this type of biomarker including pen, home range, food choice studies, as well as movement studies. Physical markers are most commonly used to mark scat. One of the most beneficial aspects of using physical markers, non-invasive sampling, may also be it most limiting. Non-invasive scat surveys can be time consuming and costly if genetic sampling or microscopy is needed to identify individuals. Applications in pen studies to measure consumption or to identify individual feces to look for parasites is also an application of physical markers. Another limitation of physical markers is their lack of persistence, although residues often remain in the intestinal track for a couple of weeks, scat is often only noticeably marked for a few days.

Another physical marker, a calciphilic biomarker, which has been used extensively in wildlife research and management to answer a variety of questions, is the antibiotic tetracycline. Tetracycline has proven to be a reliable biomarker but finding the biomarker is a laborious and an expensive undertaking. Using a compound microscope with a UV light source tetracycline deposits can be seen as a yellow ring within the cementum of the tooth. Sampling for exposure to tetracycline is a relatively invasive procedure. It is necessary to either euthanize the animal or extract the tooth of an anesthetized animal to identify the fluorescent ring deposited by tetracycline. Other limitations associated with tetracycline include the fact that older individuals, although exposed to the biomarker, may not show evidence of its

uptake due to slowed growth of bones and teeth (Linhart and Kennelly 1967). Tetracycline residues in younger animals may be lost because of reformation of bone (Johnston et al. 1987). A benefit of tetracycline is that multiple exposures to tetracycline can be observed through the teeth. For example, raccoons sampled as part of the USDA, Wildlife Services, ORV Program often show multiple tetracycline rings. These rings allow information to be gathered related to the number and time between exposures, and serve as an index of the number of baits consumed during a single vaccination period. This is because higher doses of tetracycline result in an increased intensity of the fluorescing band (Johnston et al. 1987).

Rhodamine B is another physical marker that along with marking the gut and teeth of an animal marks other growing tissue including vibrissae and fur. Rhodamine B, a dye used in the cosmetic industry in the coloration of lipstick, has been used extensively as a biomarker in Australia and has also been tested on a number of species native to the United States (Fisher 1999). Rhodamine B, when ingested, stains the oral cavity, and extremities of an animal that contacts it and it is absorbed systemically through diffusion (Clark 1953) in growing keratinous tissues such as nails, hair, and vibrissae (whiskers). Exposure to rhodamine B is easily identified in hair and whiskers as a fluorescent orange band under UV light and sometimes in ambient light. Research conducted on feral cats (Fisher et al. 1999) revealed evidence of rhodamine B in hair and whiskers under ambient light in 45% of cases, in 56% of cases under hand held UV lamps and in 100% of cases under UV microscopes. If the same is true for raccoons, field staff could easily assess whether an animal has ingested an ORV bait or similarly marked food source. This assessment could occur in

the field or an office, reducing the need for samples to be sent to diagnostic labs, thereby, reducing costs and decreasing the time it takes to obtain results. Rhodamine B is also deposited in teeth, similarly to tetracycline (Ellenton and Johnston 1975). The persistence of rhodamine B in keratinous tissues is another useful feature of this biomarker. Rhodamine B has persisted for over 24 weeks in guard hairs of coyotes (*Canis latrans*, Johns and Pans 1981) and mountain beavers (*Aplodontia rufa*, Lindsey 1983) and up to ten weeks in jackrabbits (*Lepus californicus,* Evans and Griffith 1973) with approximate doses of 15 mg/kg. Additionally, multiple exposures to rhodamine B can be observed in the hair or whiskers as long as the hair is growing at the time when rhodamine B is ingested. Finally, animals fed rhodamine B as less than 3% of the bait tended to show no taste aversion to the powdered dye.

SUMMARY

Finding an easy to use, affordable, non-invasive tool to mark animals continues to be a goal of wildlife professionals. Researchers and Wildlife Services, National Wildlife Research Center scientists are presently looking into new chemicals, new delivery methods, as well as new methods for testing biomarker presences. Recent research on the usefulness of rhodamine B as a potential biomarker for raccoons as part of the National ORV program is being completed, and analyzed by scientists at the Wildlife Services, National Wildlife Research Center. The potential of rhodamine B to satisfy many of the ideal characteristics of a biomarker, affordability, persistence, and non-invasive sampling methods are met by this dye and thus far, it appears to be safe for both the animals that ingest the dye as well as the environment. Continued research on rhodamine B and other potential biomarkers should illustrate their benefits and limitations in relation to utility in wildlife damage management.

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