

## TECHNICAL NOTE

### ALTERNATIVE METHODOLOGY FOR HANDLING AND MARKING MESO-MAMMALS FOR SHORT-TERM RESEARCH

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Keywords	Abstract
Capture; Dye; Ink; Mark-recapture; Squeeze cage.	We evaluated squeeze cages and water-soluble inks and dyes as methods for handling and marking of meso-mammals in a short-term capture-recapture study. Study animals exhibited no adverse physiological impacts from capture or marking techniques. We also observed no negative impacts on trap success for the duration of the study. We found these methods quick (5 minutes maximum hold time), safe (no observed injuries of animals or researchers) and reliable (effective marking and mark retention for a minimum of 12 days).

Handling and marking techniques may be inappropriate or cost prohibitive for short-term meso-mammal studies. These studies are often characterized by large numbers of simultaneous animal captures that must be documented and marked quickly with limited personnel, but do not require long-term or permanent markings (e.g., ear tags, branding). Meso-mammals are loosely defined as mammals larger than rodents (though sometimes including the largest rodents) up to roughly fox-sized mammals (*Vulpes* spp.) [1]. Handling and marking meso-mammals is a time intensive, expensive, and potentially dangerous undertaking that requires solid justification and methodological scrutiny. Research has linked wildlife capture, handling, handling duration, and marking with increased mortality and behavioral changes in study subjects; thus highlighting the need for safe and simplified methods [2,3,4,5]. Accepted guidelines now mandate a well-structured protocol that places emphases on the welfare of the study species and the safety of the researcher [6,7]. Current and past researchers have followed a variety of methodologies to balance the needs of the project with the obligations of ethical research. Methodologies for handling and marking generally follow similar frameworks: 1) immobilization either through physical restraint or chemical immobilization, 2) internal marking (e.g., Passive Integrated Transponders [PIT]), and 3) external marking (temporary, semi-permanent, or permanent marking [8]). Many of these techniques are unnecessary or impractical for short-term meso-mammal studies. For example, chemical immobilization often requires large time investments (60-120 minutes per animal is typical) as researchers must carefully monitor animals

through chemical application and recovery [9,10]. Additionally, research has demonstrated increased mortality and behavioral alterations may result from chemical immobilization [11,12]. These methods often require expensive equipment (e.g., chemicals) and extended preparation time, and cause increased difficulty in attaining institutional approval. These methods also become highly restrictive when researchers expect to capture many individual animals at once, the environment is intemperate (e.g., warm temperatures), or both. Therefore, researchers and managers look for handling and marking methods for meso-mammals that increase wildlife survival and researcher safety at minimum cost and maximum efficiency.

Researchers have often used squeeze cages in controlled environments (e.g., laboratory settings) to immobilize wildlife for injections or data collection, though we found little to indicate that squeeze cages have been used to immobilize wild animals in-situ for marking [13,14,15]. The concept is similar to squeeze chutes (often integrated into corrals) used to safely and temporarily immobilize large domestic animals and many ungulate species for agricultural or research purposes [16]. Although squeeze cages cause stress in captured animals; we believed handling time reduction and safe immobilization would decrease the risk of capture myopathy [17]. Squeeze cages also reduced the risk of injury to the handler by minimizing physical contact between animal and handler (i.e., animal always in trap or cage, squeeze cage manipulated through handles set back from cage surface). Additionally, temporary marking with dyes and inks has proven effective in many previous studies with no recorded adverse physiological effects [8,18,19]. We attempted fur clipping as an alternative temporary marking technique with little success. Raccoon fur is thick and requires powerful electric clippers or manual clippers. These were imprecise in marking and it was difficult to safely access and control the animal for clipping. We did not use manual clippers due to concerns of animal lacerations or punctures.

In 2008, we began researching the role of meso-mammals in water quality problems. Our overall analyses required estimation of meso-mammals population densities. As such, one of the goals of our research was to develop handling and marking methodologies for meso-mammals that fulfilled the prescribed protocols of wildlife handling and the needs of our larger research project (seasonal density estimates of meso-mammals). Since we needed only age, sex, and capture/recapture data, we emphasized short holding times. The challenge was to handle and mark conscious meso-mammals in a quick and safe manner using physical restraint and ephemeral dyes and inks. Additionally, as our data collection occurred in a grassland floodplain and our study species were likely to encounter water either through direct contact with the creek or wet grass, our markings needed to last even when wet. Many meso-mammals are large enough to cause handling difficulty outside of a trap; thereby, often necessitating chemical immobilization. We felt that the risks of chemical immobilization were not justified by the needs of our research (i.e., short-term marking). We sought to apply methods that limited handling time, subjected the meso-mammals to the minimum of stress and discomfort, provided reliable short-term marking (3-week retention), and provided safety to researchers. All capture and handling was conducted in compliance with approved permits issued by Texas Parks and Wildlife Department and Institutional Animal Use and Care Committee at Texas A&M University.

From 2008–2009, we conducted research on 2 private ranches (Property A, 518 ha; Property B, 660 ha) in Brazos County, Texas. Each ranch stocked cattle (Property A, 1 cow/10.4 ha; Property B, 1 cow/2.2 ha) on typical post oak savannah that consisted of a mix of upland/bottomland grasslands with scattered post oak woodlands (*Quercus stellata*). We determined meso-mammal density by analyzing trapping numbers in live-traps (81 cm x 25 cm x 30 cm; Tomahawk Live Trap, Tomahawk, Wisconsin, USA). We trapped on both properties using a grid-design ([20]; 42 traps total for each property) with 250-m spacing between traps that had been shown to adequately sample animals highly attracted to baits (e.g., raccoons (*Procyon lotor*), Virginia opossums (*Didelphis virginiana*)). Trapping grids and arrays were randomly placed on each property and trapped for 12 consecutive days using traps baited with canned dog food, apples, bananas and fish scent.

We immobilized Virginia opossums and raccoons using a Tomahawk squeeze cage (50 cm x 27.5 cm x 30 cm, 5.9 kg; Tomahawk Live Trap, Tomahawk, Wisconsin, USA; Figure 1). Total handling time ranged from 3–5 minutes. We decided against chemical immobilization due to concerns of capture myopathy and increased holding times [8,21]. The squeeze cage door was removed and placed against the opening of the box trap. The animal was blocked from the opening by 2 dividers (1 divider can be breached by the animal) as the door to the trap was locked open. The cage and trap were held together by holding the trap by the handle and wedging a foot behind the cage. The dividers were then removed and most animals moved voluntarily to the squeeze cage seeking exit from the trap. If unwilling, the animal was spurred by gentle prodding from the dividers. At this point, the door to the trap was allowed to close and the door to the squeeze cage was set into place. The animal was immobilized through gentle squeeze pressure from the movable wall. The squeeze cage was only used for marking and was not used for recaptured animals.

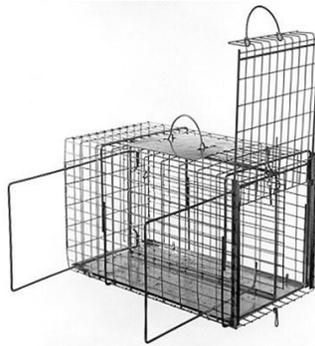


Fig. 1: Tomahawk squeeze cage (50 cm x 27.5 cm x 30 cm, 5.9 kg; photo courtesy of Tomahawk Live Trap).

We uniquely marked captured animals that were immobilized in the squeeze cage using a small paint brush to apply semi-permanent water-based hair dye (Hot Topic, San Jose, California, USA) or India ink (Dr. Ph. Martin, Golden, Colorado, USA) and released them 3–5 minutes later. All inks and dyes were bright colors (e.g., pink, magenta, blue) likely to show up on variably colored individuals. The hair dye was artificially scented by the manufacturer though we found through multiple recaptures the scent dissipated within 24 hours. We avoided colors found in the animals' fur (e.g.,

orange, black, white, yellow). Markings were placed in a variety of combinations and colors unique to each individual. Markings were limited to the back, rump, sides, or hips to reduce loss due to grooming or everyday activities (e.g., walking, swimming). Care was taken to work the colorant deep into the fur. Finally, the animal was released from the squeeze cage by removing the main door.

We grid-trapped 2,520 traps-nights during the study and used the squeeze cage to mark 192 raccoons and 23 Virginia opossums. We later recaptured 136 raccoons (80 separate individuals) and 52 Virginia opossums (15 separate individuals). We found the squeeze cage method was impractical or dangerous for nine-banded armadillos (*Dasypus novemcinctus*) and striped skunks (*Mephitis mephitis*) due to unique morphology (armadillo shell) or chemical defenses (skunk).

Although all marks were strongly recognizable for the course of the mark-recapture sessions, marks faded sooner on raccoons than opossums likely due to behavior (e.g., frequent movement in and out of water). We found an increasing percentage of captures were marked animals over the course of the trapping periods (Figure 2-A). This implied mark resilience over time as individuals marked in the first few days remained marked until the last few days. We also had relatively stable capture numbers over the same time period (summed over 3 day intervals; Figure 2-B). We observed no changes in body condition over the course of the trapping sessions. We also had no injuries to captured wildlife or researchers due to use of squeeze cage and no impacts on fur quality from dye and ink application. No animals escaped during handling and marking activities.

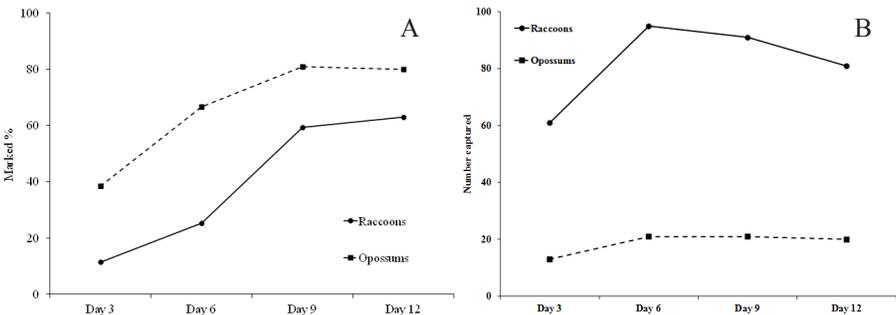


Fig. 2: (A) Percentage and (B) Number of animals captured (summed over 3 day intervals) with artificial marks, Brazos County, Texas, 2008-2009.

Due to the disjunct, seasonal nature of our research, long-term marking proved unnecessary and excessive. The replacement of permanent marks (e.g., ear tags, toe-clipping) with temporary marks (ink and dye) allowed a reduction in short-term suffering and potential long-term problems (e.g., bodily damage, behavioral changes, or survival impacts) [8]. Research indicates that properly applied dyes and inks (even brightly colored) have resulted in no reported adverse physiological affects [8]. It also reduced concerns from permitting agencies (i.e., Texas Parks and Wildlife Department and Texas A&M University's Institutional Animal Use and Care Committee) related to animal suffering and distress. The nature of marking obviated the need for lengthy restraint and placed the onus on short but productive marking and examination followed by quick release. Many hair dyes used in wildlife management

and research have a strong chemical smell, but the artificial scent supplied by the manufacturer was relatively mild and quickly dissipated. Researchers are ethically bound to impact wildlife only for the period necessary for data collection [7]. However, the size and aggression of many meso-mammal species obviates established small-mammal handling techniques (e.g., handling cones). We found that squeeze cages provided a quick, safe, and reliable method for immobilizing raccoons and Virginia opossums. The water-based hair dye and ink provided durable, but short-term marks that minimized impact on these animals. Our methods minimized handling time (3–5 minutes) and impact on animals. Although difficult to make direct comparisons due to different species, circumstances, and research needs; our handling times were less than or comparable to other studies that did not use chemical immobilization to handle wildlife [e.g., 22,23]. Additionally, we required only a single researcher to safely capture and mark meso-mammals. Finally, this methodology was faster and safer for the animal than chemical immobilization (i.e., short handling time, no mortalities or observed declines in body condition).

Our use of a relatively light-weight, commercially available squeeze cage and vibrant, water-based dyes and inks with simplified application allowed individually identifiable marking of animals with a variety of color pelages (including dark colors that generally obviate application of black dye) at a minimum of handling time and animal trauma. This simplified handling of often difficult species to control and provided the handler with the opportunity to individually mark an animal. For instance, raccoons were often very mobile within the trap and aggressive towards the handler; but use of the squeeze cage prevented excessive movement and reduced opportunities for raccoon aggression. This ultimately reduced handling time. Our technique would likely work for other meso-mammals with potential for aggression (e.g., weasels (Mustelids), feral cats (*Felis catus*)). We recommend these methodologies for short-term meso-mammal research studies with high numbers of expected captures and restricted time and monetary budgets.

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*Five “key references”, selected by the authors, are marked below (Three recommended (●) and two highly recommended (●●) papers).*

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