EVALUATION AND APPLICATION OF NOVEL TELEMETRY METHODS FOR THE STUDY OF MOVEMENTS AND ECOLOGY OF TROPICAL HYLIDS

by

ROBERT VINCENT HORAN III

(Under the Direction of John P. Carroll)

ABSTRACT

I investigated the methods and techniques used to study the movements and ecology of hylid frogs. A review of associated literature was conducted to consolidate the variety of techniques into a single, stand-alone document for researchers in the field. In order to test novel radio transmitter attachment designs, a series of controlled experiments were conducted, incorporating behavioral, morphometric and hematological parameters. The top design was then applied in a field setting to the gliding leaf frog (*Agalychnis spurrelli*) and veined tree frog (*Phrynohyas venulosa*). I determined that my method was not suitable for the gliding leaf frog, but suitable for studies of the veined tree frog. I observed partial migrations away from the breeding pond by the veined tree frog. Data on the temporal activity patterns did not support the veined tree frog being strictly nocturnal species.

INDEX WORDS: *Agalychnis spurrelli*, Barro Colorado Island, BCI, Gliding leaf frog, Methods evaluation, Movement ecology, *Phrynohyas venulosa*, Radio telemetry, Radio attachment, Telemetry, Treefrog, Veined tree frog.

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DEDICATION

This thesis is dedicated to the memory of the late Austin Stanley Rand. Stan's enthusiasm, knowledge, ideas and passion for the natural world and all of the scaly and slimy creatures within it have been an inspiration to many, myself included. Thank you Stan, I'll see you at the frog pond.

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CHAPTER 1

INTRODUCTION

Amphibian species worldwide are experiencing dramatic population declines, with up to one-third of all species currently listed as either threatened or endangered (Stuart *et al.* 2004). Anurans comprise a large portion of the loss (Stuart *et al.* 2004, Beebee and Griffiths 2005), with certain areas of the globe experiencing greater declines than others. Latin America, specifically, has experienced a dramatic decline in recent years (Lips 1999), with much of the decline being attributed to the fungal pathogen *Batrchochochytrium dendrobatidis* (Kiesecker *et al.* 2001, Collins and Storfer 2003). Even as some species disappear, our ecological knowledge of many is still lacking. Specifically, the terrestrial ecology of most pond-breeding amphibians is poorly understood (Dodd and Cade 1998a). Historically, research on this group has focused on animals found at breeding areas, in part due to the ease of monitoring anurans when they aggregate to breed and are performing conspicuous behaviors such as calling or egg laying instead of their usual silent and cryptic behaviors (Duellman and Trueb 1994). However, this approach ignores other parts of the anuran annual cycle, and a more holistic view of their life history requirements is needed to address the current conservation needs of rare frog species, and to gain a better understanding of still common species to prevent future declines.

While it has long been established that frogs migrate between breeding and terrestrial habitats, the two systems have not received equal attention. This requires expanding beyond the breeding areas to discover other essential habitats (Gibbons 2003, Trenham and Shaffer 2005, Rittenhouse and Semlitsch 2007). Many important processes occur away from breeding areas, such as juvenile dispersal (Dodd and Cade 1998b), overland migrations (Sinsch 1988), overwintering and hibernation (Bosman *et al.* 1996, Lamoureux and Madison 1999) and adult foraging (Lamoureux *et al.* 2002). However, methodological limitations have greatly hindered the study of these processes.

At its conception, the technology for radio telemetry was developed by researchers interested in the physiology of naval aviation pilots (Kenward 2000). Soon after, modifications of these techniques were used by wildlife researchers for the study of heart rate in small mammals (LeMunyan *et al*. 1959) and wing beats of waterfowl (Eliassen 1960). Modifications of the technology and techniques were soon used worldwide to investigate nearly every aspect of wildlife ecology. Thanks to such innovation, much has been gained in the disciplines of wildlife physiology, behavior, and movement (White and Garrott 1990, Millspaugh and Marzluff 2001). With the ever-growing use of radio telemetry as a research tool, more techniques are being developed to attach transmitters to previously unstudied species. No matter the faunal group, it is of the utmost importance that care is given to the evaluation of any effects caused by the attachment of such transmitters. Much work has gone into the evaluation of radio tagging of mammals and birds. Kenward (2000) and White and Garrott (1990) provide a thorough review of such studies. These studies have shown that different parameters, such as body condition, behavioral changes, and energetic requirements must be investigated to fully evaluate the different effects that may occur. With the recent miniaturization of electronic components enabling the construction of smaller radio transmitters, frog researchers have been able to use radio telemetry as a valuable research tool (Nuland and Claus 1981).

As with some other groups, frogs may be susceptible to negative effects caused by the handling, application, attachment and wear of radio transmitters; however, few studies have directly evaluated these effects.

Of the currently used and previously evaluated external radio attachment methods available for anuran research, all are similar in that the radio is positioned at the pelvic region of the tagged frogs or toads. For terrestrial species that exhibit a simple walking or hopping means of locomotion, this may be suitable; however for canopy-dwelling species that accomplish much of their movement via long distance jumps or even aerial parachuting, this shift of their center of gravity may be unacceptable. In an attempt to develop a novel radio attachment technique that centers the mass of the radio transmitter on the study animal, I used multiple biological and observational parameters to evaluate different external attachment methods with the end goal to investigate the movements and terrestrial ecology of tropical hylid frogs. Cuban tree frogs, *Osteopilus septentrionalis*, were the focus species for my captive based evaluations. Two species, the gliding leaf frog, *Agalychnis spurrelli*, and the veined tree frog, *Phyrnohyas venulosa*, were the focal species for the field-based research due to their large size and relative abundance among the species present in the study area.

CHAPTER 2

METHODS FOR THE STUDY OF ANURAN MOVEMENT ECOLOGY: A LITERATURE REVIEW WITH FOCUS ON RADIO TELEMETRY

INTRODUCTION

Amphibian species worldwide are experiencing dramatic population declines, with anurans comprising a large portion of the loss (Stuart *et al.* 2004, Beebee and Griffiths 2005). Historically, research on this group has focused on animals found at breeding areas, in part due to the ease of monitoring anurans when they aggregate to breed and are performing conspicuous behaviors such as calling or egg laying (Duellman and Trueb 1994). However, this approach ignores other parts of the anuran annual cycle. A more holistic view of their life history requirements is needed to address the current conservation needs of rare frog species, and to gain a better understanding of still common species to prevent future declines. This requires expanding research beyond the breeding areas to discover other essential habitats (Gibbons 2003, Trenham and Shaffer 2005, Rittenhouse and Semlitsch 2007). Many important activities occur away from breeding areas, such as juvenile dispersal (Dodd and Cade 1998b), overland migrations (Sinsch 1988), over-wintering and hibernation (Bosman *et al*. 1996, Lamoureux and Madison 1999) and adult foraging (Lamoureux et al. 2002). However, methodological limitations have greatly hindered the study of these processes. Therefore, the objective of this chapter is to summarize the current methodologies and techniques available to study the movement ecology of anurans. In particular, I will focus on radio tracking methods because of their unique ability to reliably locate individual anurans away from breeding grounds.

MARKING TECHNIQUES

Prior to studying the movements of individual research animals, the animals must initially be captured and marked. Many methods exist for collecting frogs from the field (Willson and Gibbons 2010), with one of the simplest being capture by hand. When effort, search time and collector experience are accounted for, this method can be standardized as a Visual Encounter Survey (VES) (Crump and Scott 1994). Another method, initially more labor intensive but increasing capture efficiency, is the use of drift fences flanked by pitfall traps built around a breeding area (Gibbons et al. 1981, Regosin et al. 2005). The use of passive refugia traps, such as standing sections of plastic pipe or bamboo or ground cover boards, is another method of sampling frog species and can be used in both terrestrial and aquatic habitats (Boughton *et al.* 2000). Similarly, the use of both commercial and custom funnel traps has been successful for the collection of many species in wetlands and uplands (Dodd 1996). Ultimately, researchers should determine the best method for collecting their study species, ensuring that the capture method properly represents the portions of the population of interest.

Once animals are collected, establishing their individual identities can often become a time intensive task. Toe clipping has long been recognized as an accepted standard for quickly marking large numbers of amphibians, whether it be for individual marks or cohort marks (Ferner 2007). Two other long used methods are skin branding and the use of temporary or permanent dyes (Ferner 2007). While not often used by contemporary researchers, past projects have relied on the marking of individual frogs via the use of radioactive isotopes, including Co^{60} and Ta^{182} and Cr^{51} (Karlstrom 1957, Kramer 1973, Freda and Gonzalez 1986, Ashton 1994). In addition to using this method as a marking technique, it may also be used as a tracking technique, effective over short distances (1-6 m). In more recent years, many researchers have used Visible Implant Elastomers (VIE), a technique originally used by fisheries biologists but now successfully adopted by herpetologists (Nauwelaerts et al. 2000). This method relies on the use of multiple marks of different colors injected in different subcutaneous locations of the body to differentiate individuals. Similar to the VIE, but requiring only a single mark, Visual Implant Alpha Numeric Tags are an inert material implanted subcutaneously into an animal and read using a Near Infra Red (NIR) light fluorescing system and have been suggested as an alternative method to toe clipping in frogs (Heard et al. 2008). Another time-efficient and highly accurate method of individually marking animals is the use of Passive Integrated Transponder (PIT) tags (Pyke 2005). Approximately the size of a grain of rice and inserted under the skin of the study animal, these tags provide a unique alphanumeric identification code when scanned by the receiver unit (range <1m). In species exhibiting individually unique coloration, patterns or markings, many individuals within a population may be recognized by establishing a photographic identification library, which can replace the need for applying an invasive mark or act as a secondary marking system to minimize error in mark recognition (Gamble *et al.* 2008, Kenyon *et al.* 2009).

The movement of animals with unique marks can be documented to a limited extend if they are later recaptured at other sites, although this is not practical for many species (Pechmann 1995). A low-tech method for mapping their movement over short distances is to attach lightweight bobbins of thread to the animal that will feed out line as they move (Tozetti and Toledo 2005). This spool-and-line technique is limited to species that are large enough to carry the attachments. An alternate technique which is similarly effective in observing detailed movements over short distance, is marking with fluorescent tracking powders (Schlaepfer 1998). Applied directly to an animal's body and incidentally dispersed during movements through the habitats, the powder is later tracked during the night with the assistance of ultraviolet lighting (Ovaska 1992). A more technologically advanced tool for tracking animals are externally attached harmonic direction finders (Leskovar and Sinsch 2005). This method allows for the study of animals as small as 18.5mm snout-vent-length (SVL), with tags being extremely lightweight and inexpensive (~$0.75), but requiring an expensive locator (~$8,000). While considerable dispersal distances were reported by using this method, low recovery rates were a concern reported by the users, and probably reflect the relatively poor detection of signals through vegetation (detected dispersal of 588m in *Bufo calamita*, only 35.9% recovery, *n*=33 and 665m dispersal in *B. viridis*, 31.6% recovery, *n*=103).

RADIO TELEMETRY

Radio telemetry offers an advantage over these other methods because VHF signals can be detected from further away, typically a few 100m for most anuran style transmitters. Starting in the 1980's telemetry began to allowed researchers to break the bonds of investigative techniques like the spool-and-line methods and provided higher recovery rates than those of the radioactive isotopes and harmonic direction finders, and has since been used with numerous species of bufonids, hylids, leptodactylids, myobatids, ranids and rhacophorids (Table 2.1). Radio telemetry requires three main components: the radio transmitter, which is attached to, or implanted in, the study animal; the radio receiver, used by the researcher to identify and isolate the radio frequency; and the antennae, used to locate the directional position of the radio transmitter's signal (Kenward 2000, Millspaugh and Marzluff 2001). Radio transmitters can be made by researchers themselves to save money, but are more often purchased from one of many commercial companies (Table 2.2). Of the studies reviewed, Holohil Systems Inc. has been the most widely used producer of radio transmitters used on anurans. An overview of general telemetry equipment selection and use is described by Kenwood (2000).

Attaching radio tags to anurans is a challenge, and there have been a variety of designs attempted (Table 2.1). External attachment methods were the first attempted (Nuland and Claus

1981) and are still preferred as they are the least invasive; however, they are not feasible for some species. Other studies have successfully forced the ingestion of a radio transmitter on the research animal (Oldham and Swan 1992) or surgically implanted them (Werner 1991).

Factors to take into consideration when deciding what type of radio transmitter to purchase include weight of the radio, which is influenced by the desired battery life of the transmitter, as related to the body mass of the study animal and whether the radio will be attached externally or inserted internally. Most previous studies have maintained a transmitter:body mass ratio of less than 10%, but using the smallest radio feasibly possible that still achieves the research objectives is highly suggested (White and Garrott 1990, Withey *et al.* 2001). External attachments require specific attachment accessories, while internal implantation of the radios may require veterinary assistance and anesthesia of each individual, often adding considerable time and cost investments, and may increase risk to the animals. Beginning with the original description of using telemetry for studying anuran movement (Nuland and Claus 1981), authors have repeatedly evaluated the effects of radio tagging on their species of focus. Methods of evaluation have varied but often included similar observations of change in mass, feeding behavior, movement and activity patterns, and the physical examination of individuals for injuries caused by the radio attachment (Bartelt and Peterson 2000, Weick *et al.* 2005, Blomquist and Hunter 2007, Rowley and Alford 2007b). Whatever the preferred method, ensuring that radio attachment does not significantly affect the behavior of the animal is key to acquiring high data quality and inferences drawn from data.

Once radio tagged animals are released in the field, data collection protocols vary greatly, depending on the objectives of the study. The majority of movement studies have reported collecting daily location points on each study animal, whereas others only locate animals 2-3 times per week. In studies focusing on landscape scale movements, locating a frog to a 1-2 m radius with the homing technique (Mech 1983) may be all that is necessary. Other studies focusing on microhabitat use or refugia locations may require the researcher to visually locate the animal within its habitat. In such situations, extreme care must be taken not to disturb the animal's behavior, which may compromise the quality of subsequent observations. Although requiring additional technological investments, the use of automated radio telemetry tools such as Automated Receiving Units (ARU) allows researchers to gather continuous data on animals that would be logistically difficult otherwise (Decoursey 1990). Such data may be used to calculate activity rates of study species (Cochran and Lord 1963), adding important natural history information to the spatial data manually gathered by the researchers (Lambert *et al.* 2009).

DATA ANALYSIS

A variety of analytical tools may be used to summarize and present the acquired movement data. When constructing home-range information from field data, the minimum convex polygon (MCP) method has long been the standard (Hayne 1949), but provides only a rough outline of the major areas inhabited by the study animals. In order to better represent information on habitat use and core areas, a method such as the kernel home range estimator may be used (Powell 2000). By giving greater importance to areas used more often by an animal, this method removes much of the unutilized space associated with the MCP. As suggested by Row and Blouin-Demers (2006), care must be taken when applying a smoothing factor to data to produce home range kernels. The researcher must ensure that the interpretations of the data properly reflect the biologically significant differences of amphibian home range use compared to more mobile species such as mammals and birds. See Millspaugh and Marzluff (2001) and White and Garrott (1990) for further details on analyses of traditionally gathered radio telemetry data.

SUMMARY

While methods may still be limited in extremely small-bodied species, the field of anuran movement ecology has been greatly advanced in the past two decades. Due to technological restraints, early movement studies were only able to incorporate large bodied, robust species with techniques such as thread bobbins and large, bulky transmitters (Dole 1972, Nuland and Claus 1981). However, the utilization of ever smaller radio transmitters has enabled the inclusion of metamorphic individuals in movement studies (Roznik and Johnson 2009). As previously reported, methods of attaching radio transmitters must be quantitatively tested prior to conducting field investigations (Rathbun and Murphy 1996, Bartelt and Peterson 2000, Weick *et al.* 2005, Blomquist and Hunter 2007, Rowley and Alford 2007b). While much work has occurred on data processing and summarization of mammals and birds, data on amphibian movements are relatively limited and appears not to meet some of the same assumptions held for prior data sets. With many anuran species using limited complimentary habitat areas outside of the migration and breeding season, the traditional home range and movement analyses methods employed for mammals and birds may not be biologically appropriate for anuran species. In the near future, though, technological advances may make it just as easy to attach a GPS or satellite tag to a gopher frog as to an elephant or an albatross.

Table 2.1. Summary of peer-reviewed anuran telemetry studies. Symbols indicate movement measurement methods reported in publications, *Cumulative straight line, **Daily straight line, ***Cumulative creek distance, ****Straight migration distance, (-) Method/Data not reported.

Family	Species	*n*	Ave. days tracked	Transmitter:body mass ratio (%)	Attachment method	Reported movement distances (m)	Study citation
Bufonidae	*Bufo boreas*	38	74	~4.2	microcatheter tubing waist band	430-2,330*	Bartelt and Peterson 2000
Bufonidae	*Bufo boreas*	18	49-119	~2.8-6.2	microcatheter tubing waist band	581-1,105*	Bartelt *et al.* 2004
Bufonidae	*Bufo americanus*	16	<126	<5	cotton/floss waist band	246-1,015*	Forester *et al.* 2006
Bufonidae	*Bufo bufo*	12	-	-	latex harness	89-430*	Gelder *et al*. 1986
Bufonidae	*Bufo microscaphus californicus*	83	31	-	microcatheter tubing waist band	>500*	Griffin and Case 2001
Bufonidae	*Bufo calamita*	43	-	<7	abdominal implant	~160*	Huste *et al.* 2006
Bufonidae	*Bufo japonicus*	26	-	<5	rubber waist band/cotton suture to back	27-260*	Kusano *et al.* 1995
Bufonidae	*Bufo calamita*	6	152	<7	peritoneal implant	2,189-4,411*	Miaud *et al.* 2000
Bufonidae	*Bufo calamita*	8	34	<4.3	polyethylene tubing waist band	567-1,540*	Miaud *et al.* 2000
Bufonidae	*Bufo calamita*	5	194	<4.7	polyethylene tubing waist band	86-934*	Miaud *et al.* 2000
Bufonidae	*Bufo boreas*	14	75	<5	wire/rubber tubing waist band	970-2,324*	Muths 2003
Bufonidae	*Bufo bufo*	-	-	6-13	latex harness	-	Nuland and Claus 1981
Bufonidae	*Bufo bufo*	23	<22	-	ingested	<108**	Oldham and Swan 1992
Bufonidae	*Bufo boreas*	10	23	<5	Velco™ waist band	25-12,000***	Schmetterling and Young 2008
Bufonidae	*Bufo marinus*	35	86	<3.5	peritoneal implant	-	Seebacher and Alford 1999
Bufonidae	*Bufo calamita*	6	-	-	abdominal implant	<985*	Sinsch 1988

Bufonidae	*Bufo calamita*	33	-	-	abdominal implant	<2,600***	Sinsch 1992
Bufonidae	*Bufo americanus*	6	12-25	-	peritoneal implant	<205*	Werner 1991
Hylidae	*Litoria nannotis*	19	9-30	<16	cotton gauze waist band	-	Hodgkison and Hero 2001
Hylidae	*Agalychnis spurrelli*	14	6.7	<6	dorsally sutured	10-245*	Horan *et al*, this manuscript
Hylidae	*Phrynohyas venulosa*	10	24	<3	dorsally sutured	<410*	Horan *et al*, this manuscript
Hylidae	*Litoria genimaculata*	9	17	<6	silicone tubing waist band	<170**	Rowley and Alford 2007a
Hylidae	*Litoria nannotis*	21	17	<6	silicone tubing waist band	<95**	Rowley and Alford 2007a
Hylidae	*Litoria lesueuri*	31	17	<6	silicone tubing waist band	<306**	Rowley and Alford 2007a
Leptodactylidae	*Eleutherodactylus augusti*	4	-	<5	aluminum bead waist band	-	Goldberg *et al.* 2002
Leptodactylidae	*Eleutherodactylus augusti*	11	-	<5	implant	-	Goldberg *et al.* 2002
Leptodactylidae	*Eleutherodactylus augusti*	1	-	<5	silicone tubing waist band	-	Goldberg *et al.* 2002
Myobatrachidae	*Mixophyes iteratus*	17	-	~2-5	surgical tape waist band	-	Koch and Hero 2007
Myobatrachidae	*Mixophyes iteratus*	10	2-5	<3	cotton gauze waist band	0-2,000*	Lemckert and Brassil 2000
Ranidae	*Rana sylvatica*	43	25.6	9	stretch bead cord waist band	102-340*	Baldwin *et al.* 2006
Ranidae	*Rana capito*	9	57	-	coelomic implant	0-286*	Blihovde 2006
Ranidae	*Bufo bufo*	6	-	-	latex harness	-	Bosman *et al.* 1996
Ranidae	*Bufo calamita*	5	-	-	latex harness	-	Bosman *et al.* 1996
Ranidae	*Rana aurora draytonii*	56	-	-	bead-chain waist band	200-2,800*	Bulger *et al.* 2003
Ranidae	*Rana luteiventris*	47	98	-	satin ribbon waist band	60-560*	Bull and Hayes 2001

Ranidae	*Rana lessonae/R. esculenta*	36	-	~6	silicone tubing waist band	<470*	Holenweg and Reyer 2000
Ranidae	*Rana clamitans*	23	117	-	abdominal implant	80-560*	Lamoureux and Madison 1999
Ranidae	*Rana muscosa*	24	21	<10	bead-chain waist band	<466*	Matthews and Pope 1999
Ranidae	*Rana pretiosa*	62	57	-	nylon ribbon waist band	-	McAllister *et al.* 2004
Ranidae	*Rana luteiventris*	87	24	-	surgical tubing waist band	200-1,030*	Pilliod *et al.* 2002
Ranidae	*Rana sevosa*	14	52	3-5	microcatheter tubing waist band	49-299*	Richter *et al.* 2001
Ranidae	*Rana capito*	49	-	<10	wire/rubber tubing waist band	<691*	Roznik and Johnson 2009
Ranidae	*Rana capito*	11	-	<10	wire/rubber tubing waist band	396*	Roznik *et al.* 2009
Ranidae	*Hoplobatrachus occipitalis*	46	30	<10	abdominal implant	<6,000****	Spieler and Linsenmair 1998
Ranidae	*Rana pretiosa*	60	57	-	nylon ribbon waist band	-	Watson *et al.* 2003
Ranidae	*Rana pipiens*	24	-	-	teflon tubing waist band	<100*	Waye 2001
Rhacophoridae	*Buergeria buergeri*	6	3	<10	rubber waist band	<71*	Fukuyama *et al.* 1988

Table 2.2. Review of radio transmitters, manufacturers, models and specifications. (-) indicates information not provided in publications.

Study Citation	Radio Manufacturer	Model	Package Weight (g)	Reported Transmitter Life (days)
Baldwin *et al*. 2006	Holohil	BD-2a	~8.1	21
Bartelt and Peterson 2000	Holohil	BD-2GT	1.85⁺	120-180
Bartelt *et al*. 2004	Holohil	BD-2GT	1.85⁺	49-117
Blihovde 2006	AVM Instrument	-	1.5	<118
Bosman *et al*. 1996	Self constructed	-	3	200
Bulger *et al*. 2003	Holohil	BD-2G	<2	140
Bull and Hayes 2001	Holohil	BD-2G	1.8	180
Forester *et al*. 2006	Holohil	BD-2G	1.9⁺	84
Fukuyama *et al*. 1988	Self constructed	-	0.9	8
Gelder *et al*. 1986	Self constructed	CVN-3	3	200
Goldberg *et al*. 2002	Holohil	BD-2T/BD-2HT/ BD-2G/BD-2H	1.55/1.45/1.4/1.55	64
Griffin and Case 2001	Holohil/Sirtrack	-	1.8⁺	-
Hodgkison and Hero 2001	Holohil	-	1.5	9-30
Holenweg and Reyer 2000	Holohil	BD-2/BD-2G/BD-2A	-	-
Horan *et al*, unpublished	Holohil	BD-2	0.8	<34
Huste *et al*. 2006	Siritrack	single stage	2.9	540
Koch and Hero 2007	-	-	2.6	105
Kusano *et al*. 1995	Self constructed	-	4.1	120-150
Lamoureux and Madison 1999	L. L. Electronics	SM-1	2.9-4.4	120-240
Lemckert and Brassil 2000	Titley Electronics	single stage	<1	-
Matthews and Pope 1999	Holohil	BD-2	1.5	30
McAllister *et al*. 2004	Holohil	BD-2/BD-2G	1/1.8	49/119
Miaud *et al*. 2000	ATS	292/293	2/2.5	34/194
Miaud *et al*. 2000	Holohil	BD-2GH	1.75	152
Muths 2003	Holohil	BD-2G	1.82	63-112
Nuland and Claus 1981	Self constructed	-	3	200
Oldham and Swan 1992	Biotrack	-	2-2.5	33-47
Pilliod *et al*. 2002	Holohil	BD-2	-	-
Richter *et al*. 2001	Holohil	-	1.44	70
Rowley and Alford 2007a	Holohil	BD-2N/BD-2NT	0.67	21
Roznik *et al*. 2009	ATS	R1625/1655	0.6/1.2	33/155
Schmetterling and Young 2008	Lotek Wireless	-	1.8	21
Seebacher and Alford 1999	Holohil	-	3.3	30-180
Sinsch 1988;1992	Custom Electronics	single stage	2.5	-
Spieler and Linsenmair 1998	Custom Electronics/ Wildlife Materials/ GFT	-	2.5-6	21-120
Watson *et al*. 2003	Holohil	BD-2/BD-2G	1.2/2	49/119
Waye 2001	Holohil	BD-2/BD-2G	1.3/3.5	-
Waye 2001	AVM Instrument	SM-1	2	-
Werner 1991	AVM Instrument	SM-1	1.8-2.3	12-25

CHAPTER 3

CONTROLLED EVALUATION OF NOVEL METHODS OF RADIO ATTACHMENT ON HYLID TREE FROGS

INTRODUCTION

At its conception, the technology for radio telemetry was developed by researchers interested in the physiology of naval aviation pilots (Kenward 2000). Soon after, modifications of these techniques were used by wildlife researchers for the study of heart rate in small mammals (LeMunyan *et al.* 1959) and wing beats of waterfowl (Eliassen 1960). The technology and techniques were soon used worldwide to investigate nearly every aspect of wildlife ecology. Much has been gained through telemetry in the disciplines of wildlife physiology, behavior, and movement (White and Garrott 1990, Millspaugh and Marzluff 2001). With the ever-growing use of radio telemetry as a research tool, more techniques are being developed to attach transmitters to previously unstudied species. It is of the utmost importance that care is given to the evaluation of any effects caused by the attachment of such transmitters. Ultimately, it is the researcher's responsibility to ensure that the applied method of radio attachment does not significantly affect the behavior of the animal in order to maintain study integrity.

Much work has gone into the evaluation of radio tagging of mammals and birds. Kenward (2000) and White and Garrott (1990) provide a thorough review of such studies. These studies have shown that different parameters, such as body condition, behavioral changes, and energetic requirements must be investigated to fully evaluate the different effects that may occur. With the recent miniaturization of electronic components enabling the construction of smaller radio transmitter, frog researchers have joined the crowd by using radio telemetry as a valuable research tool (Nuland and Claus 1981).

Frogs may be susceptible to negative effects caused by the handling, application, attachment and wear of radio transmitters; however, few studies have directly evaluated these effects. In the first study to apply the use of radio telemetry on an anuran, Nuland and Claus (1981) said little more than they observed no behavioral effects from the radio attachment, though the radio packages that they used were up to 13% of an animal's mass. The first mention of using dummy transmitters to evaluate the effects of tagging was by Oldham and Swan (1992), in their study of the forced ingestion of radios. In this experiment, the authors addressed transmitter retention time, food consumption, and body mass changes of frogs from both the laboratory as well as from the field. Again making use of laboratory animals and field trials, Bartelt and Peterson (2000) evaluated a newly developed plastic belt attachment system. Though they used time-lapse videography to quantify the effects of the attachment method on the feeding behavior and activity of their toads and found no significant effects on either. However, a large number of animals in their field evaluation developed sores from the radio attachment, while others prematurely shed their belts. Using a different technology (harmonic radar transponders; predecessors to the now commonly used PIT tags), but a similar evaluation concept, Langkilde and Alford (2002) demonstrated that frogs were not harmed by their diode attachment; however, frogs did move greater distances on the night that the transmitter was attached than during later nights. Coupling previously used evaluation parameters and experimentally introducing mock predator attacks, Blomquist and Hunter (2007) furthered the transmitter evaluation process by taking into account the frog's behavioral response to a predator while carrying a radio transmitter. This new method of field evaluation led the authors to conclude that while the attachment method they used may not have had any significant effects on the biological variables that they were measuring, the slight changes in antipredator behaviors and movements may have long term consequences for the energetics, survival and reproduction of tagged individuals.

An indirect parameter that may be used to evaluate the effects of tagging on study species is the use of leukocyte profiles to measure an animal's stress (Davis *et al.* 2008a). Although it is a non-destructive technique, it requires considerable expertise in the identification and classification of blood cells and an interpretation of the ratios present on a blood smear from the research animal. While this technique has been used as a parameter for similar evaluative studies in avian species (Davis *et al.* 2008b), it has yet to be used with anurans.

Of the currently used and previously evaluated external radio attachment methods available for anuran research, all are similar in that the radio is positioned at the pelvic region of the tagged frogs or toads. For terrestrial species that exhibit a saltorial or hopping means of locomotion, this may be suitable, but for canopy-dwelling species that accomplish much of their movement via long distance jumps or even aerial parachuting, this shift of their center of gravity may prove unacceptable. In an attempt to develop a novel radio attachment technique that centers the mass of the radio transmitter on the study animal, I used multiple biological and observational parameters to evaluate three different external transmitter attachment methods. The outcome of this evaluation was to enlist the top attachment method in the field study of the movements and ecology of a select group of tropical, tree dwelling anurans.

METHODS

Study Species

Due to the limited availability of the target field species, the gliding leaf frog (*Agalychnis spurrelli*) and the veined tree frog (*Phrynohyas venulosa*), a surrogate species, the Cuban tree frog (*Osteopilus septentrionalis*), was used as the study organism for the evaluation of radio attachment methods. Within its native range, the cuban tree frog inhabits tropical forests throughout the Caribbean and West Indies region, including Cuba, the Cayman Islands and the Bahamas (Schwartz and Thomas 1975). This species ranges from 90-140 mm snout-vent-length (SVL), with females being the larger of the sexes. As a generalist predator, its diet includes many small insects and other invertebrates, but it has also been documenting consuming larger prey items such as small lizards and other frogs (Conant and Collins 1998). The robustness of this species and its generalized diet have led to its ability to spread beyond its native range, onto the islands of Puerto Rico and St. Croix, as well as into Florida, USA, and nearby southeastern states (Schwartz and Thomas 1975). Individuals used in this study were collected within Miami-Dade County, Florida, by personnel of Glades Herp Farm (Bushnell, FL) and shipped to the University of Georgia immediately after collection.

All animals were maintained in a closed greenhouse in the Whitehall Experimental Forest of the Warnell School of Forestry and Natural Resources, Athens Georgia. The greenhouse was constructed of a cement block lower wall with a metal-reinforced glass upper wall and roof, on top of a poured concrete floor that measured approximately 5 x 20m in size. Throughout the study period, temperatures of 25-30$^{\circ C}$ and a relative humidity of 70-85% were maintained, with a low temperature of 18$^{\circ C}$ reached during an extreme weather event occurring for only a short time during the initial acclimation period. This controlled climate was maintained via the use of thermo-controlled ceiling mounted furnace units and a continuous misting system within the greenhouse. Lighting in the greenhouse was provided by a combination of natural light from the clear-glass roof and supplementary light provided from a mercury-halide lighting system. A 12:12 day/night photoperiod was maintained throughout the duration of the study. Two overhead incandescent red lamps were used for nighttime illumination of the greenhouse to provide light for the researcher while not disturbing the day/night cycle of the frogs. All animal housing and handling procedures were approved by the University of Georgia's Institutional Animals Care and Use Committee under Animal Use Permit A2009-10045 (Appendix A).

Experimental Design

Frogs were received in two separate shipments, the first shipment being used in Experiment 1 and the second shipment being used in Experiments 2 and 3. Upon arrival, individuals were sorted and paired by similarity of size. This stratification was required to avoid any incidental cannibalism, which has been known to occur with this species (Conant and Collins 1998). Pairs were randomly placed in one of 24, 65-gallon Reptarium® screen-sided enclosures (Dallas, Texas, USA). All enclosures were identically fitted with cypress mulch flooring, natural branches, artificial vines (Repti-vine®, ZooMed Laboratories, Costa Mesa, CA, USA), and a water bowl. This cage design allowed the animals to express their natural behaviors of burrowing, climbing, jumping, and feeding. The enclosures were arranged in two, parallel rows along the walls and placed on the top of two metal workbenches to maintain air circulation. This provided a central walkway with access to all enclosures for maintenance, as well as a location for the behavioral observations described later (Figure 3.1). Prior to all experiments, frogs underwent a 14-day acclimation period, during which time no handling occurred and a regular feeding schedule was established. Pairs were fed 10 large sized, captive-bred crickets per cage every three days. Crickets were dusted with a calcium/mineral supplement on every third feeding event. At the beginning of each experiment, all frog pairs underwent identical handling and processing until each individual was assigned to an experimental group.

Frogs were anesthetized in pairs by placing them in a small plastic container partially filled with a 0.5 g/L solution of sodium bicarbonate buffered MS-222 (2:1 ratio) (Finquel®, Argent Chemical Laboratories, Redmont, WA, USA) (Stetter 2007). Frogs were considered anesthetized when they were unresponsive to physical stimulation, at which point the pair was removed from the solution and placed on an aseptic workstation. Pairs were randomly split between a "control" individual and a "treatment" individual. Measurements of snout-vent length (SVL) (mm) and mass (g) were then taken. Following the administration of the experimental attachment methods described below, the pair was rinsed with a low concentration saline solution and placed in a fresh plastic container and monitored until complete recovery from anesthesia, as designated by the return of locomotive ability and response to physical stimulation. Following recovery, pairs were returned to their original enclosure. This design was used to achieve a stratified, complete block design with randomized cage location.

Experiment 1: Vest Attachment (VEST)

Experimental group 1 was received on 24 January 2008 and underwent processing on February 7. Individuals (n = 22) were fitted with an external transmitter vest constructed of Under Armour® four-way stretch material (Baltimore, MD, USA). Vests were individually cut to custom-fit each individual, and wrapped around the pectoral region of the body with holes cut to fit the front limbs of the frogs (Figure 3.2). The material was glued at a seam on the dorsal side of the frogs using Gorilla glue® (Cincinnati, OH, USA). Mock-transmitters constructed to weigh ~3% of the body mass of each treatment frog were glued to the posterior surface of the material with the same adhesive as above. This achieved a fitted design resembling a backpack or vest.

Experiment 2: Skin Attachment (SKIN)

Experimental group 2 was received on 22 March 2008 and underwent processing on April 6. Individuals (n = 12) were fitted with a mock-transmitter (~3% body mass) sutured to the skin at midline on the caudodorsal surface of the frog. To have a workable surface through which a suture could be attached, mock-transmitters were attached to the small portion of the above stretch material. With the anesthetized treatment animal lying in ventral recumbency, the transmitter was attached at two points of contact. Sutures were attached to two 10 mm-long catheter tubes, which were implanted subcutaneously, running parallel to, and situated approximately 5 mm on either side of, the vertebral column. A single strand of 3-0 PDS*II (17mm 1/2c taper) suture (ETHICON Inc., Somerville, NJ, USA) was passed through each catheter tube, secured to the transmitter and held with a double simple-interrupted knot. Points of entry and trimmed knots were covered with Nexaband S/C topical tissue adhesive (Abbott, North Chicago, IL, USA). (Figure 3.3).

Experiment 3: SKELETAL Attachment (SKELETAL)

Experimental group 3 was received on 22 March 2008 and underwent processing on April 6. Individuals (n = 12) were fitted with a mock-transmitter (~3% body mass) sutured to the caudodorsal aspect of the frog (Figure 3.4). To have a workable surface through which a suture could be attached, mock-transmitters were attached to the small portion of the above stretch material. With the anesthetized treatment animal lying in ventral recumbency, the transmitter was attached at two points of contact near the center of the posterior surface, via passing a 3-0

PDS*II (17mm 1/2c taper) suture through the skin, intercostals muscles, passing between the ribs, into the caudal aspect of the ceolomic cavity, under the vertebral column and back through the ribs, muscles and skin of the animal. Sutures were held with a double simple-interrupted knot. Points of entry and trimmed knots were covered with Nexaband S/C topical tissue adhesive.

Behavioral observations

Behavioral observations were started on the third night following the application of the experimental treatments and were repeated every three nights. Beginning one hour after sunset, two observers were each randomly assigned half of the enclosures to observe. For each observation, a 50-watt incandescent red lamp was placed on the top of the assigned enclosure to provide lighting for the observer. Ten crickets were placed at the center of the enclosure floor. On a minute-by-minute basis, for a period of ten minutes, all jumps, successful feeding events and other movements were recorded. A jump was defined as any movement that involved a frog's body being completely removed from contact with it perch. Successful feedings were recorded when a frog captured and consumed a cricket. Examples of other activities tallied as movements included failed feeding attempts, walking, changing of directional orientation or movement of the front or rear limbs, with each event being separated by a two-second interval.

Post-Experimental Data Collection

On the days concluding each of the experiments, all frogs from the respective session were placed into plastic containers and transferred to the Southeast Cooperative Wildlife Disease Study's facility, located at the University of Georgia. Frogs were deeply anesthetized with a 1.0 g/L solution of sodium bicarbonate buffered MS-222 (2:1 ratio). Upon complete anesthesia, frogs were measured, weighed and visually inspected for any injuries caused by their experimental transmitter attachment method. All injuries or malformations were photographed for later reference.

To acquire blood for the evaluation of stress-related hematological parameters, the heart was exposed by making a ventral midline incision into the coelom and extending it cranially. Intracardiac venipuncture was performed using a 0.5ml insulin syringe, as much as possible was collected. A blood smear was made immediately with fresh blood directly from the syringe, after cutting off the needle. The remaining blood was transferred to a lithium heparin laced blood collection tube to prevent clotting and maintained on ice until submission for a complete blood cell count (CBC). Immediately after blood collection, frogs were euthanized by pithing the brain with a 25 ga needle. Complete blood cell counts were performed by Dr. Shawn M. Zimmerman, DVM, of the College of Veterinary Medicine Diagnostics Lab, University of Georgia. Blood smears were evaluated by Dr. Andy Davis, Ph.D., of the Warnell School of Forestry and Natural Resources (WSFNR), University of Georgia, to develop heterophil-lymphocyte (H/L) ratios (Davis *et al.* 2008a). Following euthanasia, a complete gross necropsy was performed. Any internal injuries caused by the experimental attachment methods were recorded. The liver and fat bodies were collected and weighed (g). The intestinal tracts were removed for examination of parasite infections. The gender and reproductive status of all frogs were also noted. The entire frog, including dissected organs were fixed in 10% buffered formaldehyde for later histopathologic examination. All histopathology was performed by Dr. Kevin Keel, DVM, Ph.D., of the Southeastern Cooperative Wildlife Disease Study.

Data analysis

Behavioral observations

Behavioral data were analyzed as a repeated measures analysis of variance, using SAS 3.0. For this data set, the response variables were JUMPS, MOVEMENTS and CAPTURES, with the explanatory variables being type of attachment, cage number, observation period and gender. Due to the Poisson distribution of the response variables JUMPS and MOVEMENTS, these variables were converted by a square root transformation. This resulted in the variables being approximately normal, with constant standard deviation. For the CAPTURES variable, the number of crickets captured by each frog was constrained by the maximum number of crickets available during each observation period (n=10). Since not all crickets were always eaten, and occasionally none eaten, this made the CAPTURES variable multinomially distributed. To avoid difficulties associated with this type of data set, CAPTURES was converted to a new variable, DIFFCAPS, which is the difference between the number of food items captured by the control animal and the treatment animal in a single enclosure. Using these variables, this data set was analyzed as a repeated measures experiment with each frog being the unit of measure. For comparison of experimental attachment methods, analyses from all experiments are presented together, with control frogs from the three experiments being pooled. Behavioral analyses were performed in SAS (SAS Institute Inc., Cary, NC, USA).

Biological parameters

Standard morphometric and body mass measurements were gathered from each animal, both at the initiation and conclusion of the experimental periods. These measurements were then transformed into DELTA LENGTH and DELTA WEIGHT variables to calculate changes at the individual level. Additionally, the liver, intestinal tract and any visible fat bodies were removed from each frog during post-experiment necropsies. Livers and fat bodies were weighed to develop a liver:body mass ratio and fat/body mass ratio. Intestinal tracts were dissected and an intestinal wash performed by Dr. Michael Yabsley, WSFNR, to determine parasite presence and infection rates. A one-way ANOVA test of significance was performed for each of the biological parameter variables, with the explanatory variables being the experimental group, sex and cage. These analyses were performed in Statistica (STATISTICA 6.1, Stat Soft, Inc., Tulsa, OK, USA).

Hematological parameters

From the blood smears, the ratios of neutrophils to lymphocytes (N/L ratio) were determined for each individual. This variable was square root transformed to approximate a normal distribution. Differences in N/L ratios between groups were analyzed with a one-way ANOVA in Statistica (STATISTICA 6.1, Stat Soft, Inc., Tulsa, OK, USA). Complete blood count evaluations were not included in the analyses.

RESULTS

Experiment 1: Vest Attachment (VEST)

One day after the application of the VEST method, 11 of the 22 treatment individuals prematurely shed their transmitters. During the observation period, 4 additional individuals also removed their transmitters, with only 7 individuals from this group continuing to hold their transmitters until the experimental observations were terminated. On day 17 of the experiment, multiple treatment frogs were found with swollen limbs and skin excoriations located at the contact points of the harness and the arms of the frogs. Due to animal welfare concerns, Experiment 1 was immediately terminated, and all remaining frogs euthanized and processed. Six observational periods were completed.

Experiment 2: Skin Attachment (SKIN)

Beginning approximately 4 days following the application of the experimental attachment method, multiple frogs began shedding their transmitters. The skin at the points of attachment appeared to be sloughing due to the pressure of the internal stent. By day 12 of the experiment, all treatment frogs had dropped their transmitters or were in the process. The skin of individuals that had dropped them early in the experimental period was already healing, some to the point that it was difficult to distinguish the treatment individuals from the control individuals. At this point, observations on Experimental group 2 were terminated and all frogs euthanized for post-processing. Four behavioral observation periods were completed.

Experiment 3: SKELETAL Attachment (SKELETAL)

Individuals from this group did not display any externally visible injuries or other effects from the attachment procedure. All frogs remained in the experiment for the full period. On May 3, 2009, all frogs were euthanized for post-processing. During the post experimental necropsy, three individuals displayed skin abrasions underneath the mock transmitters. These injuries appeared to have been caused by the continued rubbing of the material housing the mock transmitter. Ten observational periods were completed.

Behavioral observations

Jumps and movements

From the Repeated Measures analysis, the response variables demonstrated an increased trend over time during the SKIN and SKELETAL trials, no matter if they were from treatment or control groups. This was not the case for the VEST treatment group, which displayed decreasing trends following the second observation period (Figure 3.5). For the VEST experiment, the number of both JUMPS and MOVEMENTS was different ($P < 0.001$and $P = 0.001$, respectively). For the SKIN experiment, neither JUMPS nor MOVEMENTS were different from the control groups ($P = 0.26$ and $P = 0.60$, respectively). In the SKELETAL experiment, the number of JUMPS was different, but the number of MOVEMENTS was insignificant ($P = 0.03$and $P = 0.60$, respectively) (Table 3.1). Only the treatment effect was important in the MOVEMENTS analysis ($F_{1, 18} = 12.83$, $P = 0.001$), with treatment ($F_{1, 18} = 28.77$, $P < 0.001$), cage ($F_{6, 18} = 2.90$, $P = 0.014$), and gender ($F_{1, 18} = 5.92$, $P = 0.017$) being significant in the JUMPS analysis. (Table 3.2)

Prey Capture

In all three experiments, the DIFFCAP variable averaged to a negative value (Figure 3.6). This indicates that the experimental animals for all groups consumed fewer prey items than their respective control animals, overall. This difference was significant for the VEST experiment ($t_{174, 522} = -2.83$, $P = 0.006$), but not different for the SKIN ($t_{174, 522} = 0.05$, $P = 0.96$) and SKELETAL experiments ($t_{174, 522} = -1.86$, $P = 0.06$) (Table 3.3). In the VEST experiment, the downward trend of the DIFFCAPS variable began almost immediately following the first observation. During the SKIN experiment, both groups were consuming near the same amount of prey early in the experiment.

Biological parameters

Snout-vent-length

The DELTA LENGTH variable was significantly different between experimental attachment types ($F_{5, 32} = 3.16$, $P = 0.02$), with cage number and gender having no effect. Among the attachment types, both the treatment and control animals from the SKELETAL experiment had negative values, indicating a decrease in the snout-vent-length measurement from the initial and final measurement periods (Figure 3.7)

Mass

The experimental attachment type and cage variables were important in the analysis of the DELTA WEIGHT variable (attachment: $F_{5, 32} = 2.49$, P = 0.05; cage: $F_{23, 32} = 2.09$, P = 0.03), with gender having no effect. These differences appear to be due to the animals from the SKIN experiment gaining relatively more weight during the observation period than the animals from the other experiments (Figure 3.8). Further analysis of the cage variable indicated that cage number 18 from the SKIN attachment experiment had a lower DELTA WEIGHT value than all other cages, causing the overall difference (Figure 3.9).

Liver:Body mass %

The Vest control group had a greater liver:body mass ratio than all other experimental groups ($F_{5, 32} = 6.23$, P < 0.001) (Figure 3.10). Cage and gender had no effect.

Fat:Body mass %

Attachment method had an effect on the fat:body mass percentage variable ($F_{5, 32} = 9.08$, P = 0.001), with both treatment and control groups from the SKELETAL experiment have little to no visible fat bodies, all animals from the SKIN experiment and the treatment individuals from the VEST experiment having slightly more, and the control individuals from VEST experiment having the most (Figure 3.11). Cage and sex did not have any significant effect.

Hematological parameters

The experimental group had an effect on the N/L ratios of frogs ($F_{5, 28} = 17.66$, P < 0.001). While the square root transformed values greatly differed between the treatment and control groups within the VEST experiment, as well as between the SKIN and SKELETAL experiments, they were not significantly different within the treatment and control groups of the respective experiments (Figure 3.12).

DISCUSSION

Of the three attachment methods evaluated, all resulted in a shift in behavior from that of the control individuals. Although the VEST attachment method appeared to be a suitable technique early in the experiment, it later proved to be an unacceptable method due to the later effects that it had on both the behavioral and biological parameters measured. The SKIN technique also appeared to be a suitable attachment method, but did not provide the required longevity of a method suitable for field application. While the SKELETAL method appeared to have a slight negative effect on the study animals early in the experiment, it provided the only tested method that maintained its integrity for the entire study period. Though the behavioral parameters differed between the control and experimental animals during the first 10-12 days of observations, the remaining observations did not show any differences between the parameters of the two groups, inferring that if used in the field, caution should be exercised when analyzing data from this early period.

Table 3.1. Repeated Measures Analysis for the Movement and Jump variables among the control and treatment groups of 3 experimental attachment methods applied to Cuban tree frogs, *Osteopilus septentrionalis*, presenting differences and P values for VEST, SKIN and SKELETAL experiments (*n* =7 for both groups in VEST experiment, *n* =12 for both groups in SKIN experiment and *n* = 12 for both groups in SKELETAL experiment).

Effect: Treatment		Movement			Jumps		
		LS means	diff	P	LS means	diff	P
VEST	Control	3.13	-0.98	0.0011	2.40	-1.14	<0.0001
	Treatment	2.15			1.26		
SKIN	Control	2.08	-0.12	0.60	1.86	-0.28	0.26
	Treatment	1.96			1.58		
SKELETAL	Control	2.66	-0.09	0.53	2.50	-0.441	0. 03
	Treatment	2.57			2.06		

Table 3.2. ANOVA table for the response variables when taking into account the gender effect and the interaction of gender and treatment of 3 experimental attachment methods applied to Cuban tree frogs, *Osteopilus septentrionalis*. Only the treatment effect was important in the MOVEMENTS analysis ($F_{1, 18}$ = 12.83, P = 0.001), with treatment ($F_{1, 18}$ = 28.77, P < 0.001), cage ($F_{6, 18}$ = 2.90, P = 0.014), and gender ($F_{1, 18}$ = 5.92, P = 0.017) being significant in the JUMPS analysis (*n* =7 for both groups in VEST experiment, *n* = 12 for both groups in SKIN experiment and *n* = 12 for both groups in SKELETAL experiment).

	Source	**df**	**Type III SS**	**Mean Square**	**F Value**	**P**
Movements	Trt	1	21.778	21.778	12.830	<0.001
	Obs	1	5.153	5.153	3.040	0.09
	Cage	6	12.125	2.021	1.190	0.32
	Gender	1	1.704	1.704	1.000	0.32
Jumps	Trt	1	34.155	34.155	28.770	<0.001
	Obs	1	3.697	3.697	3.110	0.08
	Cage	6	20.627	3.438	2.900	0.014
	Gender	1	7.032	7.032	5.920	0.02

Table 3.3. Estimate values of difference in prey captures and the p-value of comparing the difference with zero in the Differential Capture variable of 3 experimental attachment methods applied to Cuban tree frogs, *Osteopilus septentrionalis*. This difference was significant for the VEST experiment (*n* =7 for both control and treatment groups) but not different for the SKIN (*n* = 12 for both groups) and SKELETAL experiments (*n* = 12 for both groups).

Experiment	**Estimate**	**Error**	**df**	**t Value**	**P**
Vest	-2.16	0.763	174	-2.83	0.005
Skin	0.04	0.763	174	0.05	0.96
Skeletal	-1.10	0.590	174	-1.86	0.06

Overhead Heating Unit	Misting System	Enclosure 13
Enclosure 12		Enclosure 14
Enclosure 11		Enclosure 15
Enclosure 10		Enclosure 16
Enclosure 9		Enclosure 17
Enclosure 8		Enclosure 18
Enclosure 7		Enclosure 19
Enclosure 6		Enclosure 20
Enclosure 5		Enclosure 21
Enclosure 4		Enclosure 22
Enclosure 3		Enclosure 23
Enclosure 2		Enclosure 24
Enclosure 1	Misting System	Overhead Heating Unit

Figure 3.1. Diagram of Whitehall Forest experimental green house, floor plan. Overall dimensions for the greenhouse were approximately 5 x 20 m. Lighting was provided by both natural and artificial light and maintained for a 12:12 photoperiod. Overhead heating was provided by furnace units and humidity was maintained by a continuously operating misting system.

Figure 3.2. Photograph of a Cuban tree frog, *Osteopilus septentrionalis*, fitted with a VEST attachment of a radio transmitter. Transmitter and attachment materials were equal to approximately 3% of the frog's mass.

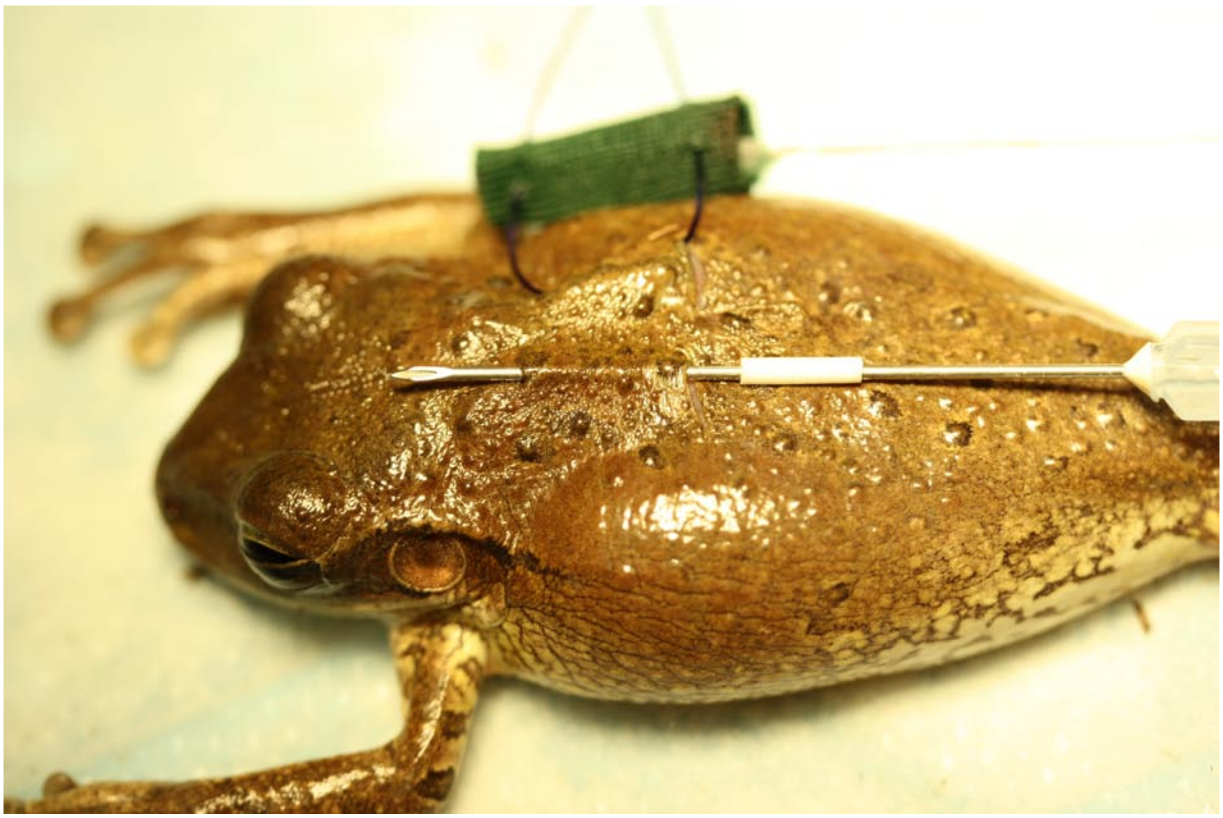
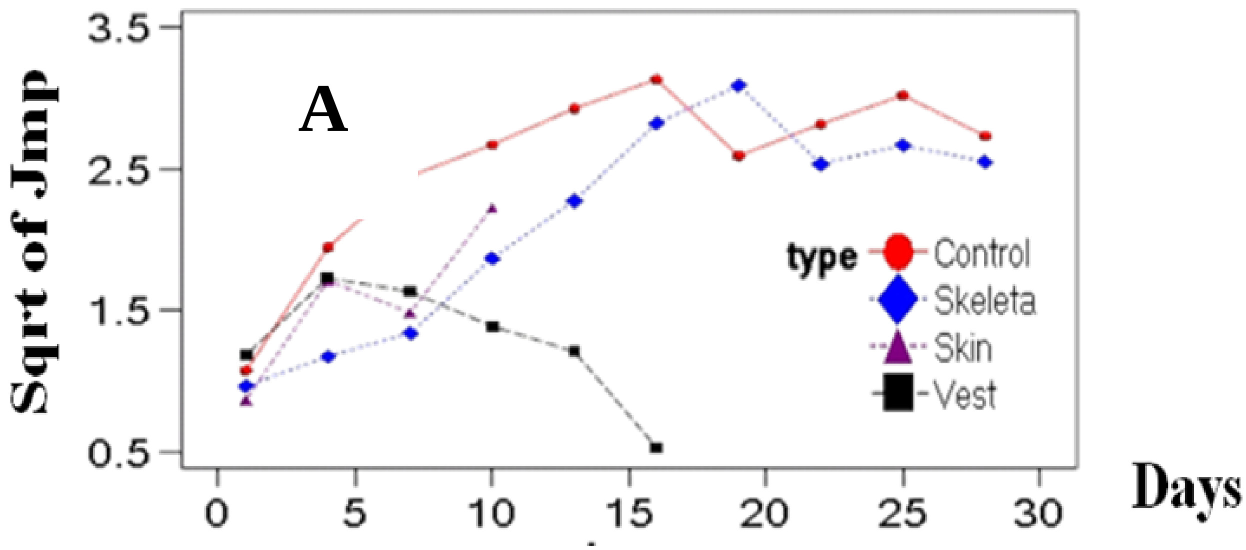


Figure 3.3. Photograph of a Cuban tree frog, *Osteopilus septentrionalis*, being fitted with a SKIN attachment of a radio transmitter. Transmitter and attachment materials were equal to approximately 3% of the frog's mass.

Figure 3.4. Photograph of a Cuban tree frog, *Osteopilus septentrionalis,* fitted with a SKELETAL attachment of a radio transmitter. Transmitter and attachment materials were equal to approximately 3% of the frog's mass.



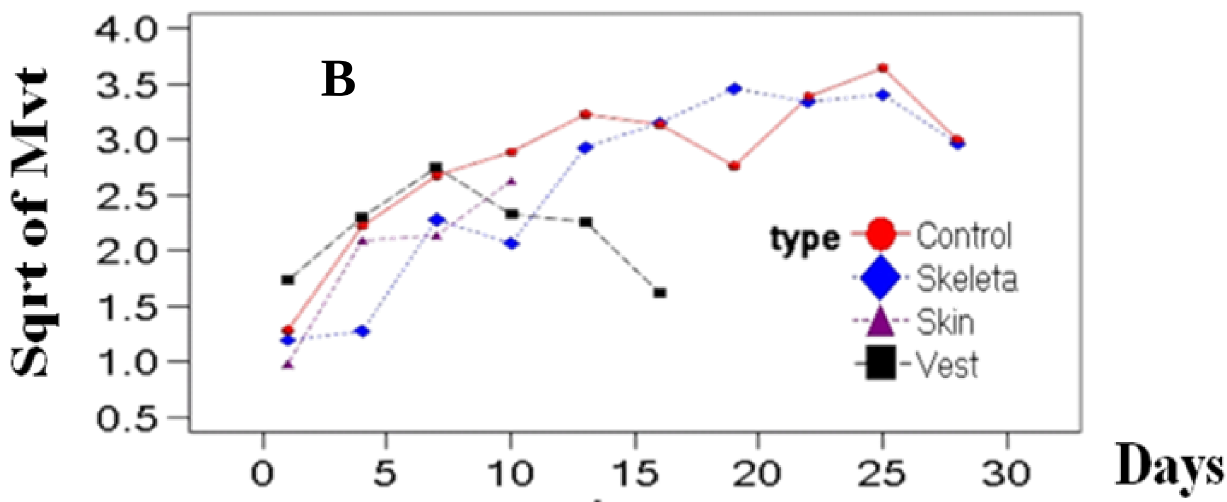


Fig 3.5. Repeated Measures Analysis Time series plot of Squarer root Jumps (A) and Square root Movements (B) variables among the control and treatment groups of 3 experimental attachment methods applied to Cuban tree frogs, *Osteopilus septentrionalis*, presenting trends for VEST, SKIN and SKELETAL experiments (n =7 for both groups in VEST experiment, n = 12 for both groups in SKIN experiment and n = 12 for both groups in SKELETAL experiment). For demonstration purposes, the values from control groups are pooled.

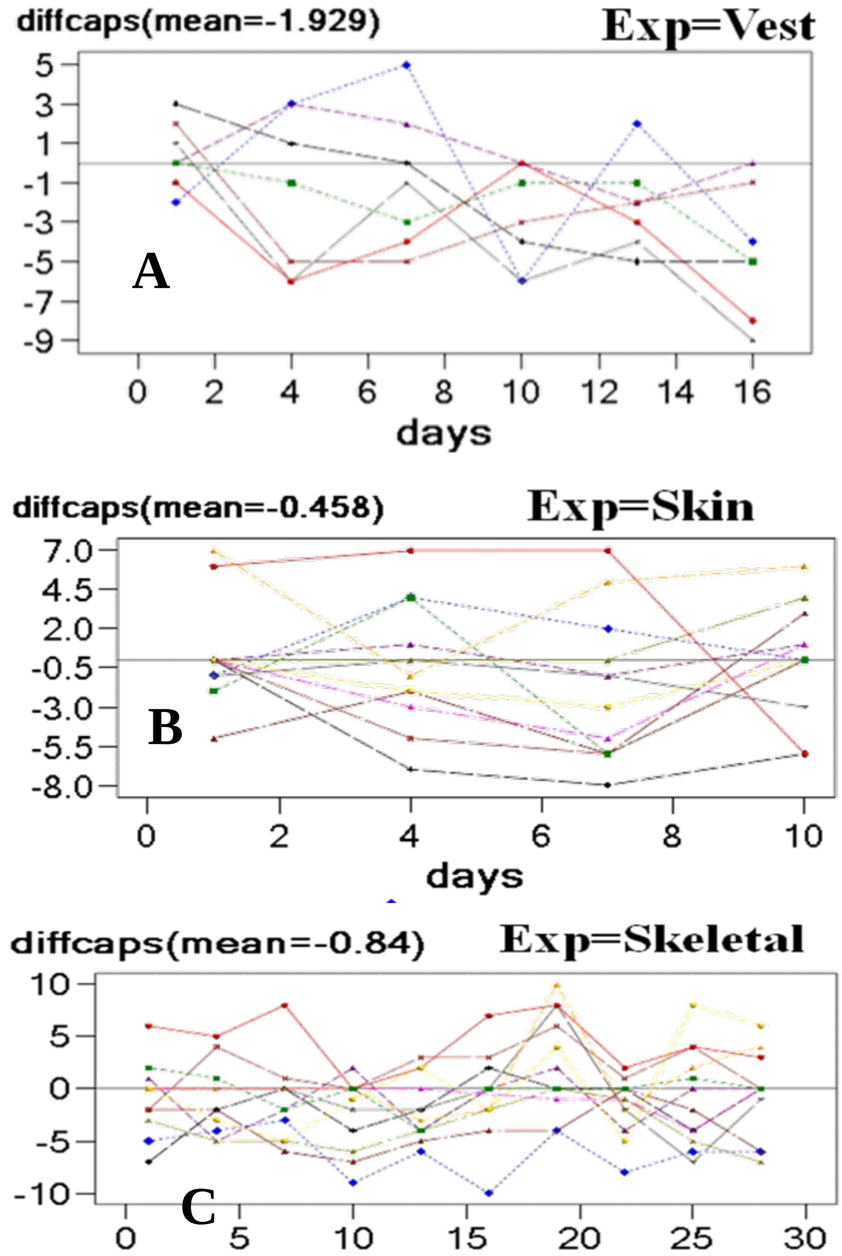


Figure 3.6. Repeated Measures Analysis Time series plots for the differences in number of food items captured between treatment and control individuals of 3 experimental attachment methods: VEST (A) (*n* = 7 pair), SKIN (B) (*n* = 12 pair) and SKELETAL (C) (*n* = 12 pair). Each line represents a pair of frogs in one cage.

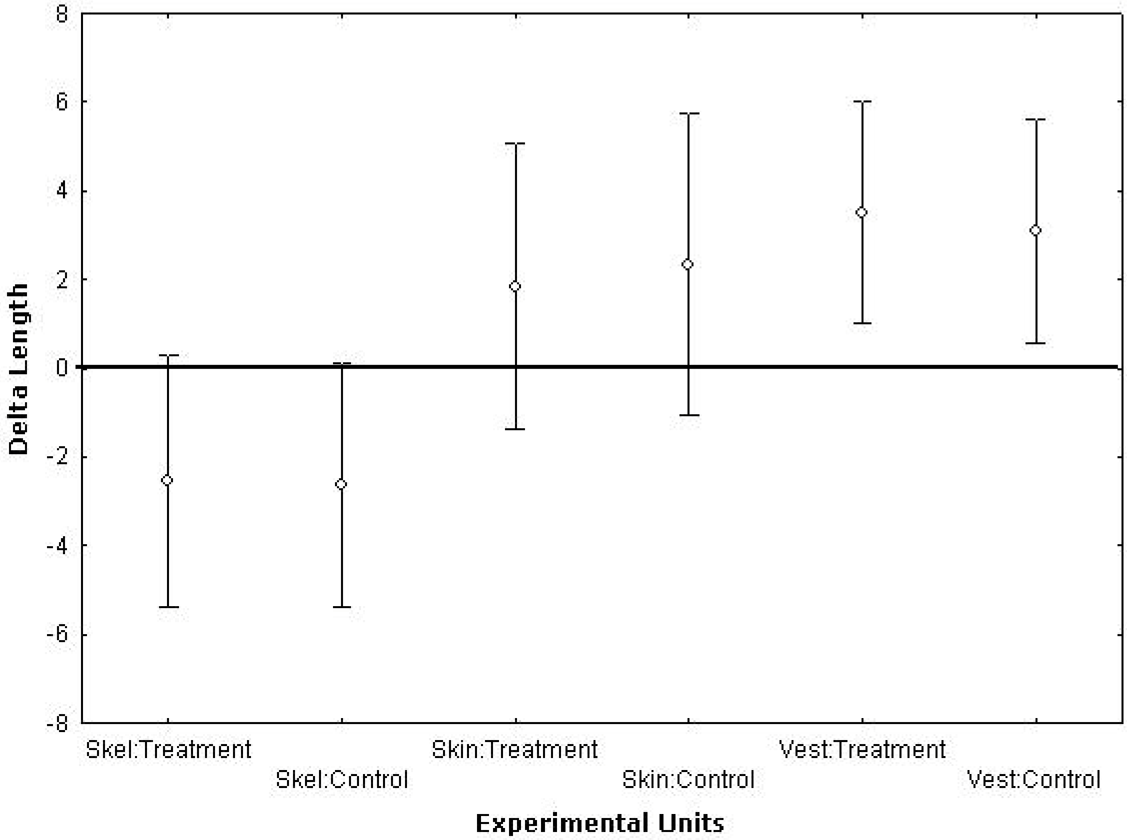


Figure 3.7. Delta Length variable of Cuban tree frogs, *Osteopilus septentrionalis*, from treatment and control frogs from VEST, SKIN, and SKELETAL experiments (n = 7 for both groups in VEST experiment, n =12 for both groups in SKIN experiment and n = 12 for both groups in SKELETAL experiment). Error bars represent 95% CI.

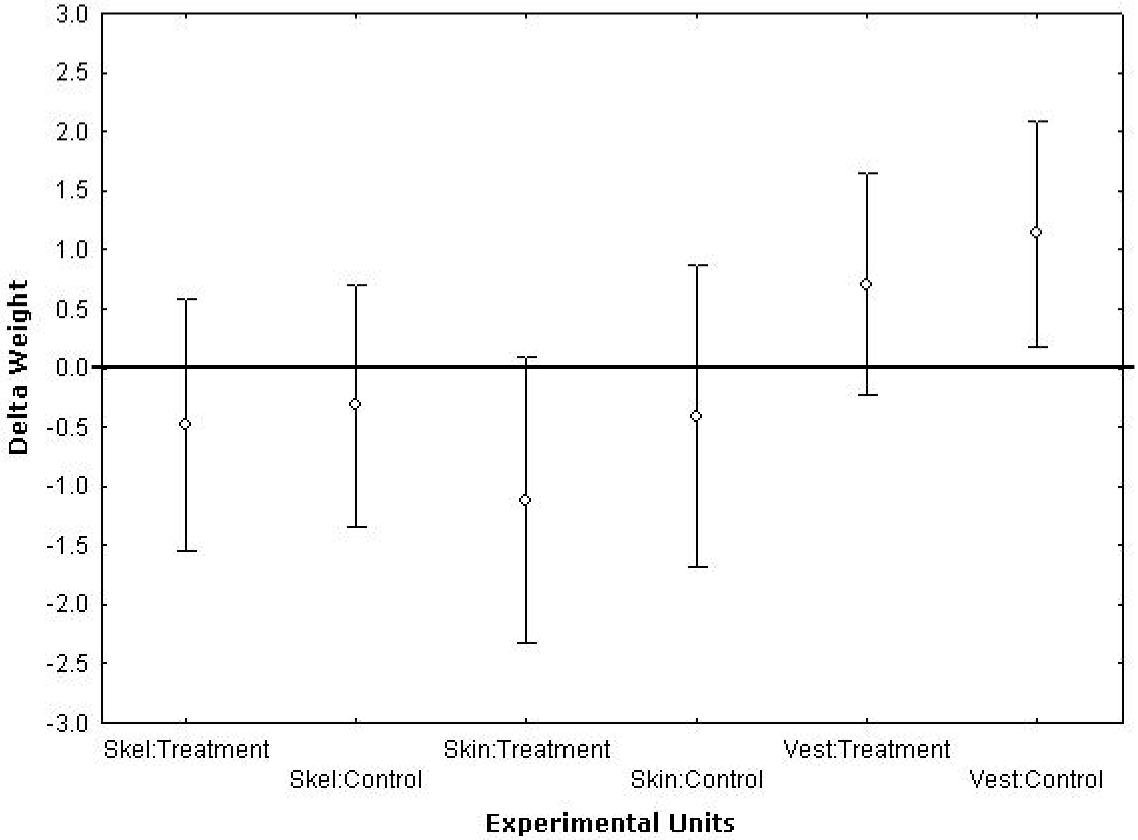


Figure 3.8. Delta Weight variable of Cuban tree frogs, *Osteopilus septentrionalis*, from treatment and control frogs from VEST, SKIN, and SKELETAL experiments (n = 7 for both groups in VEST experiment, n = 12 for both groups in SKIN experiment and n =12 for both groups in SKELETAL experiment). Error bars represent 95% CI.

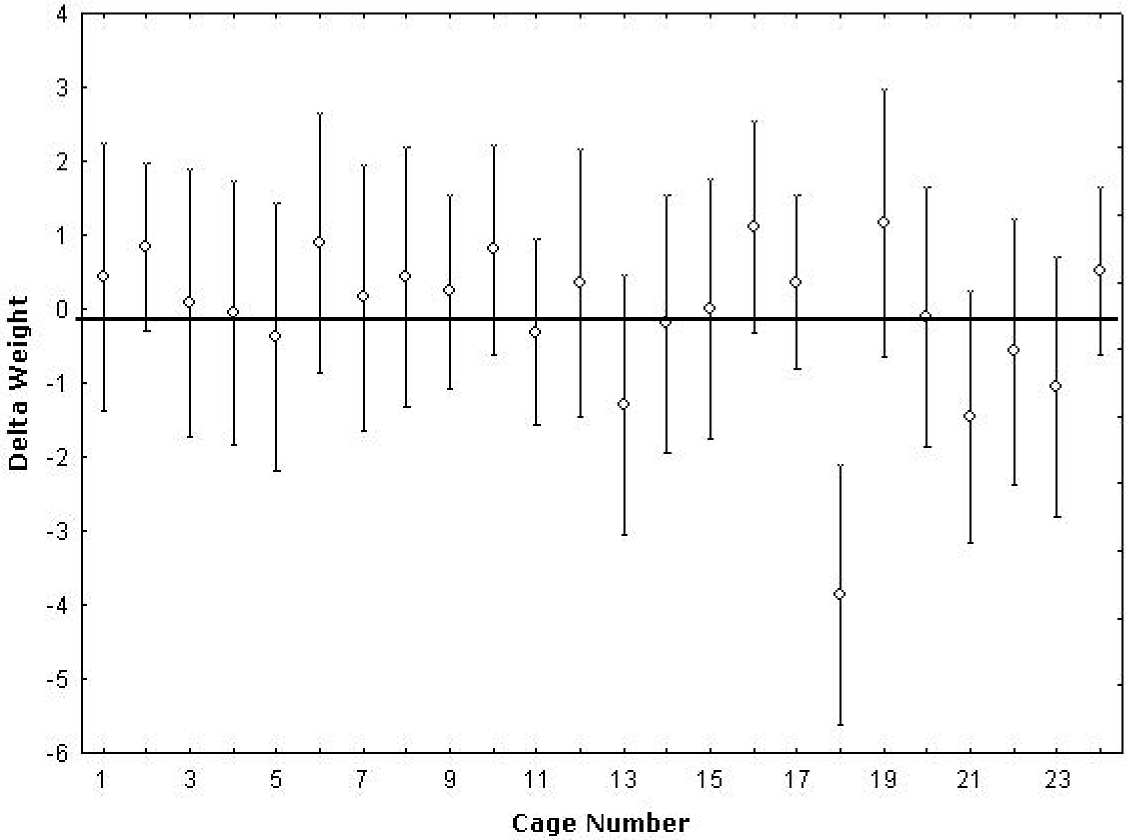


Figure 3.9. Cage by cage breakdown of the values from the Delta Weight analysis of Cuban tree frogs, *Osteopilus septentrionalis*, from treatment and control frogs from VEST, SKIN, and SKELETAL experiments (n = 7 for both groups in VEST experiment, n =12 for both groups in SKIN experiment and n = 12 for both groups in SKELETAL experiment). Error bars represent 95% CI.

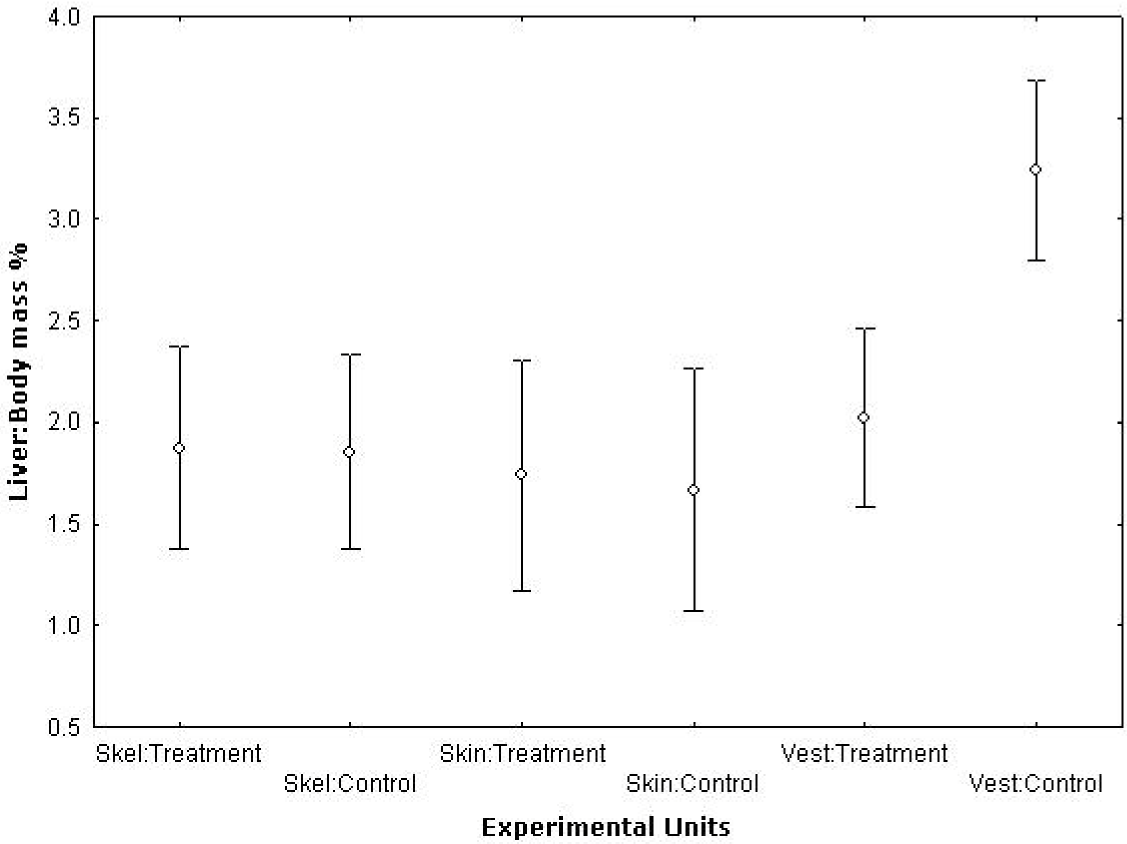


Figure 3.10. Liver:body mass % variable of Cuban tree frogs, *Osteopilus septentrionalis*, from treatment and control frogs from VEST, SKIN, and SKELETAL experiments (*n* = 7 for both groups in VEST experiment, *n* = 12 for both groups in SKIN experiment and *n* = 12 for both groups in SKELETAL experiment). Error bars represent 95% CI.

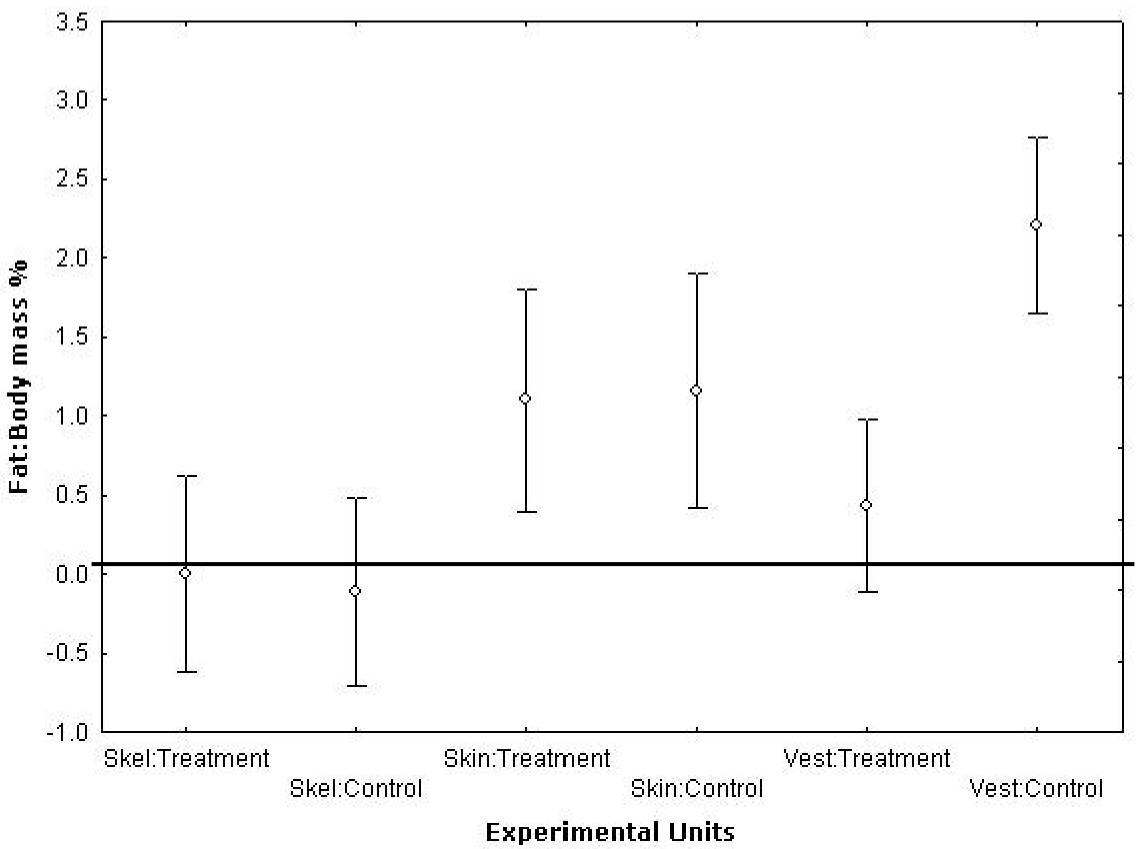


Figure 3.11. Fat:body mass % variable of Cuban tree frogs, *Osteopilus septentrionalis*, from treatment and control frogs from VEST, SKIN, and SKELETAL experiments (n = 7 for both groups in VEST experiment, n = 12 for both groups in SKIN experiment and n = 12 for both groups in SKELETAL experiment). Error bars represent 95% CI.

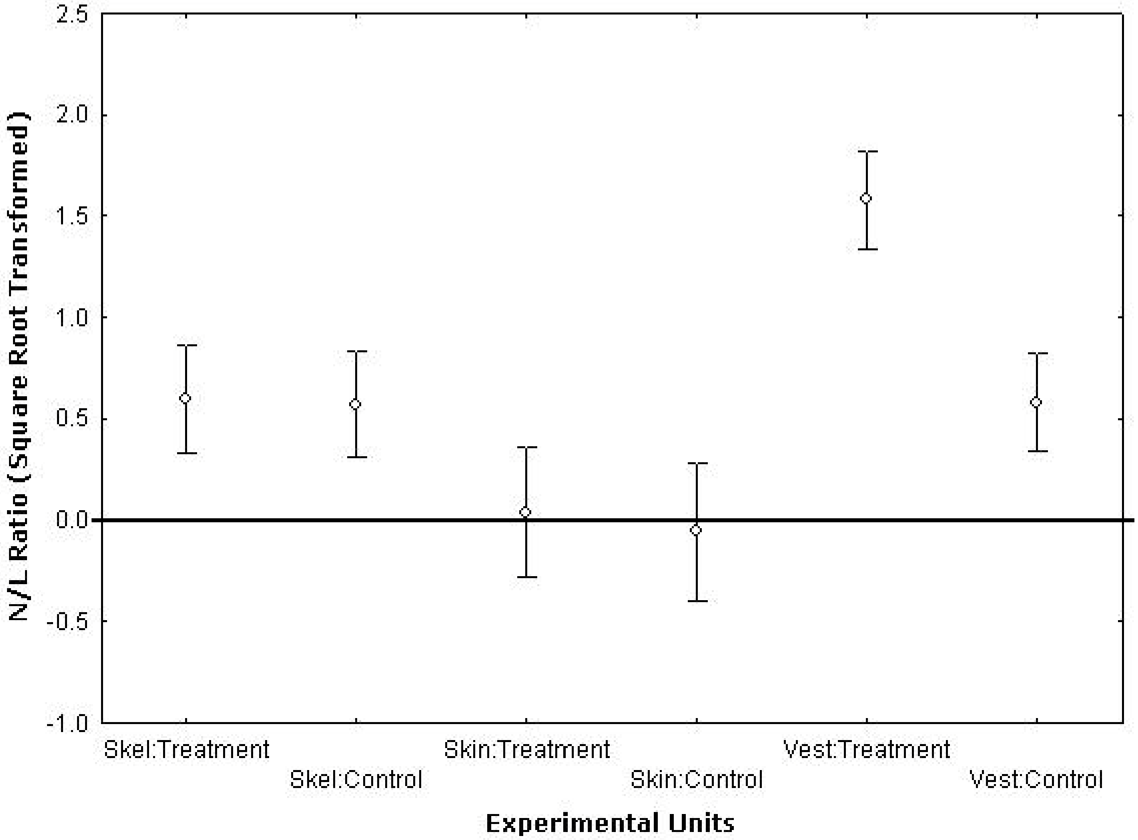


Figure 3.12. Neutrophil/lymphocy (N/L) ratio of Cuban tree frogs, *Osteopilus septentrionalis*, from treatment and control frogs from VEST, SKIN, and SKELETAL experiments (n = 6 for both groups in VEST experiment, n = 11control and n = 12 treatment in SKIN experiment, n = 11control and n = 12 treatment in SKELETAL experiment). Error bars represent 95% CI.

CHAPTER 4

FIELD APPLICATION OF NOVEL TRANSMITTER ATTACHMENT TECHNIQUES TO STUDY THE MOVEMENTS AND ACTIVITY PATTERNS OF HYLID TREE FROGS AT KINGFISHER POND, BARRO COLORADO ISLAND, PANAMA

INTRODUCTIONS

Amphibian species are declining worldwide, with up to one-third of all species currently listed as either threatened or endangered (Stuart *et al.* 2004). Latin America, specifically, has experience dramatic declines in recent years (Lips 1999), with much of the decline being attributed to the fungal pathogen *Batrchochochytrium dendrobatidis* (Kiesecker *et al.* 2001, Collins and Storfer 2003). Even as some species disappear, our ecological knowledge of many is still lacking. Specifically, the terrestrial ecology of most pond-breeding amphibians is poorly understood (Dodd and Cade 1998a). While it has long been established that frogs migrate between breeding and terrestrial habitats, the two systems have not received equal attention. To better understand the evolutionary history, population ecology, and ultimately, conservation management strategies of these species, equal emphasis should be placed on both habitats (Baker 1978, Semlitsch and Bodie 1998). Currently, the estimates of a landscape to maintain species that require multiple habitat types to complete their life cycle are grossly underestimated, as they rely on research that focuses on the breeding period alone (Lamoureux and Madison 1999, Pope *et al.* 2000). The highest diversity of amphibian species occurs within the tropics (Savage 2002). Major die-offs in the tropics have prompted greater interest in research on tropical amphibians ecology; however, the terrestrial component of the life cycle is still being overlooked, often due to the difficulties associated with accessing such systems and tracking small animals.

The objective of this research was to investigate the terrestrial ecology of two species of arboreal hylid, the gliding leaf frog, *Agalychnis spurrelli*, and the veined tree frog, *Phyrnohyas venulosa*.

METHODS

Study Site

The study was conducted within the forests of Barro Colorado Island (BCI), Colon Province, Republic of Panama. BCI is an isolated island of 1,500 hectares, with forest characterized as a diverse lowland tropical forest (Leigh *et al.* 1996). On the average, BCI receives *ca* 2600mm of precipitation annually, with a pronounced dry season lasting from January – April of each year (Windsor 1990). Specifically, the focal site on the island was centered on Kingfisher Pond (KFP), an approximately 0.25 hectare ephemeral pond located east of Standley trail marker 5. The forest around KFP is designated as old growth and characteristic of that found on the western portion of BCI (Croat 1978), with the exception of the *Elaeis* palms that grow at the pond margin (Figure 4.1). For a detailed account of the fauna and flora of BCI, see Leigh *et al* (1999) and Croat (1978).

Agalychnis spurrelli Boulenger, 1913, and *Phrynohyas venulosa* Laurenti, 1768, are both nocturnal, arboreal canopy-dwelling species, which move through the canopy by climbing vines and branches using a hand-over-hand locomotion and parachuting or gliding while leaping to move horizontally (Savage 2002). Similarly, both species are characterized as explosive breeders, breeding in isolated pools during the first heavy rains following the dry season. Much of the challenge in gaining critical demographic data on this group is due to their arboreal behavior, as is similar with the majority of the hylids. As canopy dwelling genera, *Agalychnis* and *Phrynohyas* are usually observed at breeding sites, and rarely observed in bromeliads and felled trees (Savage 2002). Although observations of explosive breeding clusters of both of these species have been documented in the literature as long ago as 60 years, little has been learned about the behavior of the species in the canopy, where presumably they spend the rest of year (Scott and Starrett 1974, Gray 1997, Savage 2002). All animals included in this study were captured at night by hand grab at the breeding pond. All animal housing and handling procedures were approved by the University of Georgia Institutional Animals Care and Use Committee under Animal Use Permit A2008-10141 (Appendix B) and Smithsonian Tropical Research Institute Animal Use Protocol 2008-08-8-24-08 (Appendix C).

Radio Telemetry

Due to the gliding behavior of the study species, the commonly used radio transmitter attachment methods used on most frog species, which positions the transmitter on top of the pelvic region of the frog, were considered unsuitable. In hopes of positioning the weight of the transmitter in a more centralized location the body of the frog, multiple attachment methods were attempted.

-*Glue on method*

The first method attempted was to directly attach the transmitter to the center of the frog's dorsum using medical grade adhesive, Nexaband S/C topical tissue adhesive (Abbott, North Chicago, IL, USA) (Figure 4.2). This technique was attempted on two occasions in June 2008, using male *A. spurrelli* and mock-transmitters. The animals were physically restrained by hand and gently wiped with a cotton tipped applicator to remove any debris or loose mucus from the skin. Three drops of glue were applied to the mounting surface of the mock-transmitter, as well as to the center of the frogs' dorsum. The glue was allowed to become tacky by waiting approximately 30 seconds and then the transmitter was pressed to the animals' dorsum and held for one-minute, while the glue was allowed to dry. These animals were placed in separate 65-

gallon Reptarium® screen-sided enclosures (Dallas, TX, USA) held in an out-doors laboratory space and observed hourly. Enclosures were identically setup with cypress mulch flooring, natural branches, artificial vines (Repti-vine®, ZooMed Laboratories, Costa Mesa, CA, USA), and a water bowl. This cage design allowed the animals to express their natural behavior .

-*Harness method*

The second attachment technique attempted was a modification of a technique described by Rappole (1991) (Rappole and Tipton 1991) for the external attachment of radio transmitters to passerine birds. This technique was used on 10 *A. spurrelli* (5 male: 5 female) during July 2008. This technique utilizes a harness of stretchable elastic thread, which is threaded through the manufacturer-installed eyelets built into the radio transmitter (BD-2, 0.7g, Holohil Systems Ltd., Ontario). The thread was tied into a loop, individually fitted for each frog to which it was attached (Figure 4.3). The frogs fitted with this attachment method were anesthetized with a 0.5 g/L solution of sodium bicarbonate buffered MS-222 (2:1 ratio) (Finquel®, Argent Chemical Laboratories, Redmont, WA, USA) (Stetter 2007). Once unresponsive to physical stimuli, a frog would be placed on its back with the radio transmitter located directly underneath. The loop of elastic thread was pulled around the arms of the animal and the two opposing ends of the loop glued together (Nexaband S/C topical tissue adhesive), at the center of the thoracic region of the frog.

-*Skeletal method*

The third attachment method was a surgical technique, which relied on suturing the transmitter to the dorsum of the animal. This technique was used on 4 individual *A. spurrelli* (2 male: 2 female) and 10 *P. venulosa* (5 males: 5 females; 1 female refitted with second transmitter) during August 2009 (Figures 3.4 and 3.5, respectively). Individuals were fitted with a transmitter (BD-2, 0.7g) sutured to the center of the posterior surface of the frog. Frogs were anesthetized with a 0.5 g/L solution of sodium bicarbonate buffered MS-222 (2:1 ratio). Frogs were considered anesthetized when they were unresponsive to physical stimulation at which point, they were removed from the solution and placed on an aseptic workstation. With the anesthetized treatment animal lying in ventral recumbency, the transmitter was attached at two points of contact near the center of the posterior surface, via passing a 3-0 PDS*II (17mm 1/2c taper) suture through the skin, intercostals muscles, passing between the ribs, into the caudal aspect of the ceolomic cavity, under the vertebral column and back through the ribs, muscles and skin of the animal. Sutures were held with a double simple-interrupted knot. Points of entry and trimmed knots were covered with Nexaband S/C topical tissue adhesive. Following this procedure, frogs were rinsed with a 0.9% saline solution, placed in a fresh plastic container and monitored until complete recovery from anesthesia, as designated by the return of locomotive ability and response to physical stimulation. Following recovery, individuals were transferred into separate 65-gallon Reptarium® screen-sided enclosures held in an out-doors laboratory space. All individuals were released on the night following their capture, approximately 6-10 hours following the attachment procedure.

Upon release, all individuals were located daily via the homing technique (Mech 1983). An R-1000 Telemetry Receiver (Communication Specialists Inc., Orange, CA, USA) was used with an RA-14K rubber ducky "H" antenna (148-152MHz) (Telonics, Meza, AZ, USA). In order to locate frogs in arboreal locations, single-rope technique was used to ascend into the midstory or canopy, when possible (Maher 2004). Daily point locations were recorded with a Garmin 60CSX handheld GPS receiver (Olathe, KS, USA).

In order to collect continuous signal strength data from the frogs, an Automated Radio Telemetry System (ARTS) was established in the forest near KFP. Three understory, portable towers were established (Figure 4.1). Each tower was composed of a tower segment for vertical structure, 6 mounted 3-element Yagi antennas, an Automated Receiving Unit (ARU) (Sparrow Systems, Dewey, IL, USA) and powered by a 12-volt deep-cycle car battery. All towers were programmed to record maximum signal strength of each frog in the field at 3-8 minute intervals (interval dependent on cycle program date). ARU memory modules were exchanges on a weekly biases and batteries changed as needed. For a more complete description on the BCI ARTS Initiative, see Crofoot *et al.* (2008).

Data Analysis

Daily point locations were plotted for visualization in ArcGIS 9.3 (ESRI, Redlands, CA, USA) using the Animal Movement extension (USGS, Anchorage, AK, USA). Dispersal directions for post-breeding movements were manually calculated by taking the bearing of the generalized direction of movements from the center of KFP. Movement distance parameters were calculated in ArcView 3.3 (ESRI). Activity data from the ARU's were reformatted, and calculated, using Excel 2010 (Microsoft, Redmont, WA, USA). Data sequences were censored so that sequential points for each frog were no greater than eight minutes apart from one another. Once in this format, maximum signal strength from each reading was compared with its respective prior data point, creating a Delta Signal Strength data set. Data points with a delta value of 400 or greater were designated as active periods, those of less than 400 were designated as inactive. Using a previously designed Excel spreadsheet function (C. Collins, *pers. comm.*, 2010), active data points were graphed in individual actograms for visualizing data over an extended time frame (see example in Appendices B). The same data were graphed using the Circular Histogram function of Program Oriana 2 (Kovach Computing Services, Anglesey, Wales, UK) in order to visualize patterns of activity during specific hours of the day. Circular statistics were performed using the Rayleigh's Z test in Program Oriana 2 to test for clumping of activity during one-hour periods.

RESULTS

In both of the replications of the glue-on technique, the transmitters dropped off of the animals in less than six hours. No further trials were conducted with this technique. Using the external harness method, 10 individual *A. spurrelli* were tagged during the 2008 field season. Of these individuals, six were tracked to distances of greater than 80 meters away from their point of release, with two males moving 130 and 245 meters. From this same study group, though, multiple animals were observed with lacerations and abrasion directly caused by the attachment method. Of these, 6 prematurely dropped their transmitters, one was found dead with injuries caused by the attachment method, another was euthanized due to injuries caused by the attachment method, one individual had its transmitter removed by the researcher due to attachment related injuries and one was depredated by a parrot snake, *Leptophis ahaetulla*.

Of the *A. spurrelli* that were tagged with the skeletal suture method during the 2009 field season, all four individuals were found dead within five days of release. No visual observations were made on these individuals due to the limited access of their locations in liana tangles and palm thickets. In each case only skeletal remains were found, with transmitter attachment materials still intact. With the lack of further information, the cause of their deaths can only be speculated.

Of the 10 individual *P. venulosa* that were tagged via the skeletal attachment method (including one female that was tagged twice), all survived through the observation period, with no signs of injury of effects from the tagging procedure. Five individuals (2 males: 3 females) were captured, tagged and tracked during the time period immediately prior to their breeding event. Six individuals (3 males: 3 females) were captured, tagged and tracked during the time period immediate following their breeding event. The tracking period of individuals ranged from 16-45 days (mean = 20.5 days). During the pre-breeding period, males made average daily movements of 34 m and a maximum daily movement of 137 m. During this same time period, females made average daily movements of only 9 m, and a maximum daily movement of 151 m. During the post-breeding tracking period, the males daily average movements were 16 m, with a maximum daily movement of 116 m, females average daily movement 19 m, and a maximum daily movement of 153 m (Table 4.1). During the pre-breeding and post-breeding movement periods, frogs were encountered in a variety of habitat types, including the forest floor, understory vegetation, midstory palms, liana tangles, tree trunks, tree holes and canopy vegetation. Dispersal directions of the six frogs tracked during the post-breeding period were approximately 45°, 132°, 220°, 260°, 300° and 345° (Figure 4.6).

Of the 11 radio transmitter deployments monitored by the ARTS, 10 individuals produced data that could be plotted in actograms and circular histograms. A data management error occurred with the 11th individual, so it was unavailable during the statistical analysis. Of the 10 frogs that provided activity data, only two individuals exhibited a pattern of temporal activity that was clumped enough to produce a confidence limit that could be considered reliable (Figure 4.7, D - nocturnal and H - diurnal). From the Rayleigh's test performed on each of the frogs, only one individual demonstrated a temporal activity pattern with a clumped distribution that was statistically different from random (Table 4.2).

DISCUSSION

Identifying a suitable radio attachment method for these species presented a unique challenge. Unfortunately, none of the methods tested here were suitable for tracking the movements of *Agalychnis spurrelli*. While the glue-on method did not appear to negatively affect the animals, it would not secure a transmitter for a long enough period to gather any relevant movement data. The external harness technique appeared suitable at the time of release, but soon after was found to cause unacceptable injuries to the animals, hence making it unsuitable. The same was true of the skeletal suture method when applied to *Agalychnis spurrelli*. The skeletal attachment method did not cause any injuries or observable affects when applied to *Phrynohyas venulosa*, which seemed to be thriving during field observation. The observed movements by this species fall well within the spectrum of movement distances reported from other frog and toad species from across the globe (Kusano *et al*. 1995, Bulger *et al.* 2003, Forester *et al*. 2006, Huste *et al.* 2006). While multiple frogs were located in and near tree holes, both in the midstory and canopy, no frog was ever encountered within bromeliads as would have been expected based on reports from Duellman and Savage (Duellman 2001, Savage 2002). This may simply be a result of the limited number of observation that were able to be made in the high canopy.

While limited in quantity, the results of the activity data were surprising in that they did not support that accepted biology of this species being strictly nocturnal. From the activity histograms, the majority of the frogs appeared to be active in a near equal distribution of all hours of the day. Observations of frogs in the canopy were limited due to access, so it is not certain whether the frogs were truly active in terms of foraging and moving during these periods or simply changing their resting postures, which could cause the same changes in transmitter signal strength that were used for the activity interpretations.

Table 4.1. Summary of spatial movement distances of veined tree frogs, *Phrynohyas venulosa*, at Kingfisher Pond, Barro Colorado Island, Panama, 2009.

	Female: Pre-breeding (3)	Males: Pre-breeding (2)
Av. *N* Movements	26	17
Min. Daily (m)	0	0
Max. Daily (m)	151	137
Av. Daily (m)	9	34
Total movement (m)	58-438	155-819
	Female: Post-breeding (3)	Males: Post-breeding (3)
Av. *N* Movements	23	16
Min. Daily (m)	0	0
Max. Daily (m)	153	116
Av. Daily (m)	19	16
Total movement (m)	112-496	155-506

Table 4.2. Rayleigh's test of the temporal activity patterns of the veined tree frogs, *Phrynohyas venulosa* at Kingfisher Pond, Barro Colorado Island, Panama, 2009. The Rayleigh's test is a test for the significance of the mean direction in a period histogram. As indicated by the P values, only 1 animal demonstrated a temporal pattern that could be characterized as clumped.

Comparison	*n*	*Z test statistic*	P
Females, Pre-breeding	3319	32.133	0.000
	5096	21.541	<0.001
	378	7.004	<0.001
Females, Post-breeding	3421	197.763	0.000
	5731	19.912	<0.001
	4016	68.314	0.000
Males, Pre-breeding	177	0.479	0.619
	336	40.929	0.000
Males, Post-breeding	3974	35.882	0.000
	3595	22.529	<0.001

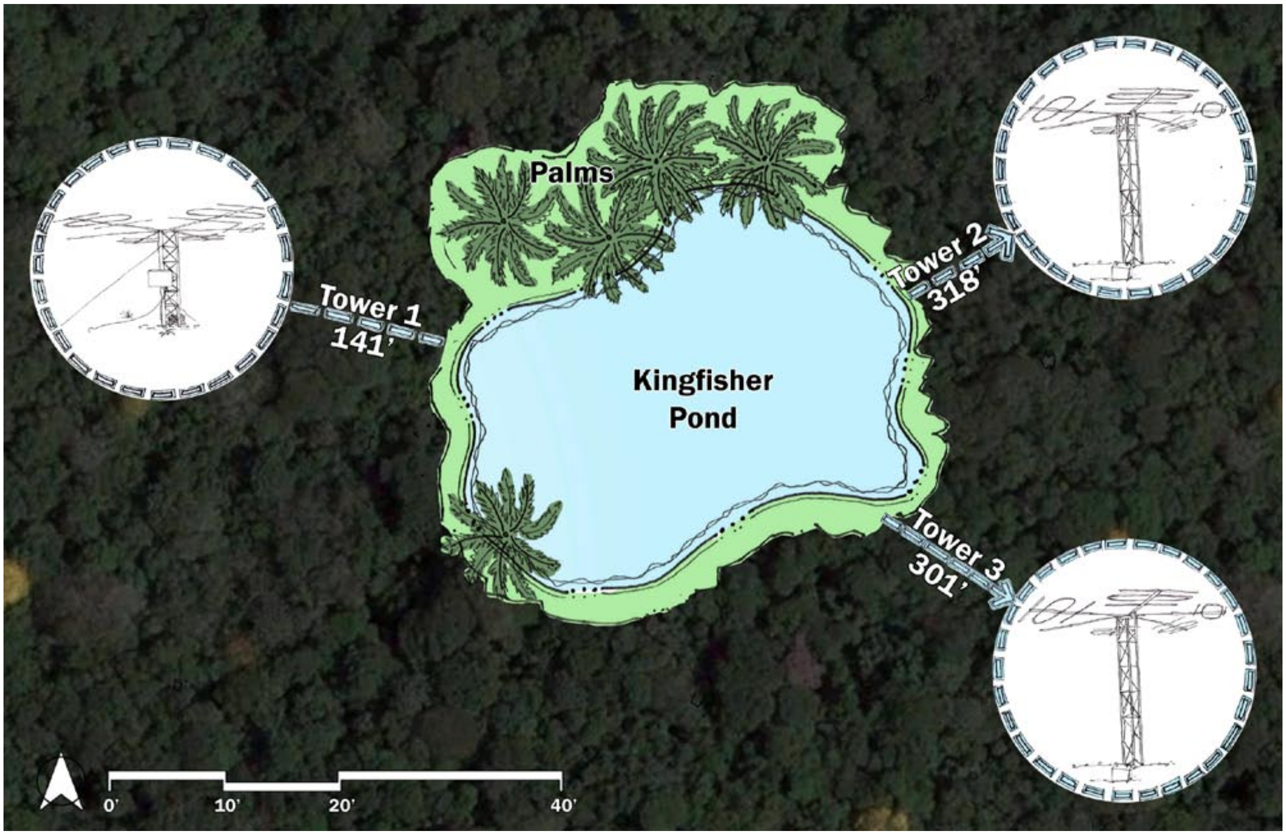


Figure 4.1. Schematic of the arrangement of the understory tower units of the Automated Radio Telemetry System established at Kingfisher Pond, Barro Colorado Island, Panama during the 2009 field season. Diagram by Willie Horan, ©2009.

Figure 4.2. Male gliding leaf frog, *Agalychnis spurrelli*, with "Glue-on" attachment of mock radio transmitter (~3% of body mass). Though this attachment method did not cause any observable effects in the study animals, the adhesive stayed intact for less than 6 hours before falling away from the animal. Barro Colorado Island, Panama, 2008.

Figure 4.3. Female gliding leaf frog, *Agalychnis spurrelli*, with external harness attachment, shown here with a Holohil BD-2 radio transmitter (0.78g). This attachment method proved unsuitable due to the harness material causing severe abrasions and laceration to the study animals. Barro Colorado Island, Panama, 2008.

Figure 4.4. Female gliding leaf frog, *Agalychnis spurrelli*, with skeletal suture, shown here with Holohil BD-2 radio transmitter (0.78g). Attempts at field observations were unsuccessful following release of this study group. All 4 animals tagged with this technique were found dead and skeletonized within 5 days of release. Barro Colorado Island, Panama, 2009.

Figure 4.5. Female veined tree frog, *Phrynohyas venulosa*, with skeletal suture, shown here with a Holohil BD-2 radio transmitter (0.78g). This appeared to be a successful and suitable attachment method for this species. Zero mortalities or injuries were observed with this study group, with frogs being tracked for up to 45 days. Calling males and amplexed and ovipositing females were observed in the field. Barro Colorado Island, Panama, 2009.

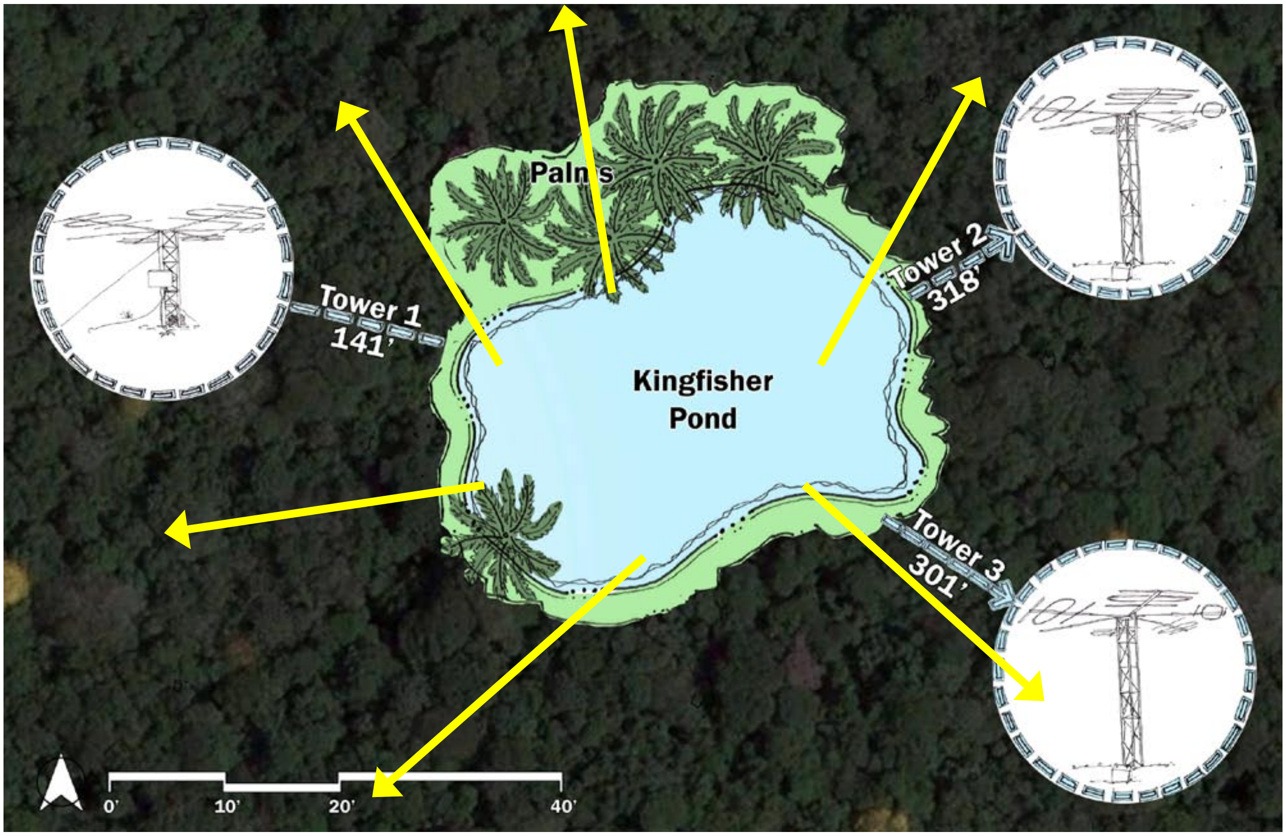


Figure 4.6. Schematic of the generalized directions of post-breeding dispersal of 6 veined tree frogs, *Phrynohyas venulosa*, at Kingfisher Pond, Barro Colorado Island, Panama during the 2009 field season. Diagram by Willie Horan, ©2009.

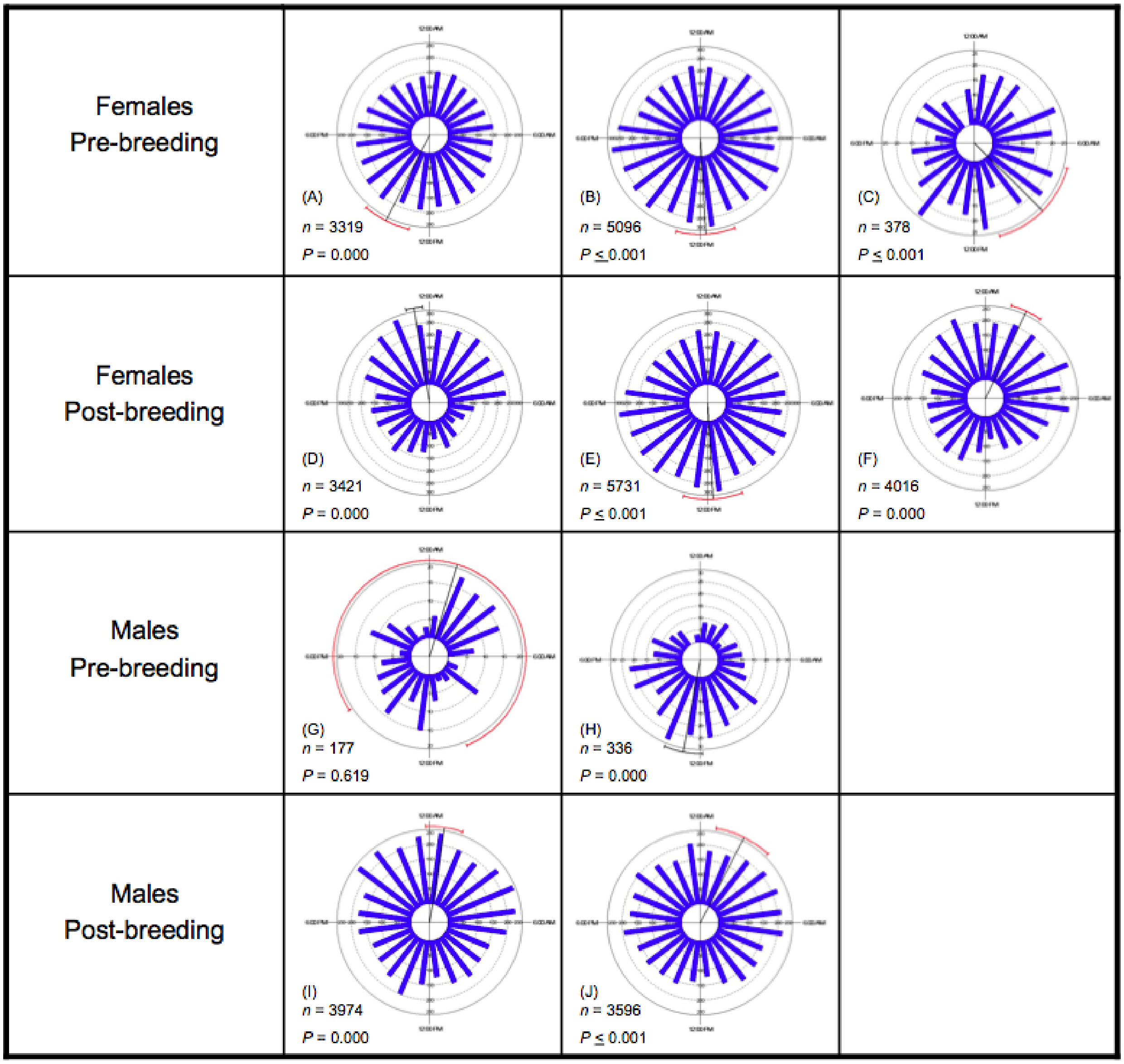


Figure 4.7. Circular histograms of activity distributions for veined tree frogs, *Phrynohyas venulosa* on Barro Colorado Island, Panama, 2009. Diagrams are in the arrangement of a circular 24-hour clock, with midnight at the top-center and noon located at the bottom-center of the wheel. The black line running from the center of the diagrams to their outer edge represent the mean angle of the data. The arcs extending to either side represent the 95% confidence limits of the mean. Note that sometimes the confidence limits can be unreliable when the combination of the sample size and the concentration is low. In these cases the confidence limit arc is displayed in red rather than black.

CHAPTER 5

CONCLUSIONS

While field methods may still be limited in extremely small-bodied species, the field of anuran movement ecology has been greatly advanced in the past two decades. Due to technological restraints, early movement studies were only able to incorporate large bodied, robust species with techniques such as thread bobbins and large, bulky transmitters (Dole 1972, Nuland and Claus 1981). However, the utilization of ever smaller radio transmitters has enabled the inclusion of recently metamorphic individuals in movement studies (Roznik and Johnson 2009). As previously reported, methods of attaching radio transmitters must be quantitatively tested prior to conducting field investigations (Rathbun and Murphy 1996, Bartelt and Peterson 2000, Weick *et al.* 2005, Blomquist and Hunter 2007, Rowley and Alford 2007b). While much work has occurred on data processing and summarization of mammals and birds, data on amphibian movements are relatively limited and appears not to meet some of the same assumptions of prior data sets. With many anuran species using limited complimentary habitat areas outside of the migration and breeding season, the traditional home range and movement analyses methods employed for mammals and birds may not be biologically appropriate for anuran species.

Of the three attachment methods evaluated during the controlled laboratory Alhough the vest attachment method appeared to be a suitable technique early in the experiment, it later proved to be an unacceptable method due to the later effects that it had on both the behavioral and biological parameters measured. The skin suture technique also appeared to be a suitable attachment method but did not provide the required longevity of a method suitable for field application. While the skeletal suture method appeared to have a slight negative effect on the study animals early in the experiment, it provided the only tested method that maintained its integrity for the entire study period. Although the behavioral parameters differed between the control and experimental animals during the first 10-12 days of observations, the remaining observations did not show any differences between the parameters of the two groups, inferring that if used in the field, caution should be exercised when analyzing data from this early period. Unfortunately, none of the methods tested in the field were suitable for tracking the movements of *Agalychnis spurrelli*. The glue-on method did not appear to negatively affect the animals, but it would not secure a transmitter for a long enough period to gather any relevant movement data. The external harness technique appeared suitable at the time of release, but soon after was found to cause unacceptable injuries to the research animals, hence making it unsuitable in the long run. The same was true of the skeletal suture method when applied to *A. spurrelli,* but was not so for *Phrynohyas venulosa*. This species did not appear to receive any of the injuries or negative effects from the skeletal suture and seemed to thrive during the observations periods. Though the movements of this species were shorter than were expected by the researcher, they fall well within the spectrum of movement distances reported from other frog and toad species from across the globe (Kusano *et al*. 1995, Bulger *et al*. 2003, Forester *et al*. 2006, Huste *et al.* 2006). Multiple frogs were located in and near tree holes, both in the midstory and canopy, but no frog was ever encountered within a bromeliad as would have been expected based on reports from Duellman and Savage (Duellman 2001, Savage 2002). While this may be a result of the limited number of observation that were able to be made in the high canopy, it may also be explained by a difference in the forest type of BCI and the areas of study by the previous authors. Though limited in quantity and having been interpreted by a method never before used to analyze anuran activity patterns, the results of the activity data were surprising in that they did not support the accepted biology of this species being strictly nocturnal. From the activity histograms, the majority of the frogs appeared to be active in a near equal distribution of all hours of the day.

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Appendix A. University of Georgia, Institutional Animal Care and Use Committee, Animal Use Permit for Chapter 2 evaluation protocols.

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The University of Georgia
Office of The Vice President for Research

Animal Care and Use
608A Boyd Graduate Studies Building
Athens, Georgia 30602-7411
(706) 542-5933
Fax No. (706) 542-5638

Date: **October 15, 2008**

To : **JOHN CARROLL**

From : **Tina Tornambe, IACUC Coordinator**

Regarding : **Initial Approval of Animal Use Protocol**

AUP Approval Date : **2008-10-15**

Title : **Safety and Utility of an External Radio Transmitter System**

AUP # **A2009-10045-0**
Highest Use Category : **B**

Species :
Green Tree Frog, 90

Source of Support: Smithsonian Tropical Research Inst.
UGA Animal Welfare Assurance # A3437-01

In accordance with the procedures of The University of Georgia Institutional Animal Care and Use Committee (IACUC), your proposal involving the use of animals was approved.

If your funding agency requires notification of animal use approval, please forward a copy of this approval letter to them.

Please let us know if there are any anticipated changes in the use of animals for this proposal. Any significant changes in animal use methodology or numbers of animals must be reviewed by the Committee before implementation.

Appendix B. University of Georgia - Institutional Animal Care and Use Committee - Animal Use Permit for Chapter 3 protocols.

The University of Georgia
Office of The Vice President for Research

Animal Care and Use
608A Boyd Graduate Studies Building
Athens, Georgia 30602-7411
(706) 542-5933
Fax No. (706) 542-5638

Date: **March 12, 2008**

To : **JOHN CARROLL**

From : **Tina Tornambe, IACUC Coordinator**

Regarding : **Initial Approval of Animal Use Protocol**

AUP Approval Date : **2008-03-12**

Title : **Treefrog telemetry study**

AUP # **A2008-10141-0**
Highest Use Category : **A**

Species :
Gliding frog, 250

Source of Support: Smithsonian short-term fellowship
UGA Animal Welfare Assurance # A3437-01

In accordance with the procedures of The University of Georgia Institutional Animal Care and Use Committee (IACUC), your proposal involving the use of animals was approved.

If your funding agency requires notification of animal use approval, please forward a copy of this approval letter to them.

Please let us know if there are any anticipated changes in the use of animals for this proposal. Any significant changes in animal use methodology or numbers of animals must be reviewed by the Committee before implementation.

Appendix C. Smithsonian Tropical Research Institute - Institutional Animal Care and Use Committee - Animal Use Permit for Chapter 3 protocols.

Smithsonian Tropical Research Institute
Instituto Smithsonian de Investigaciones Tropicales

TO: Robert Horan
Warnell Scholl of Forestry and Natural Resources
University of Georgia

FROM: Georgina de Alba, STRI IACUC Chair
Oris Acevedo, STRI IACUC Co - Chair

DATE: September 20, 2008

RE: PROTOCOL REVIEW APPROVAL

Your IACUC protocol has been reviewed by the Committee on August 24, 2008 and has been approved.

Please provide the IACUC committee with updated report on the result of the applied methodology and any changes in the protocol need to be reported.

If you have any questions, call me at (507) 212 8901.

PRINCIPAL INVESTIGATOR: Robert Horan

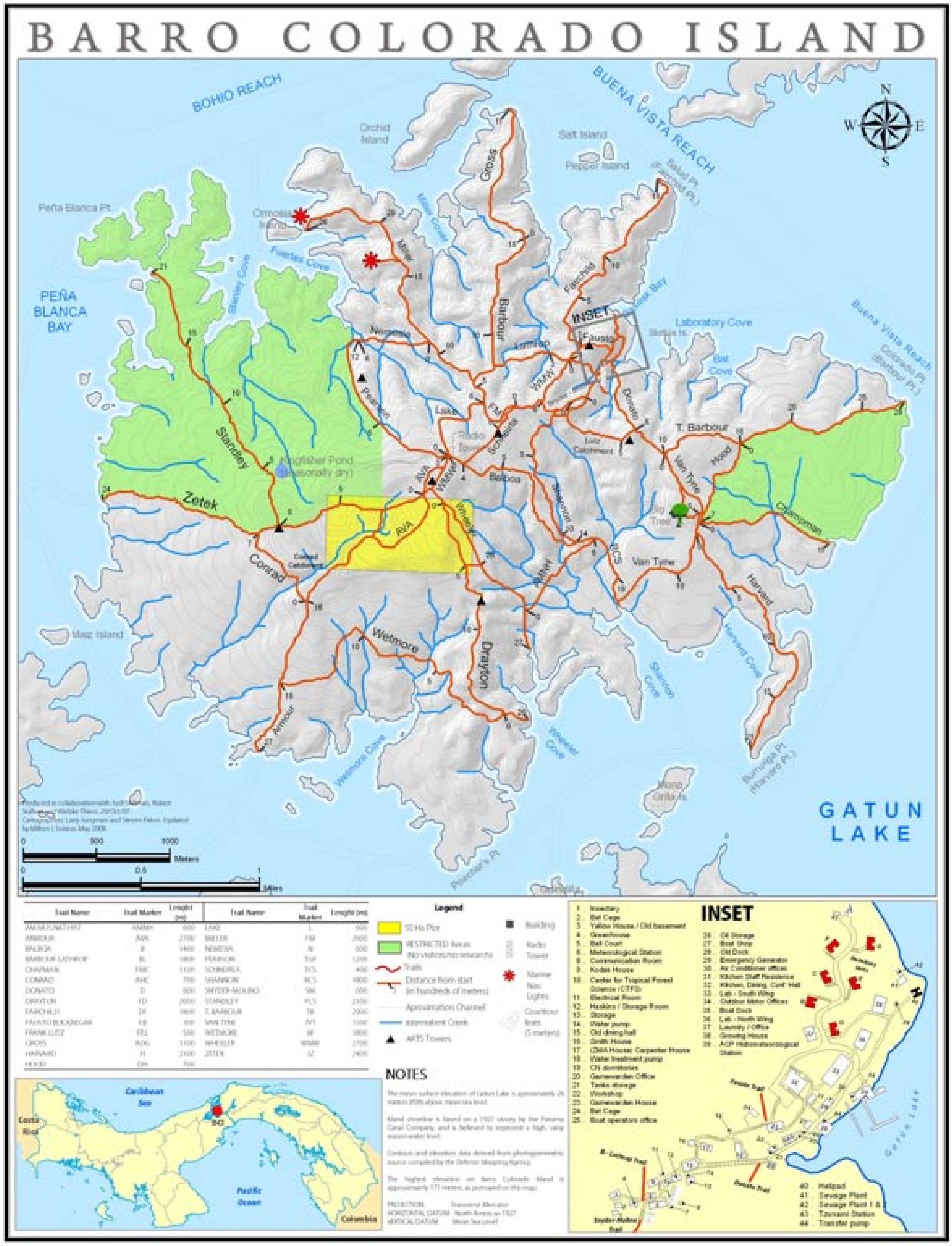
PROJECT TITLE: A telemetry study on the frogs Agalychnis spurrelli, BarroColorado Island, Panama.

ANIMAL SPECIES: Agalychnis spurrelli

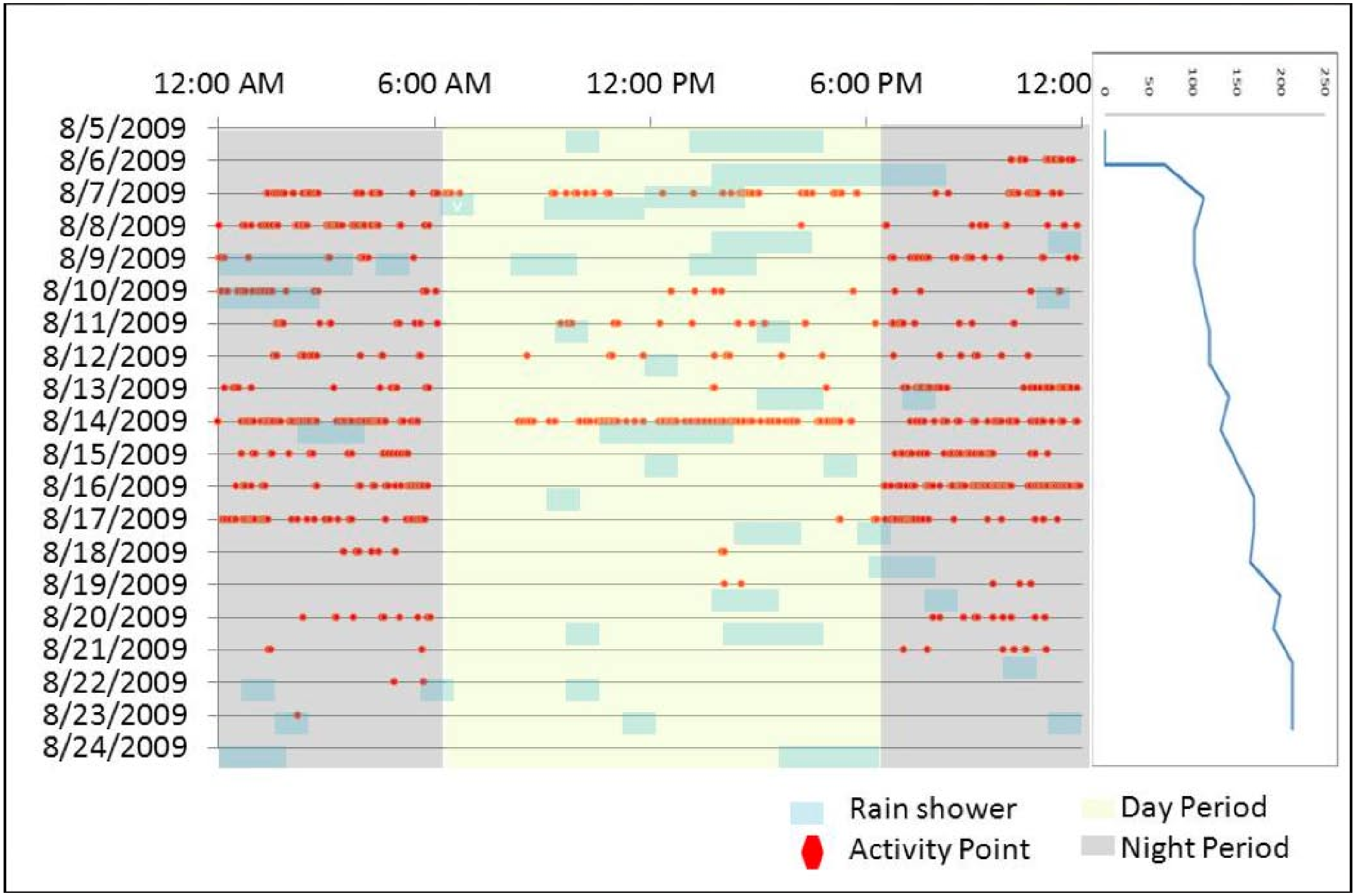
PROTOCOL CODE NUMBER: 2008-08-8-24-08

This protocol requires an annual review.

Appendix D. Trail map of Barro Colorado Island, Panama. Kingfisher Pond is located on the western side of BCI, near Standley trail marker 5.



Appendix E. Actogram of a female veined tree frog, *Phrynohyas venulosa*, during a 19 day post-breeding period in August 2009, at Kingfisher Pond, Barro Colorado Island, Panama. Red dots represent periods in which the frog was determined to be active. Blue blocks represent periods of precipitation. The line graph to the side of the actogram represents the distance of the frog from the center of Kingfisher Pond, following the same time scale as that of the actogram.



Appendix F. Summary of project funding sources, amounts and uses.

Source	Type	Amount	Use
Daniel B. Warnell School of Forestry and Natural Resources	Graduate assistantship	33 % stipend	Living expenses
Warnell Graduate Student Association	Presentation award	$250	Field materials
UGA Latin American and Caribbean Studies Institute	Travel Grant	$1,200 (2009) $850 (2008)	Airfare and travel
Smithsonian Tropical Research Institute	Short-term fellowship	$4,000	On-site housing expenses
East Texas Herpetological Society	Research Grant	$500	Telemetry equipment
Riverbanks Zoo Conservation Fund	Research Grant	$4,984	Lab expenses, field housing, animal care
Max Planck Institute for Ornithology, Department of Migration and Immuno-ecology	In-kind donation	20 Holohil BD-2 radio transmitters	Radio transmitters
New York State Museum, Mammalogy Department	Travel Grant	$1,000	Research travel, conference attendance
Southeast Cooperative Wildlife Disease Study	In-kind donation	Lab usage, Expert knowledge, sampling materials	Histopathology, animal processing, chytrid sampling
Project Orianne	Travel Grant	$100	Conference attendance
TWS Spatial Ecology and Telemetry Working Group	Travel Grant	$500	Conference attendance