Reproductive Behavior of Male *Hyla versicolor*

During the 1991 Breeding Season

BIOS 569 - Practicum in Aquatic Biology

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1991

ABSTRACT

This study attempted to determine a number of aspects of H. versicolor territoriality. A mark-recapture experiment was established to gather data on male breeding behavior. By recording behavioral interactions, calling sites and success of males in attracting females, I aimed to determine the nature of territoriality, and to characterize the microhabitat of the most successful territories for Hyla versicolor at UNDERC. The literature on various anuran prolonged breeding taxa suggest that there are different behavioral reproductive strategies for males of different taxa. Individual distancing, clustering and orientation, and lekking are strategies suggested for taxa other than H. versicolor. Territoriality is the accepted description for the male H. versicolor behavior. The frogs had a much shorter breeding period than anticipated. The data and observations collected seem to indicate that the behavioral reproductive strategy used by the Hyla versicolor males was not territoriality, but individual distancing instead.

INTRODUCTION

In recent years, both evolutionarily and historically speaking, the number of amphibians on earth has been declining. This has been as a result of the disappearance of the habitat that amphibians require to live. Most recently, this habitat destruction has been through the filling of wetlands and the acidification of freshwater habitats on earth. In order to prevent the unnecessary disappearance of more amphibians, it is important to gain an understanding of the ecology and physiology of these animals. Studies of these animals will hopefully lead to understanding of the importance of maintaining the fragile habitat on which these animals depend for life. Behavioral studies should constitute an important focus of the study of amphibian life.

For many vertebrates, the focus of much of a species' social behavior involves and includes reproductive behaviors, attracting a mate. Specific reproductive behaviors enhance an individual's chance to contribute genetically to the next generation. Anuran species do practice specific behaviors evolved to enhance their chance of reproductive success. Studies of the reproductive behaviors of anurans have been conducted in the past. One of the ways to categorize anuran reproductive behavior is according to temporal patterns of behavior. The relationship between the temporal patterns of anuran behaviors and the behaviors particular to the temporal patterns has been the subject of much study.

There are two basic temporal patterns in anurans: explosive and prolonged. Explosive breeders are defined as those which breed from a few days to a couple weeks. Species which breed for a month or more are referred to as prolonged breeders. Behavioral characteristics typical of explosive breeders are: spatial clumping, active search for mates by both sexes, and intense competition for females. Prolonged breeders maintain individual spacing, do not have active searching by males for mates, and often practice territoriality. These distinctive behaviors are adaptive for their particular temporal breeding pattern. Vocalization by males is a behavior common to both explosive and prolonged breeders. "Males of many species vocalize in choruses that probably advertise the locations of breeding sites to females in surrounding areas" (Wells, 1977). In the species Hyla versicolor, "females use sound as at least one cue in selecting mates," (Fellers, 1979). Hyla regilla, H. cinerea, Rana clamitans, and R. catesbeiana females also use auditory cues in locating prospective mates (Whitney & Krebs, 1975; Garton & Brandon, 1975; Wells, 1978; Emlen, 1968). It is not the vocalizations alone which account for the reproductive success of individuals, but the combination of vocalizations with behaviors typical of the temporal breeding pattern of the particular species.

Territoriality is a behavioral pattern practiced by males of many prolonged breeding species of anurans. In these species, females are not sexually receptive to males for the entire season. Individual females become receptive at different times during the course of the mating season. Males, on the other hand, are sexually active throughout the season. This situation results in a high ratio of males to receptive females at any particular point of the season. Reproductive success of males, therefore, depends on their ability to attract females and prevent interference of other males (Wells, 1977). The practice of territoriality is an effective mechanism for enhancing chances of reproductive success in this situation. Territoriality infers that an individual will use aggressive means to acquire and maintain a locality which would be considered a desirable resource by the opposite sex. The locality or territory is likely to have particular characteristics: size, location, occupant, which are highly attractive to the opposite sex. Thus territoriality enhances an individual's attractiveness while providing a reproductive resource. For prolonged breeding anurans, territory provides the opportunity for a male to distinguish himself from the others in the chorus.

This study was an attempt to determine if Hyla versicolor practice true territoriality, returning to the same site each night of the breeding season. If so the males in the "good" territories would be expected to mate more times during the breeding season than other males. Determination of what constitutes a "good" territory will indicate what characteristics of a territory are most attractive to females.

MATERIALS AND METHODS

The experiment was conducted at the University of Notre Dame Environmental Research Center (UNDERC) during the summer session (May-July) 1991. Dr. Berg showed me where populations of Hyla versicolor had actively bred in the past. At night I went to investigate the different breeding habitats. I chose an experimental population located approximately 75 m from the maintenance building at the camp on the property (See Map 1-Property). (Place Map 1 Here). The males call at night during the breeding season, the females do not. Because the frogs must be located by sound at night, females are rarely seen unless they are with a calling male. Thus, observations and hypotheses about Hyla versicolor are necessarily based primarily on male behavior.

Reproductive Behavior of H. versicolor

The breeding area was a vernal pond on the side of the road close to camp (See Map 1- Property). (Place Map 1 Here). I could hear the frogs from camp when they began to call at night. Before beginning the study, I first mapped then marked off the pond. Trees along the perimeter of the pond were numbered and tagged with colored plastic ribbon. With a group of 4-5 volunteers I went to the pond nightly after dusk when the frogs were calling. The volunteer waded in and around the pond in search of calling males while I set up a processing station. The volunteers carried flashlights covered with red plastic wrap, and a net weighing bag. The frogs were undisturbed by the light and continued to call even when a light was shone directly on them. When a frog was found, its location was noted with reference to the nearest marked tree (See Maps 2-4). (Place Maps 2-4 Here). The frog was brought to me where it was weighed, measured, and marked by toe clipping and tagging. Tags made of numbered, colored pieces of balloon were attached to the forelegs of the toe-clipped frogs. The number on the tag coincided with the toe-clipping number. The tags did not stay on the frogs and were soon abandoned as a marking strategy; the toe-clipping, however was very effective. After processing the frog, it was returned to its calling site by a volunteer. The volunteers were told to be alert for behaviors such as fighting and amplexus which would indicate the territoriality and reproductive success of particular individuals and territories. I returned to the pond on successive nights of the breeding season to record locations and activities of new males as well as previously captured and marked individuals (See Maps 2-4). (Place Maps 2-4 Here). Each night, air and water temperature and humidity were measured and recorded. During the day, I searched the pond for egg masses and tadpoles which would also indicate the reproductively successful territories.

DATA AND OBSERVATIONS

By Monday, May 27, the Hyla versicolor had begun breeding as evidenced by the calling of various populations of males. Most were found in vegetation along the edge of the pond. Sites at the water's edge were the most popular. They ranged in weight 2g-7g and in length 3.8cm-5.5cm. They called for three days, stopped, resumed three days later and called for two more days before quitting for the remainder of the summer. I was able to complete four nights of the mark recapture study. During this time I recorded 31 captures of different males. We were also able to recapture 14 males over the 4 nights. Each night of the study we observed one instance of amplexus (See Map 5). (Place Map 5 Here).

Reproductive Behavior of H. versicolor

Spring Peepers, Hyla crucifer, had a breeding season which coincided with that of the Hyla versicolor. The calls of the two were easily distinguished; the H. crucifer's coming from the trees above, and the H. versicolor's coming from the pond's edge. The Hyla versicolor were never heard calling in the absence of the Hyla crucifer. In fact, the H. versicolor appeared to take cues from the H. crucifer. The two called in simultaneous chorus. When the pond was disturbed, the frogs fell silent. After the disturbance ceased, the H. crucifer would commence calling. Approximately 30-60 sec. after the H. crucifer commenced, a single H. versicolor would initiate the chorus again. It took 2-3 calls from the chorus leader before the entire chorus rejoined the calling. This sequence was repeated several times each night we were out capturing. The chorus leader was usually quite distant from the disturbance, and on at least one night the leader, though never caught, sounded like the same individual after each disturbance. The light from flashlights was never enough to be considered a disturbance by the frogs. Splashing by the volunteers,on the other hand did constitute a disturbance.

On the afternoon of May 28, while tagging trees around the pond, I found two egg masses floating in the pond. The first egg mass was found in the water approximately 1m from site #35. The mass measured 6.2cm X 4.0cm across the top, and approximately 12 eggs were visible from above. Another egg mass was found about 1m from site #47. It measured 6.9cm X 7.0cm across the top and approximately 33 eggs were visible from above. I was unable to identify the species of the eggs, but their presence indicated the reproductive success of the pond.

Returning to the pond two and a half weeks after the last night of calling, I collected tadpoles and salamander larva. Sixteen of the specimens were anuran larva of undetermined species, and fifteen specimens with the trademark, external, feathery gills of salamander larva were captured. The population of amphibian larva were found primarily in the shallow water along the edge of the pond, however, no particular area of the pond had a higher concentration of the organisms. Rana catesbeiana were seen sunning on logs floating in the pond. One female was captured for identification and was obviously gravid. Rana catesbeiana were never heard calling from the study pond.

DISCUSSION

Whitney and Krebs have studied vocalizations and territoriality in Hyla regilla. They assert that vocalizations function in maintenance of spacing of individual males within the population. Hyla regilla spacing is not territory, but "individual distance". Individual distance is related to sexual selection because, like territoriality, it reduces interference of other males and facilitates localization of individuals by females. Hyla regilla individuals usually occupy a certain calling site for one night or less, thus the distinction between "individual distance" and territoriality (Whitney & Krebs, 1975). In a study of Hyla cinerea by Garton and Brandon, males were observed returning nightly to the same general area of the breeding habitat, but not necessarily to the same calling perch. "Males do not defend a particular spot, but rather and area about themselves wherever they are" (Garton & Brandon, 1975). This would seem to indicate that Hyla cinerea also maintain individual distances rather than territories.

Rana clamitans have been studied by Martof (1953) and Wells (1978). Martof defined territoriality as the, "active defense of an area which is used in some phase of the breeding cycle" (Martof, 1953). The males in Martof's study population did not form a stationary population in one site for the entire season. There was not random mixing of males, however, but organization as a result of two social phenomena: clustering effect and orientation. The clustering effect means that certain frogs tended to remain together. That individuals consistently maintained the same spatial relations relative to other individuals is called orientation by Martof. A period of aggression at the beginning of the season followed by one of social stability as a result of repeated recognition of neighbors characterized male Rana clamitan behavior (Martof, 1953). This social phenomenon means that if a cluster moves from one site to another within the breeding habitat, spatial relations will be maintained. Wells also states that males do not stay in the same spot for the entire season, however, he maintains that Rana clamitans are territorial. The spacing relationships between neighbors keeps fighting among males at a minimum; spatial relationships help males distinguish between neighbors and intruders. Males will engage in physical competitions if aggressive vocalizations do not deter an intruder (Wells, 1978). According to Nice, an animal has territory if it exhibits: advertisement, isolation, and intolerance (Martof, 1953). Rana clamitans do all these things: calling, spacing, and resisting intruders.

Emlen suggests that the organization of Rana catesbeiana males is analogous to lek organization in birds. Males establish territories within a communal display ground or area; these choruses are central to female attraction. Each male has a discrete territory or calling station within the chorus, and females will ignore calls of non-chorus males en route to a chorus site. Females come to the area and select a male with which to copulate. Therefore, there is a strong selective premium on rapid attraction of the opposite sex, and possession of a territory increases the chance of successful mating. Sites at the center of the chorus are occupied by the older, larger, most successful males. Females allow male-male interactions which determine territory to do much of their selection (Emlen, 1976). Although it does not occur in all R. catesbeiana populations, some studies have observed male choruses relocating every 4-5 nights during the breeding season. The relocation offers the opportunity for different males to vie for the most desirable sites within the chorus. A male's mating success is dependent on his ability to control high quality territories because females of this species use the male territories as oviposition sites (Howard, 1978).

Hyla versicolor maintain their territories with calling, and if needed, fighting. The call advertises the presence and position of the male within the chorus to conspecific males and females. It also aids in the uniform spacing of males. Fellers found that there was no relation between male body size and reproductive success. However, there is a direct relation between call intensity and female mate choice with females choosing the most intense calls. There is strong evidence to suggest that perches and territories affect call intensity; vegetation and other objects can block or absorb sound. Thus perches and territories may be the most important factors in females' mate choice (Fellers, 1979).

It is clear that the definition and determination of territory and territoriality is dependent on the author. Everyone agrees that territory, along with vocalizations, greatly enhances a male's attractiveness to females. Because prolonged breeders, like H. versicolor, do not actively seek out females, which are few in number on any given night, they must have something that makes them attractive to the females once they have been called. It is inefficient for males to actively search out mates which arrive unpredictably. Prolonged breeders form choruses to call females to the mating site. Territory quality is used by females to select an individual out of the chorus for a mating partner. What characteristics of the territory are most attractive to the female: size, type of vegetation, proximity to oviposition sites, location within the chorus, or something else?

Fellers' study of Hyla versicolor breeding behavior focused primarily on the influence of female selection on male reproductive success. The male practice of territoriality was assumed without considering the possibility of other, similar strategies which might be used by the males. Territoriality was not adequately defined, and the behavior observed may be more adequately described by individual distance, clustering and orientation, or lekking behavior. This confusion demonstrates the necessity of adopting a clear, widely accepted definition of territoriality, and a close analysis of male behavior to determine under which system it falls. My observations indicate that the male behavior may best described as an individual distancing system. All of the males gathered at a collective display area (the pond), and attempted to distinguish themselves by calling from favorable sites to attract the females. Certain sites were used more often than others, and it appeared that each frog tended to maintain an individual distance from the others. Territories were not used consistently by particular frogs, although calling sites were all relatively similar in characteristics. This inconsistency in territory seems to eliminate territoriality as the primary reproductive strategy of the H. versicolor males. Lekking is not an acceptable explanation because lekking implies that there are no resources attractive to females that are defended and advertised by males. Oviposition sites are valuable reproductive resources defended by the male H. versicolor. There was not enough specific data collected to determine if there was a system of clustering and orientation. All of this evidence seems to point to individual distancing as the most likely description of male Hyla versicolor behavior. Although these questions could not be definitively answered by the present study, it hints at a focus for future research. What is the best description of male H. versicolor breeding behavior, and how does this behavior contribute to reproductive success?

Hyla chrysosceles, the sibling species of Hyla versicolor, is found primarily in the southeastern United States. Because of their genetic similarity it could be hypothesized that there would be a similar display of reproductive behaviors. Restartis and Wilbur have studied the influence of predators and competitors on females' choice of oviposition sites. The choice of oviposition site is extremely important in determining the reproductive success of amphibians which breed in temporary ponds. The study found that ponds treated with Ambystoma larva or conspecifics were avoided by female H. chrysosceles. Ambystoma larva are major tadpole predators, and conspecifics would result in intraspecific competition, thus adversely affecting the reproductive success of breeding individuals. The presence of predators and competitors along with the pond's age, temperature and vegetation influence the suitability of breeding sites, and therefore the hatchling success and parental fitness (Restarits & Wilbur, 1989). Another study found that male calling sites were influenced by the presence of other species. Both males and females avoid conspecific tadpoles and Enneacanthus, however, only the females avoid Ambystoma. These observations suggest that each sex responds independently to the environment, with the interaction between the responses resulting in the H. chrysosceles breeding system being dominated by female choice

(Restarits & Wilbur, 1991). These results indicate that the presence of Ambystoma or conspecific tadpoles at a Hyla versicolor breeding pond might influence females' mate and oviposition site choices.

Ambystoma are found on the UNDERC property, and it is likely that the salamander larva found at the study site are Ambystoma. Their presence would affect male calling site and female oviposition site and mate choice if H. versicolor are analogous to H. chrysosceles in behavior as well as genes. All of the tadpoles found at the site were the same in size and appearance. If they were indeed H. versicolor tadpoles, then the presence of these conspecifics may have also influenced the females choice of breeding site and mate.

This study presents many questions which might be pursued in further research. If the frogs cooperate with a normal-length, prolonged breeding period, it would be possible to answer some questions about territoriality. Do the Hyla versicolor males practice territoriality, and what kind? Is their behavior better explained by "individual distances", clustering and orientation, or lekking behavior than by territoriality. If the males are territorial, what is an adequate definition of a territory or territoriality? Do males with access or control over certain resources have higher reproductive success? Does the presence of predators or competitors affect the reproductive success of individuals or an entire population?

There are other factors which might influence Hyla versicolor breeding behavior. My observation of the close interaction between the H. crucifer and H. versicolor populations at the study site, raises a question of just how closely related their behaviors are. Further research could explore this relationship. Another question would involve the reason for the shortened breeding season of Spring 1991. Measurements of water-level in the pond, rainfall, and changes in photoperiod may all have an effect on the breeding season.

There are some hints which would, if followed, would make the mechanics of future studies go much more smoothly. I would recommend having a Colman lantern or headlamp available when processing the frogs at night. It was difficult to balance a flashlight while handling the frogs and still have enough light to see what I was doing. If the same site was used, the lantern could be placed on the road without disturbing the calling frogs and still be convenient enough for quick processing. Colored leg bands designed for use in bird identification in the field, would provide a quick and easy way to mark and recognize individuals. The nets designed for use with the balance were very handy for carrying the frogs from capture site to processing site. In addition to numbering and marking trees along the perimeter of the pond, I would recommend staking the pond into quadrants for more precise determination of frog location. The site used for this study was a very manageable size. There was another site not far down the road (indicated on the Property Map) that also had a lot of activity, but was much larger.

ACKNOWLEDGEMENTS

This project could not have been completed without the help of many people. First, I would like to thank the Bernard J. Hank Family Endowment for providing the opportunity for me and other undergraduates to participate in the unique UNDERC learning experience. The program was an extremely important growing experience for me as a student and a biologist. Thanks also to Dr. Sunny K. Boyd and Dr. Martin Berg for their help and guidance during the course of the project. And a very special thanks to the UNDERC class of 1991. Without their support and assistance in the field, and their friendship, the experience would have been half of what it was.

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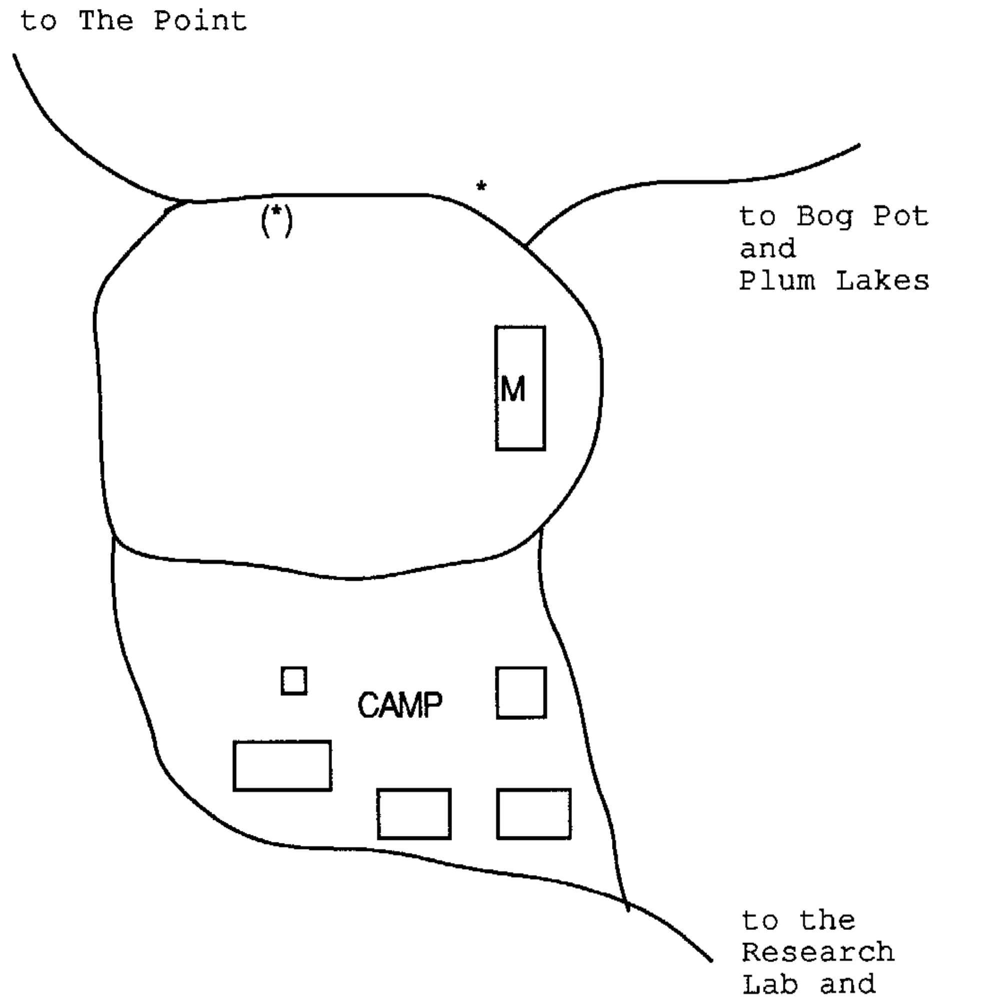
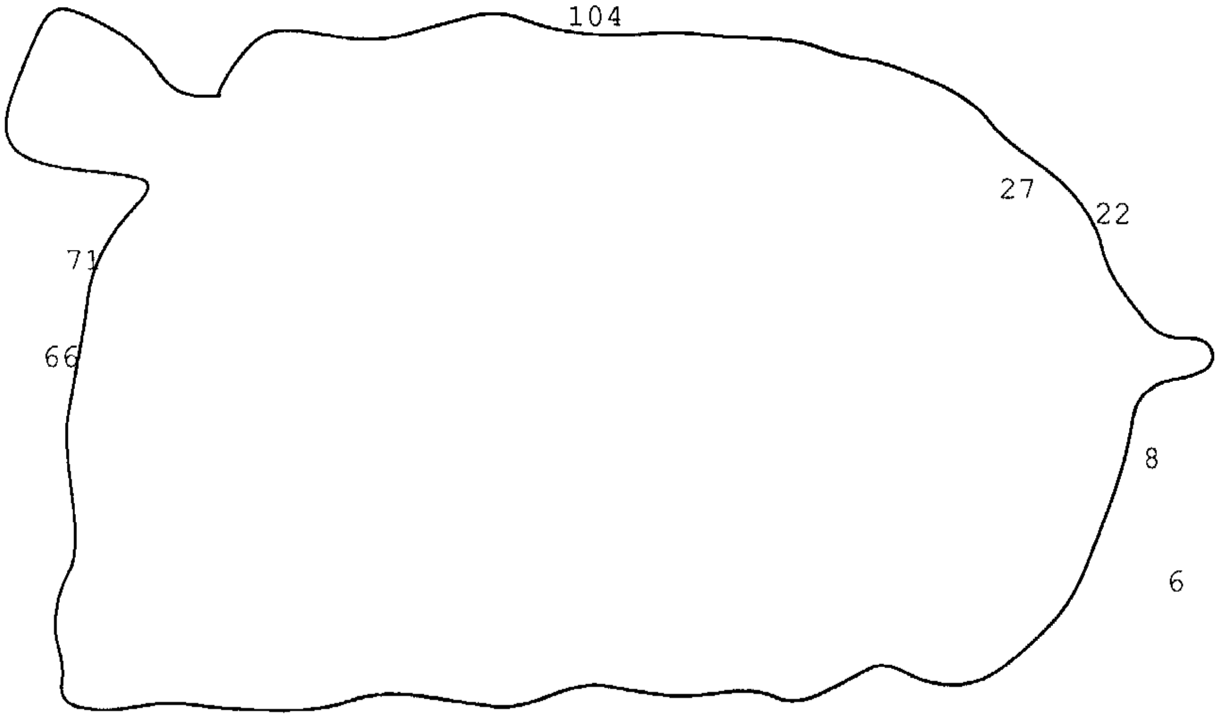


Map 1

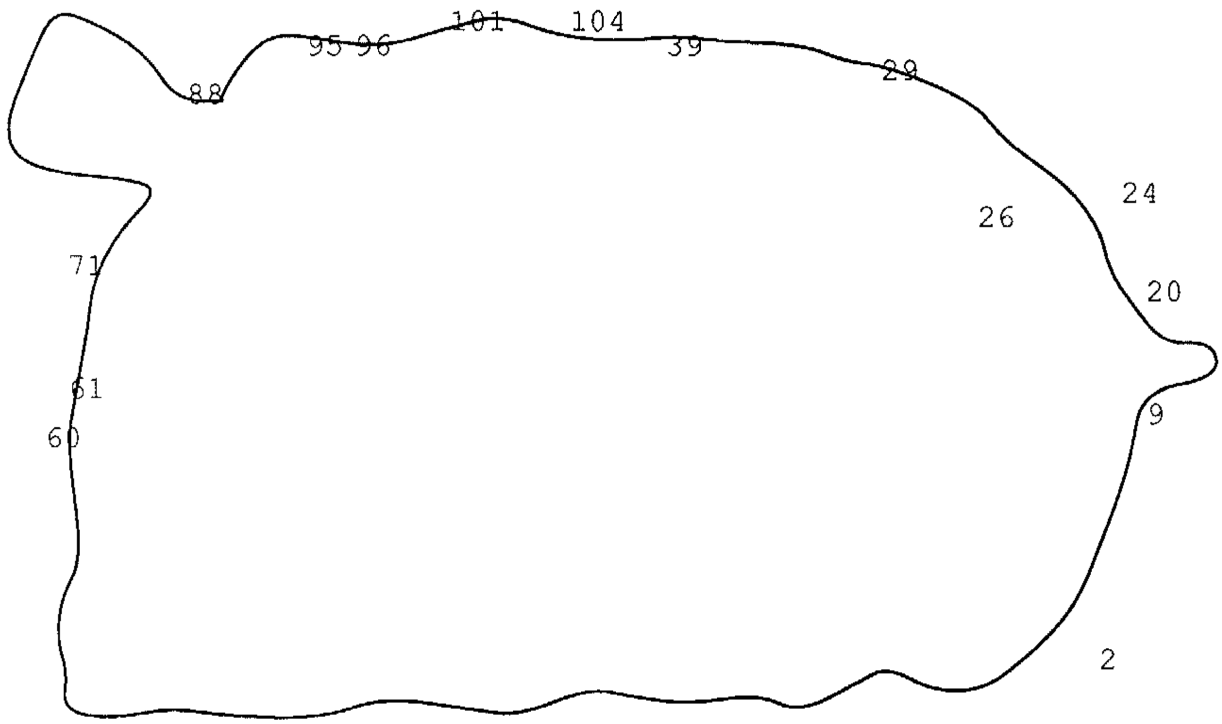
Map of the property near camp. M=Maintenance building.
* = Study site. (*) = possible future study site.



Map 2

Night of May 29, 1991. Site numbers where frogs were captured on map. Details below.

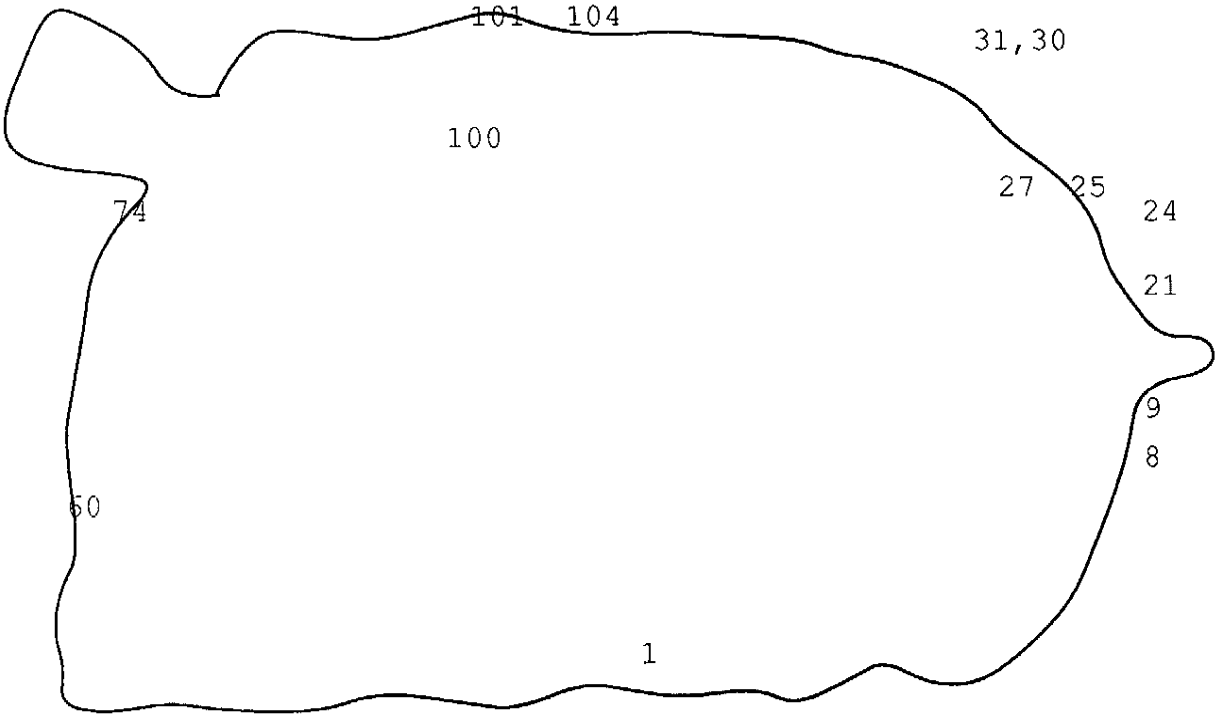
Location#	Location Description	Frog#
6	base of tree, 1m from water's edge	4
8	sm. tree, ferns, grass at water's edge	8
22	base of tree, water's edge	1
27	lg. fern at water's edge	3
66	sm trees at water's edge, lg. tree sticking into water	6
71	sm. fir at water's edge	2,7
104	fallen tree w/ evergreen cover, grass at water's edge	5



Map 3

Night of June 1, 1991. Air temp.=19.1C, Water temp.= 20.4C, Humidity =78%. Site numbers where frogs were captured on map. **Bold print** indicates recapture. Details below.

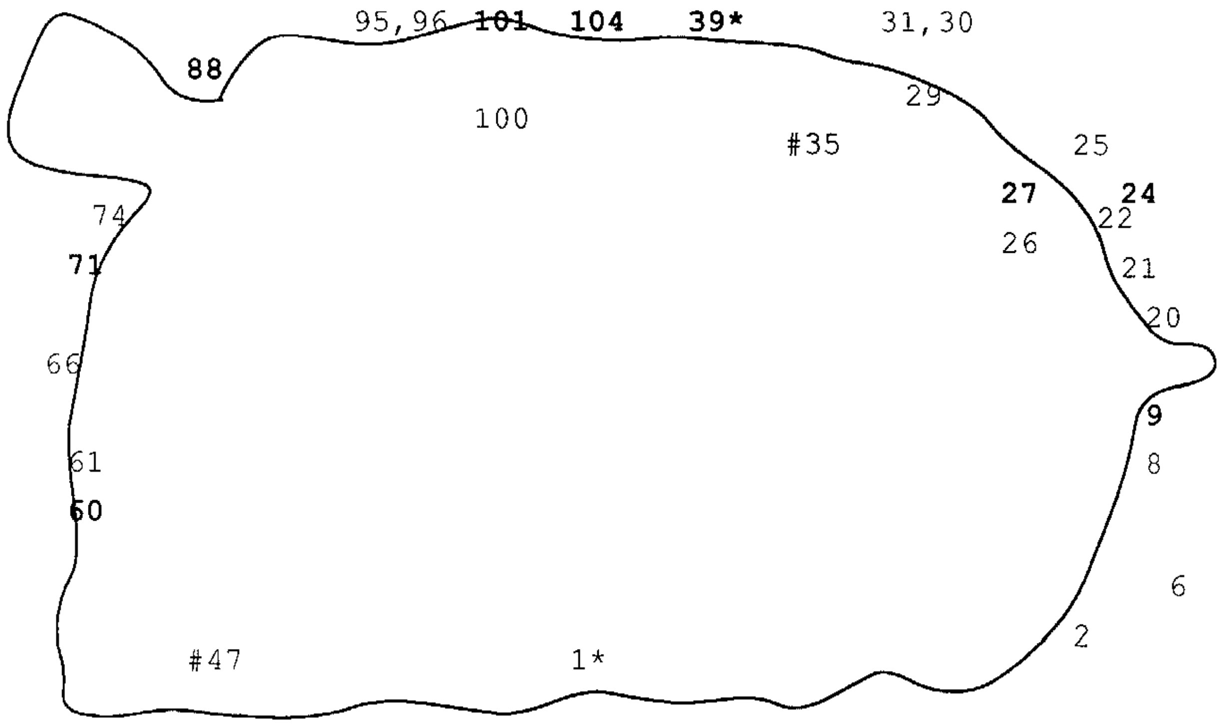
Location#	Location Description	Frog#
2	base of tree at water's edge	**16**
9	sm.tree,ferns, and grass at water's edge	**16**
20	base of large tree at water's edge	18
24	gp. of sm. trees, .5m from water's edge	**1**
26	3 sm. trees growing at water's edge	16
29	base of tree at water's edge	17
39	tree and grass at water's edge	*8
60	trees and grass at water's edge	**6**
61	trees and grass at water's edge	**6**
71	sm. fir atwater's edge, 1m from fallen tree in water	20
88	bush at water's edge	12,13 14,21 **4**
96	sm. tree, ferns, and grass at water's edge	15
95	sm. bush at water's edge	**5**
101	sm. tree and grass at water's edge	11
104	fallen branches w/ evergreen cover, and grass at water's edge	**8**



Map 4

Night of June 2, 1991. Air temp.= 19.0C, Water temp.= 21.9C, Humidity =78%. Site numbers where frogs were captured on map. **Bold print** indicates recapture. Details below.

Location#	Location Description	Frog#
1	adjacent to ~ 20 saplings in water	**1,*1**
8	in grass at water's edge	**13**
9	water's edge, sm. tree, ferns,grass	23
21	base of large tree, .5m from water's edge	25
24	gp. of sm. trees 1m from water's edge	**2**,22
25	gp. of sm. trees .5m from water's edge	**22**
27	lg. fern at water's edge	28
30	base of tree, 1m from water's edge	**20**,27
31	base of tree, 1m from water's edge	**11**
60	tree and grass at water's edge	31
74	in grass at water's edge	**14**
100	in saplings in water, 1.5m from shore	26
101	grass and sm. tree water's edge	**14**
104	fallen branches w/ evergreen cover and grass at water's edge	30



Map 5

Capture sites of male Hyla versicolor during Spring 1991 breeding season. Bold print indicates sites of more than one capture. Astricks (*) indicate sites of amplexus. Sites where egg masses were found are indicated by (#).