Monitoring and Excluding Frogs from Agricultural Fields in South Florida

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INTRODUCTION

Veg Pro International, headquartered in Québec, Canada, produces spring-mix lettuces and other leafy vegetables. During the winter months Veg Pro operates in Florida and grows crops in muck fields leased from sugarcane growers near Belle Glade. Frogs were entering Veg Pro's fields and were sometimes found in the final lettuce product. In 2004, Veg Pro contacted the University of Florida for advice to exclude these frogs from the fields and prevent them from appearing in the final lettuce products. In December of 2004, Dr. Steve Johnson of the University of Florida committed to investigating methods for excluding frogs from Veg Pro agricultural fields. Dr. Johnson and his staff worked during the 2004-05 growing season to determine the species of frogs in an around the fields and find methods to exclude and monitor frogs. The findings of that first season were presented in a report to Veg Pro in September 2005. Dr. Johnson and his staff were again hired by Veg Pro to continue their research during the 2005-06 growing season, but in a more limited basis. The objectives of the '05-'06 season's project were:

1) refine aquatic trapping methods developed last season,

2) test the effectiveness of PVC pipe refugia along canals within the lettuce fields,

3) explore the use of cover objects to monitor Greenhouse Frogs,

4) train Veg Pro personnel to use monitoring and trapping methods, and

5) develop standard operating procedures (SOPs) for Veg Pro to use in subsequent seasons to monitor and exclude frogs.

This report details the findings of the '05-'06 growing season and includes standard operating procedure and recommendations to exclude and monitor frogs in subsequent seasons. The report covers Veg Pro's winter season in Florida from December 2005 through March 2006 (2005/2006 Season or Season 2). Unlike in Season 1, when we made monity visits to conduct fieldwork, at the request of Veg Pro we only made three visits during Season 2. During periods when we were not on site, Veg Pro personnel monitored traps and maintained barrier fences.

METHODS

For this second season, we used several different methods for either monitoring frogs or preventing frogs from entering fields: barrier fencing, PVC pipe refugia, cover boards, and aquatic traps.

Barrier Fences

As in the first season, barrier fences were used around all canals adjacent to Veg Pro agricultural fields. These physical obstructions reduced frog access (particularly Southern Leopard Frog, *Rana sphenocephala*) to the fields from canals. The fencing was installed by Veg Pro personnel prior to our initial visit in

December 2005. Barrier fencing this season was constructed of a more durable shade cloth material that was supported by rebar and metal guide wires (Fig. 1). Barrier fencing in 2005 was constructed of a less durable shade cloth material supported by wooden stakes. Although unavoidable damage by agricultural equipment and machinery was inflicted on the fences, the materials used in Season 2 were superior to the first season. This season we did not use funnel traps in conjunction with the barrier fences. We felt their use was not warranted since they only resulted in a few amphibian captures in Season and are labor intensive to build, deploy, and maintain. As in Season 1, the barrier fences were angled toward potential source canals in order the make potential trespass of frogs less likely.

PVC Pipe Refugia

PVC pipes (3.2 cm diameter, thin-walled, approx. 1 m long) were again installed in the ground at roughly 30 m intervals along the main canals at the borders of the fields to provide refuges for treefrogs. PVC pipes were also placed along canals bordering the main road in the middle of each field. PVC pipes do not actually trap treefrogs, but instead they are attractive refuge sites for treefrogs during the day, providing protection from the sun, wind, and predators. This allows researchers to easily capture the frogs by removing them from the pipes. PVC pipes were used to monitor the spatial and temporal activity of treefrogs. Target species for PVC pipes are treefrogs (genus *Hyla*). Only species in this genus are able to use the pipes because of their ability to climb.

Cover Boards

This season we also used cover boards to monitor for frogs in fields. Cover boards were 0.5 m² plywood pieces approximately 1.5 cm thick. Cover boards serve as refugia to many vertebrates, including Greenhouse Frogs (*Eleutherodactylus planirostris*), Southern Toads, snakes, and lizards. We targeted Greenhouse Frogs with this method in Season 2. We placed cover boards at the ends of each lateral canal within each field for a total of 56 boards in both sections. Twenty-six cover boards were placed in Section 3/10 and 30 were placed in Section 9. We checked Cover boards for frogs during each of our three visits in Season 2.

Aquatic Trapping

We used aquatic traps (called minnow or Gee traps) to sample for tadpoles in lateral canals in Veg Pro fields. Gee traps proved just as effective as three other minnow traps we tried in Season 1, but they are less expensive and/or more durable than the other models. During each visit we sampled every lateral canal within each field using ten Gee traps per canal. Aquatic traps were deployed and spaced evenly the entire length of the canal if water was present throughout. If water was not present the entire length of the canal, the ten traps were concentrated in the areas where water was present. Traps were checked daily. All frogs and tadpoles captured by us and Veg Pro personnel in Season 2 were transported well away from Veg Pro's field and released in similar habitat.

RESULTS

We made three visits to the Veg Pro fields in Season 2: 12-17 December, 2005; 17-20 January, 2006; and 13-17 March 2006. During these visits we captured three different species of amphibians: Green Treefrog (*Hyla cinerea*) in PVC pipes; Southern Toad (*Bufo terrestris*) under coverboards; and Southern Leopard Frog (*Rana sphenocephala*) tadpoles in aquatic traps.

As with Season 1, the most commonly encountered species were the Southern Leopard Frog (tadpoles) and the Green Treefrog (adults). We captured only three Southern Toads: two were found under cover boards on two separate dates and the third was hand-captured in the fields by a Veg Pro employee. All frogs and tadpoles that we captured were released in similar, suitable habitat at least 8 km away from the fields.

Barrier Fences

Carlos and Veg Pro personnel installed barrier fences around the outside borders of the fields as well as around the main canals in the center of the fields prior to our arrival. Barrier fencing likely prevented frogs from entering the fields from adjacent, deep canals. Because of their ineffectiveness in Season 1, we did not deploy wire-screen funnel traps along the barrier fences. We assume barrier fences were effective at excluding Sothern Leopard Frogs from the fields but have no way of determining how effective they are without additional research, such as radio tracking frog movements.

PVC Pipe Refugia

Veg Pro personnel installed all PVC pipes prior to our visits. All PVC pipes were checked for treefrogs once during each of our visits. Veg Pro also checked pipes for frogs during our absence. This season we captured a total of 63 Green Treefrogs in PVC pipes in Section 9 and Section 3/10. All frogs were adults (i.e., greater than 2 cm) indicating that either breeding activity did not occur during Veg Pro's growing season or that Veg Pro completed operations prior to metamorph emergence from breeding areas (as appeared to be the case in Season 1). Forty-two treefrogs were captured in Section 3/10 and twenty-one treefrogs were from Section 9. Of the 42 treefrogs from Section 3/10, 14 were found along the east side, nine were found along the north side, five were found along the middle canal separating the field (Figures 2 & 3). Of the 21 Green Treefrogs captured in Section 9, none were found along the east side, three were found along the north side, five were found along the north side, five were found along the west side, and eight were found along the south side, five were found along the west side, and eight were found along the south side, five were found along the west side, and eight were found along the middle canal (Figures 2 & 4)

Cover Boards

In all three visits, we captured only two Southern Toads (*Bufo terrestris*) from underneath the cover boards—we found no Greenhouse Frogs under the boards. Cover boards either were not successful at providing refuge for Southern Toads and Greenhouse Frogs or there were not sufficient numbers of these animals in the fields to use the cover boards. Alternatively, cover boards may have been unsuccessful due to high numbers of fire ants using the boards and potentially deterring amphibian use of the boards as refugia. As with all amphibians captured in Veg Pro fields, these individuals were removed to similar habitat at least 8 km from Veg Pro fields. Because of the lack of captures and issues with fire ants, we do not recommend that Veg Pro use this method in the future.

Aquatic Trapping

We used Gee minnow traps to sample and capture tadpoles in the lateral canals in the fields. Last season we used four different aquatic traps to sample tadpoles but found Gee traps the most effective, durable, and cost-efficient. During each visit we sampled every lateral canal in both sections one time using ten Gee traps per canal. During all of our visits we captured only 23 Rana sphenocephala (Southern Leopard Frog) tadpoles. All 23 tadpoles were removed from only two canals. Twenty of the tadpoles were removed from canal 3/4 in Section 9 and three tadpoles were removed from canal 2/3 in Section 3/10. Most of the canals in Section 9 were rather shallow, requiring us to concentrate aquatic traps at canal ends where the water was deep enough to deploy traps. Tadpole captures this season were dramatically less than captures in Season. We captured 2,039 Southern Leopard Frog tadpoles last season, of which 1,327 were from canal 8/9 in Section 9. In Season 2 this canal was completely dry during the last visit and almost completely dry during our January visit to Belle Glade. The significant reduction in tadpole numbers thus far may be a result of one or many factors. The barrier fences are likely preventing or reducing Southern Leopard Frog access to the canals, which results in fewer breeding opportunities. Although the effects canal dredging following of Hurricane Wilma, and lack of rain subsequently on tadpole and frog presence cannot be quantified, these events likely negatively impacted frog populations in the fields and canals, thus resulting in greatly reduced frog and tadpole presence.

Our frog and tadpole data from Seasons 1 & 2 demonstrate the extreme variability in frog populations from season to season and underscore the need to continued trapping and monitoring over a many more seasons in order to understand the dynamics of amphibian populations in the area.

STANDARD OPERATING PROCEDURES (SOPs)

During the two seasons of fieldwork at Veg Pro's fields the UF team tried many different methods to exclude, monitor, and capture amphibians: dip-netting, seine-netting, aquatic traps, barrier fencing, aural surveys, cover boards, funnel traps, and PVC pipes. Some methods were more effective than others, and it is

these effective methods that we suggest should form the basis of standard operations procedures (SOPs) to be followed by Veg Pro to continue to exclude and monitor amphibians at their fields.

Based upon capture success of amphibians, cost, durability, ease of use, and potential to exclude amphibians from the fields we recommend that Veg Pro personnel use PVC pipes, barrier fencing, and aquatic trapping to further monitor and exclude amphibians in their fields. The following sections detail the SOPs involving these methods as well as an annual strategy that we recommend for Veg Pro's Florida operation. It is important that Veg Pro take an integrated approach simultaneously employing all of the methods in order to realize the most effective strategy to exclude and monitor frogs.

Barrier Fencing

Barrier fences (aka drift fences) act as a physical barrier for frogs, making it difficult for adults to gain access to the fields from the canals. It also may limit access of frogs into some canals, thereby reducing the potential for reproduction. The fence likely acts as a barrier to other animals as well, such as rodents and snakes, further reducing the likelihood of animals in the fields. Barrier fences therefore reduce adult numbers in the fields as well as tadpole numbers in the canals.

Barrier fences should be erected along as many of the canals as possible each season. Our initial aural surveys as well as opportunistic observations show that Southern Leopard Frogs, and potentially other species of *Rana* inhabit the main canal bordering the fields. These canals are also suitable breeding habitat for Green Treefrogs. Thus, barrier fences should be placed along all of the main canals each season, at a minimum. Our aquatic trapping showed, particularly in Season 1, that tadpoles grow and develop in the lateral canals that run through the fields. Therefore we also suggest that barrier fences be erected along as many of the lateral canals as possible, if not all of them.

Barrier fence should be installed as early as possible during each growing season, preferably prior to winter operations each year. If this is not feasible, the fencing needs to be in place as soon as possible to prevent frog access to the fields. The material chosen by Carlos Restrepo the second season was superior to fencing material used the first season. Season 2 shade cloth was thicker, making it more durable and less likely to tear. Furthermore, the use of rebar supports with a guide wire in Season 2 versus wooden stakes in Season 1, provided a more structurally sound barrier. We recommend that Veg Pro continue to use the same fencing materials as used in Season 2 in the future.

The way the barrier is erected in important. The shade cloth should be supported by guide wire and rebar supports with the supports spaced about 7-10 meters apart. The supports should be close enough together that the top of the barrier does not sag. The barrier fence should be attached to itself along the guide wire and this is easily accomplished with cable ties. To help prevent trespass of frogs by climbing over the barrier material, the top of the material should be folded over the guide wire and secured so that a pocket is formed on the side of the fence toward the canal. The bottom of the barrier material must be buried underground to a depth of 20 cm to prevent trespass of animals underneath the fence. When the substrate prevents burying the material this deep it should still be buried as deep as possible and/or secured tight to the ground by some means.

Regular maintenance of the barrier fences is very important. During both seasons we frequently observed tears in the barrier fences a gaps at the bottom of the fence where is had been pulled up on purpose (e.g., water pipe access) or inadvertently. Gaps under the fence are potentially a serious problem because frogs will move along the barrier and when they find a gap they will be able to access the fields. Although the barrier fencing in Season 2 was less likely to tear, machinery from the day-to-day operations was still able to inflict major and minor damage to the fence. While damage to barrier and formation of gaps under the barrier are unavoidable, damage and gaps need to be repaired as soon as possible to maintain the physical barrier, reduce frog presence in the fields, and, ultimately, frog appearances in the final product. We suggest a thorough inspection of the barrier fences on a weekly basis to identify damage and gaps. Repairs to ensure the integrity of the barrier fence should be made immediately.

We do not recommend covering canals with material to exclude frogs (Figure 5). This method is cumbersome and maintaining the fence without gaps underneath the material is likely to be very difficult because of the muck soils, which are shallow and light in weight. An undesirable side effect of this method is that is would like inhibit movements and foraging of snakes and wading birds, which eat frogs.

PVC Pipes

PVC pipes proved to be an effective, durable, and cost-effective method to monitor treefrog activity around the fields. PVC pipes serve as daytime refugia for treefrogs only, since they are the only type of frogs able to climb and use the pipes. Frogs using the PVC pipes can easily be captured and transported off site, reducing the potential for this species to show up in the fields. They offer and added benefit from a monitoring perspective—by regularly checking these pipes and recording frog captures, Veg Pro personnel are able to notify workers in the packing plants when to be on heightened alert for potential contamination of frogs in the product.

We suggest that PVC pipes continue to be used on a regular basis to monitor and capture treefrogs. As with barrier fencing, PVC pipes should also be in place prior to Veg Pro's winter operations, or as early as possible once a season begins. PVC Pipes should be 3.2 cm in diameter, thin-walled, and cut to a length of 1 m. One end of each pipe should have a flat, straight cut while the other end should be an angled cut making it a sharp end that is easier to place into the ground. PVC pipes should be placed approximately 30 m apart from each other along the main canals at field borders (Figure 6) and along the middle canals in each field (middle canals are those adjacent to roads). Because of the need for machinery to access fields to plant and harvest the spring mix lettuces, we do not suggest installing PVC pipes along all of the lateral canals in the fields. Pipes can be alternately placed inside and outside of the fencing (i.e., field side and canal side) about 1m from the barrier fencing. Frog presence inside and outside of the fields can then be monitored by alternating on which side of the barrier fencing pipes are located. Be sure that PVC pipes are at least 1m from the barrier fences when at all possible to prevent frogs from being able to trespass the barrier fence by jumping from the top of the PVC pipes. Pipes should also be erected around each work area ramp.

PVC pipes should be checked weekly by Veg Pro personnel. Each frog encountered should be removed by placing a Ziploc bag over one of the pipe and evicting the frog into the bag with a 'plunger'. Frogs should then be transported to similar habitat at least 5 km from the fields and released. Furthermore, pipes should be checked the day after any significant rainfall occurs since frog movement typically increases after rain events. If metamorphs (hatchling frogs, ≤ 2 cm in length from snout to vent) are found in any pipes, then pipes should be checked daily until no metamorphs are captured (i.e., metamorph emergence from canals has ended). Pipes should be uniquely numbered within and between each field. Any time a frog is captured it should be removed and the following data recorded: date of capture, species of frog, exact or approximate snout-vent length (SVL) of frog, and pipe number. If possible, the coordinates of each frog capture should be recorded and analyzed periodically to determine if treefrogs are being captured more in certain areas. Accumulated over the years, these data may enable Veg Pro to further tailor their trapping dates.

Similar to barrier fences, maintenance of the PVC pipes is important. We had problems occasionally with pipe being blown over by wind or destroyed or knocked down by farm machinery. Maintenance of pipes can be conducted each week when the pipes are checked for frogs. Broken pipes should be replaced immediately.

Aquatic Trapping

Our research showed that using aquatic traps is a very effective method for capturing and monitoring tadpoles in the canals around Veg Pro's fields. In Season 1 we tested four types of traps and found that "Gee" minnow traps were as effective as or more so than the three other types of traps we tried. Gee traps are very durable and relatively inexpensive too. We recommend that Veg Pro continue to use aquatic traps to monitor and remove tadpoles in the lateral canals within fields. By regularly trapping for tadpoles, personnel can determine tadpole locations in lateral canals as well as the developmental stages of the tadpoles. Determining developmental stages of tadpoles will enable Veg Pro personnel to estimate when metamorph frogs may be emerging from the canals

and thus identify periods of increased probability of finding frogs in the spring mix fields. Removing tadpoles on a regular basis prevents metamorphs from getting into the fields and so reduces the likelihood of frog contamination in Veg Pro products.

Veg Pro should continue to use Gee traps since they are cost-effective, durable, and can be easily stored. All lateral canals (main canals are too deep and steep– sided for the traps to work) should be sampled regularly with at least ten, evenlyspaced Gee traps placed in each canal. Veg Pro personnel should decide which of the following sampling schedules would be most suitable:

- 1. deploy traps in all canals over a 1-3 day period every three weeks, or
- 2. deploy traps in a few canals at a time on a rotating basis so that all canals are sampled over a three week period.

Both of these potential trapping scenarios should be an effective means of monitoring tadpoles in the lateral canals. Initial deployment of traps should be as early in the growing season as possible and trapping should continue throughout the entire season. When Gee traps are deployed in the canals, they should be checked daily for tadpoles and to remove non-target species, such as non-venomous water snakes, invertebrates, fish and aquatic salamanders. Canals should be sampled every three weeks since the target animals for the Gee traps are Southern Leopard Frog (*R*. sphenocephala) tadpoles. Tadpoles of this species requires several months to reach metamorphosis (timing is largely dependent on water temperature and availability of food), therefore sampling every three weeks is sufficient to detect tadpole presence. In the event that tadpoles are found in the canals during one of the monitoring scenarios outlined above, trapping should be intensified in the canal(s) (e.g., more traps deployed continuously) until no or very few tadpoles are captured.

Gee traps are easy to deploy and check. Clip both halves of a Gee trap together and tie a 3-5 m line to the trap clip. Toss the trap in a canal at an appropriate location then secure the line to a stake or post and mark the post with flagging tape. The traps do not need to be baited, but baiting may increase trap success (we did not test this during our work). The trap should sit flat on the bottom and be sure the canal is deep enough that water covers the interior openings of the funnels on either end of the trap. Check traps by retrieving them from the canal with the line. Disconnect the halves of the trap and dump tadpoles into a bucket containing canal water. Release tadpoles in similar habitat well at least 5 km from the fields.

Harvesting Considerations

There are several considerations from a harvest and operations perspective that should also help reduce the potential for a frog occurring in the processed product.

- In the event that metamorph frogs are found (in PVC pipes or in the aquatic traps) the packing house should be notified to be extra vigilant when processing the spring mix. Juvenile frogs have a natural tendency to disperse, and as a result may end up in the fields. Due to their size, they are likely to be difficult to find in harvested spring mix, especially juvenile Green Treefrogs
- Do not harvest during or immediately following rain. Frogs are most active during and prior to rain, so rain increases the potential for a frog to be in the fields.
- Store pallets, containers, and equipment indoors at the Cypress Cooling plant when possible. This will reduce the likelihood of treefrogs being inadvertently transported from the cooler into the fields.

The "Frog Crew"

Installing barrier fences and PVC pipes as well as and conducting trapping and monitoring is a labor and time intensive job. Regularly maintaining the barrier fences is crucial and also takes a lot of time. Additional time, not to mention expertise, is required to deploy and check aquatic traps and PVC pipes, and to transport and release frogs and tadpoles. Because of these demands, we strongly suggest that Veg Pro hire a person each year, or train current personnel, whose primary duty is to conduct frog exclusion and trapping activities and coordinate these efforts with the University of Florida, as long as UF (e.g., Dr. Steve Johnson) and Veg Pro continue some type of agreement to work together. Although Carlos Restrepo did a commendable job in both seasons, he simply did not have enough time to devote to the task of frog exclusion and monitoring.

Summary SOPs

- Erect barrier fencing as described above along all canals as early as possible each season and maintain the barrier fences on a regular basis.
- Continue to use PVC pipe refugia to monitor and capture treefrogs as noted above—check pipes once a week. Release captured frogs well away from the canals and be sure to maintain the pipes.
- Be alert for metamorph (very young frogs) frogs. If found, increase the frequency of checking pipes and trapping in canals and notify the packing house to be on heightened alert for small frogs, especially little Green Treefrogs.
- Continue to monitor and trap tadpoles by deploying Gee aquatic traps in lateral canals. Initial trap deployment should be as early in the season as possible and monitoring should continue throughout the growing season.
- Trap in all canals once every three weeks following one of the two protocols suggested above and release tadpoles off site.

- Increase sampling effort in canals where tadpoles are captured until none or few tadpoles are trapped.
- Assign a permanent person to handle the daily monitoring and trapping of frogs and tadpoles as well as to maintain the barrier fences.
- Store pallets, containers, and vehicles indoors at the Cypress Cooling plant whenever possible.

FROG NATURAL HISTORY

There are several species of frogs that are known to occur, or are suspected to occur, in Veg Pro's fields in Florida and Canada. They fall into several groups, with members of each group sharing similar physiology and ecology. The groups include toads, treefrogs, and 'true frogs'. In Florida there is another group represented by a single species—the Greenhouse Frog. Toads have warty, dry-ish skin and are quite terrestrial. They are poor climbers and cannot jump very high. The treefrogs have suction pads on the ends of their toes, making them excellent climbers. The 'true frogs', genus *Rana*, have webbed rear toes and are primarily aquatic. However, they can disperse long distances in terrestrial habitats, such as crop fields. The Greenhouse Frogs in terrestrial and lays its eggs on land. It prefers to hide under objects in moist places.

What follows are brief 'species accounts' of frogs known or suspected to occur in Veg Pro's fields in Canada and Florida. Because of the variation in their ecology, a variety of methods to trap and monitor these species is required.

Florida Fields

Southern Toad (*Bufo terrestris*)

Like other toads, Southern Toads are squat frogs with short legs, warty skin, and obvious parotoid (poison) glands on their shoulders. They have prominent cranial crests or knobs on the top of their heads. They vary in color from reddish, to gray, to brown with darker spots and splotches. They are medium-sized frogs, with adults normally ranging from 5-10 cm SVL (snout to vent length). Males are usually smaller than females. During the breeding season, roughly March-August in southern Florida, males have darkened throats and relatively rough pads on their thumbs for grasping females during breeding. The most similar looking toad in southern Florida is the non-native Marine Toad. The Marine Toad lacks the prominent cranial crests of the Southern Toad, has very enlarged parotoid glands, and grows much larger than the Southern Toad—Marine Toads can reach lengths in excess of 20 cm.

Southern Toads may be found throughout the Southeastern Coastal Plain from Virginia to Louisiana. They occur in a great variety of habitats ranging from pinelands and hammocks to suburban neighborhoods and agricultural fields. They prefer loose soils in which to burrow and are often found well away from

water. They are primarily nocturnal and feed on a wide variety of insects. They are preyed upon by snakes, mammals such as raccoons, and giant water bugs.

Southern Toads are most active during warm, wet periods, but may be active year-round in south Florida. They are spring and summer breeders and have a call that is a loud, high pitched, drawn out trill. Females lay their eggs in long strings or coils of up to 4,000 eggs. They prefer to lay their eggs in shallow areas along the edges of lakes, streams, canals, ditches and ponds. They will event lay eggs in tire ruts that hold water. Depending on the temperature, eggs develop into tadpoles and then small toadlets in 30-55 days. Recently metamorphosed Southern Toads are about the size of a raisin. They disperse into upland areas away from breeding sites.

Because of their propensity to live away from water and burrow, Southern Toads may be found in Veg Pro's Florida fields. Because they burrow, the lettuce fields may be suitable places for them to spend extended periods of time. Like other toads, because they are poor climbers they should be excluded from fields by barrier fences.

Green Treefrog (Hyla cinerea)

Green Treefrogs are recognizable by their green color and a distinct white line down each side of their body. Their green color may range from a dull, olive green (almost brown) to the bright lime green color of fresh vegetation. The lines on either side of a Green Treefrog are very well demarcated and may be bordered by a thin line of black. Some individuals have small yellow flecks on their backs. Green Treefrogs have an elongate body and a somewhat pointed snout. They grow up to 7 cm SVL. Like other treefrogs, they have pads on the ends of their toes and are excellent climbers.

Green Treefrogs have a large geographic range and occur throughout much of the Southeast, from Texas to the Chesapeake Bay region of the Delmarva Peninsula. They are habitat generalist and live in a wide variety of places. Although they are terrestrial, they usually do not stray far from permanent bodies of water, such as lakes, ditches, canals, swamps, and marshes. They are commonly found in suburban neighborhoods and agricultural fields close to a water source. They prefer to live in areas of dense, shrubby vegetation, but will also live on and around homes and buildings. They feed primarily on insects.

Green Treefrogs, like many species of frogs in southern Florida, are most active during the spring and summer months, especially during periods of rain. They breed primarily in late spring and early summer in permanent bodies of water that support fishes. Males call from perch sites in shrubs, usually above or very near water, to attract females. Females lay 500-1000 eggs, which take 25-45 days to develop into small frogs. Recently transformed Green Treefrogs are very cryptic and difficult to see because of their color and small size. Young animals will disperse from breeding sites, especially during rain.

Because of they are very camouflaged, are good climbers, and are somewhat terrestrial, Green Treefrogs are a hazard in Veg Pro's Florida fields. It is important to monitor and capture Green Treefrogs by erecting PVC pipes around the fields. Learning more about this species ecology could lead to better refined methods to monitor and capture them.

Southern Leopard Frog (Rana sphenocephala)

Southern Leopard frogs are related to the 'true frogs', which include Bullfrogs, Pig Frogs, Green Frogs, and their allies. Southern Leopard Frogs range in size from 3 cm for a young juvenile to 10 cm for an adult. Although they vary in color greatly, they usually are a combination of greens and browns. They have numerous spots in their back and two obvious yet thin ridges of skin down each side of their back (e.g., dorsolateral ridges). They have a relatively pointed snout and webbed rear feet. During breeding periods males developed enlarged thumbs with dark excrescences for grasping females. Males have paired vocal sacs that are obvious on the sides of the head of calling individuals. They have strong legs and are excellent jumpers

The Southern Leopard Frog has a large geographic range and may be found throughout the eastern US, from Texas north to Illinois, east to New Jersey, and south to the tip of the Florida peninsula. They are found in just about any aquatic habitat, ranging from lakes and rivers to small ponds and ditches. They are most active at night and during rain. Although they are highly aquatic, Southern Leopard Frogs are capable of moving hundreds of meters away from wetland habitats. This is especially true of recently metamorphosed frogs that are dispersing. They are preyed upon by primarily by alligators, snakes, and various wading birds. They eat insects and other invertebrates mostly.

Although they are active all year, Southern Leopard Frogs breed primarily during the winter months in Florida. Males call from the water to attract reproductive females. Females lay round masses of up to several thousand eggs, which take about three months to develop into small frogs. Young leopard frogs frequently leave the habitat in which they developed as tadpoles and disperse to colonize new areas.

Because Southern Leopard Frogs are somewhat terrestrial, they are likely to move through Veg Pro's fields, especially during rain and when young frogs are dispersing. Because of their ability to jump, and potentially trespass barrier fences, it is important to maintain the fences regularly.

Greenhouse Frog (Eleutherodactylus planirostris)

Greenhouse Frogs are not native to Florida, but were accidentally introduced via shipments of crops about 150 years ago. They are a small brown frog, seldom exceeding 4 cm SVL. They have thin, unwebbed toes. Some have what look like two light stripes on their backs and others do not—this varies among individuals.

These nonnative frogs are found throughout peninsular Florida and in isolated locations in the panhandle. They occur in a diversity of natural habitats as well as suburban and urban settings. They are common in the yards and gardens of suburban neighborhoods. They prefer to stay hidden under logs, rocks, and debris. Although there have been few studies of their diet, in Florida they are known to eat ants, roaches, beetles, and other small invertebrates. They in turn are eaten by small snakes.

Unlike Florida's native frogs, Greenhouse Frogs do not require water in which to lay their eggs or for tadpoles to develop. They lay eggs terrestrially, in moist places under objects like rocks, logs, and leaves. The number of eggs ranges from 3-26 and they take 2-3 weeks to hatch. Tiny, fully formed froglets emerge from the eggs, having gone through the tadpole stage in the egg. In southern Florida, Greenhouse Frog breeding activity occurs from April-September, with most activity in May and June. Their call has an insect-like quality and sounds like soft chirping.

Because of their small size and the fact that they are exclusively terrestrial, this species may be expected to be found in Veg Pro's fields. Erecting barrier fences and keeping them well maintained, especially preventing gaps under the fences, is probably the most effective means to exclude them.

Canada Fields

American Toad (Bufo americanus)

The American Toad, like other members of the genus *Bufo*, is a rather fat bodied amphibian with dry, warty skin. The large parotoid glands on the shoulders, also characteristic of this genus, are separate from the prominent cranial crests or connected only by a short 'spur.' American Toads range in length from 5-10 cm SVL with females generally larger than males. Coloration is also variable, ranging from gray to olive or reddish brown, with patches of yellow/tan and brown/black spots that contain only 1-2 warts. The chest is usually spotted with dark pigment and the vocal (throat) sac of males is often dark in color. During breeding season, males may also be distinguished by the presence of dark 'nuptial pads' on the "thumb" of the forelimb and are used to grasp the female during mating. Notably, the Canadian Toad (*Bufo hemiophrys hemiophrys*) is highly similar in appearance although a cranial bump or 'boss' makes crests less obvious, and the ranges of these two species only overlap in extreme southeastern Manitoba.

The range of the American Toad extends from the Maritime Provinces to southeastern Manitoba and continues throughout the eastern U.S. (with the exception of the southeastern coast). These toads are abundant in a variety of suburban and natural habitats, preferring open wooded areas, forest edges and fields that provide moist hiding places in close proximity to shallow water breeding sites. Like most toads, they are nocturnal, foraging for invertebrate prey (beetles, slugs, worms, etc.) by night and returning to depressions under grass or rocks by day. Although toads secrete distasteful toxins, they are preyed upon by many snakes and nocturnal animals such as owls, raccoons and skunks. Remarkably, adults of this hardy species are not highly sensitive to human impacts such as habitat fragmentation and readily repopulate clearcut or burned areas, although the tadpoles are less resilient and suffer negative effects of chemical/pesticide contamination.

American Toads are active from mid-late spring into the fall, although the timing of the activity period varies with latitude and elevation. This species is not freeze tolerant and hibernates during winter months, burrowing into the soil below the frost line when temperatures drop below 9°C. Upon emerging from hibernacula in mid-April/May, males move to shallow (5-10 cm, often temporary), fish-free wetlands, pools, ditches or streams that will serve as breeding sites. Males establish territories and compete for mates, calling females to the breeding site with long, whistle-like trills. Females lay 2-20,000 eggs in two long strings amid emergent vegetation, which rapidly hatch, producing schools of dark-colored tadpoles. In approximately two months, tadpoles metamorphose into small, terrestrial toadlets and migrate *en masse* to adult habitats where they will remain for 2-4 years before reaching sexual maturity.

Because of their preferred breeding and nonbreeding habitat, American Toads are likely to be encountered in Veg Pro's Canadian fields. They also occur in the forested areas bordering the fields. They will breed in small, shallow depressions in and around the fields. Because they burrow, the lettuce fields may be suitable places for them to spend extended periods of time.

Gray Treefrog (Hyla chrysoscelis and H. versicolor)

Gray Treefrogs exhibit the long limbs and disk-like adhesive toepads characteristic of the genus *Hyla*. The Gray Treefrogs are actually two "look-alike" species that share a range and can only be distinguished in the field by their calls. The coloration of these small treefrogs (2.5-5 cm SVL) varies from brown to gray/green to nearly white with irregular black markings on the warty skin of the back, enabling them to camouflage on rough trees and mossy or lichen encrusted fences. A distinctive light spot is present beneath the eye, and the hidden portion of the hind leg (e.g., thigh) is a bright orange-yellow with black splotches.

The range of the Gray treefrog extends from southeastern Manitoba through southern Ontario and Quebec (with isolated populations in New Brunswick), and continues throughout the eastern U.S. (with the exception of northern Maine, peninsular Florida and coastal Louisiana). These treefrogs are found on the ground or at the water's edge only when breeding, preferring trees and shrubs near water where they "sit and wait" for unwary beetles, caterpillars and other insects that make up their diet. There are a variety of frogs, turtles, snakes, birds and mammals that might potentially prey upon the Gray Treefrog, which exudes a foul-tasting, irritating mucus, and has even been reported to feign death to discourage predation. Human impact upon this species is limited and it is at least moderately tolerant of human habitat disturbance and chemical/pesticide contamination.

A freeze tolerant species, Gray Treefrogs are active from spring through the fall and are capable of surviving temperatures of -6°C for up to two weeks, although they may hibernate in the soil or leaf litter at the bases of trees during winter months. Breeding occurs from March to July, peaking in May and June, and each individual will mate approximately three times during this period. At dusk, males begin to solicit mates, calling in musical trills from high in the trees near the edges of ponds, ephemeral wetlands or ditches, and move gradually lower as the evening progresses. Females lay packets of 30-40 eggs that form a surface film (10 x 12 cm), usually loosely attached to emergent vegetation. Interestingly, there are reports of Gray Treefrogs laying eggs in the water-filled furrows between cornrows, although these sites may not retain water for long enough (about 60 days) for the tadpoles to metamorphose and leave for terrestrial habitats. Young Gray Treefrogs generally do not ascend into the trees during the first year, rather utilizing sedges, reeds or saplings, and will require approximately two years to reach sexual maturity.

Gray Treefrogs, preferring forested habitats, are a common frog in the forests bordering Veg Pro's fields in Canada. They breed in shallow ponds within these habitats, but may also breed in ditches and pools in and at the edges of lettuce fields. It is unlikely, given their habitat preference, that Gray Treefrogs will be a problem in the fields. However, during metamorphosis, young Gray Treefrogs may disperse through crop fields, especially at field edges bordering forested areas.

Wood Frog (Rana sylvatica)

The Wood Frog, a member of the genus *Rana*, has the smooth skin, long legs, webbed rear toes, paired 'dorsolateral' ridges along the body and large 'tympanum' or eardrum (larger than the eye in males) characteristic of this genus. These frogs range in length from 2.5-7 cm SVL and vary in color from pink-brown to nearly black, with a distinctive dark "bandit mask" behind the eye. In the far northern areas of its range, Wood Frogs often have a light stripe down the back and move with a toad-like hopping gait due to shorter hind legs.

The range of the Wood Frog extends from Labrador to Alaska and continues south through the northeastern U.S. to the southern Appalachian Mountains, with the western edge of the range following a diagonal from Alaska southeast to the Virginia/Tennessee border. These frogs are typically found in or near moist wooded areas in a variety of habitats (tundra, alpine woodlands, thickets, meadows, bogs, temperate forests), often a great distance from water. Wood Frogs prey upon a myriad of insects and other invertebrates and are themselves prey to a variety of snakes, birds and mammals. Human activities have also affected this species, which is particularly sensitive to reduced cover and edge effects caused by forest cutting.

The moderately freeze tolerant Wood Frog is active from late winter/early spring through the summer, although the timing of activity varies with latitude and elevation. Adults synchronously emerge from shallow terrestrial hibernacula located near breeding ponds and converge upon seasonal, semi-permanent breeding sites (wetlands, ponds, ditches, etc.), often while ice is still present on the surface of the water. Males attract females with duck-like "quacks" (produced by paired vocal sacs) to communal egg laying sites where each female lays 1-3000 eggs in a loose mass. Within a few days, the breeding frenzy ends and adults disappear from the ponds, to which they will faithfully return in subsequent years. After an extended developmental period of approximately 60-120 days, tadpoles metamorphose and disperse to terrestrial habitats, where they will remain until reaching sexual maturity (1-3 yrs).

Because of their ecology, Wood Frogs are not likely to be a problem in Veg Pro's Canada fields. They are most active when they emerge from winter hibernacula to breed. They are explosive breeders, laying eggs over a short period of time in early spring. This breeding activity should be over by the time Veg Pro moves operations back to Quebec each summer. However, considering the extended larval period, young Wood Frogs may be leaving ponds and dispersing after Veg Pro has established operations in Canada in some years. Also, given their sensitivity to disturbed areas, they likely will avoid crop fields, preferring to remain in forested habitats around the fields.

Green Frog (Rana clamitans)

The Green Frog, like other members of the genus *Rana*, has smooth skin, long legs and webbed rear toes, dorsolateral ridges and a sizeable tympanum (larger than the eye in males). Green Frogs range in length from 5-10 cm SVL, with females slightly larger than males. These frogs are typically green or greenish-brown in color with gray-brown spots on their backs (especially in young frogs) and dark spots on their white bellies. Notably, the throats of males are often bright yellow in color.

The range of the Green Frog extends from the Maritime Provinces west through the southern regions of Quebec and Ontario to extreme southeastern Manitoba, and continues throughout the eastern U.S. (with the exception of southern peninsular Florida and portions of Illinois and Indiana). This species has also been introduced in areas of British Columbia and Newfoundland in Canada, and in Washington and Utah in the U.S. These frogs are often found in abundance in or near (within 1 m) ditches, wetlands, creeks, ponds or other shallow bodies of water, where they opportunistically feed on a variety of invertebrates, fish and other frogs. In turn, they fall prey to various birds, other Green Frogs or Bullfrogs, Garter Snakes, water snakes and even humans. Undoubtedly, human activities such as hunting (game species in some areas), shoreline development and chemical/pesticide contamination have had marked effects on Green Frog populations.

Green Frogs are active from spring through fall, overwintering in silt and debris at the bottom of flowing, highly oxygenated springs, creeks or streams that do not freeze. These frogs typically breed for an extended period from mid-late spring through summer in the permanent wetlands, ponds and lakes in which they reside. Males utilize paired vocal sacs to emit a distinctive call, attracting mates to their territories (clumps of vegetation) with a sound that can only be compared to the plucking of a loose banjo string. Females lay 1-7000 eggs in a foamy surface film (~30 cm diameter) among the emergent vegetation in shallow water. Notably, the tadpoles require approximately 90 days of development prior to metamorphosis, and tadpoles hatched from late season eggs may overwinter as tadpoles. After metamorphosis occurs, the young move to nearby meadows or woods under rainy conditions, dispersing up to 5 km during the time required to attain sexual maturity (~1 yr).

Green Frogs, being largely aquatic, will most often be found in ditches bordering Veg Pro's Canadian fields. When tadpoles are undergoing metamorphosis, as well as during wet periods, Green Frogs may disperse through Veg Pro's lettuce fields. They are unlikely to remain in the fields for extended periods, however.

SUGGESTED RESEARCH

Although we developed several effective methods to exclude, trap, and monitor frogs, there are still questions that need to be answered in order to further refine the techniques and learn more about frog behavior. This information will enhance the ability of Veg Pro to keep frogs out of their product.

Will frogs dispersing through the fields remain in the fields for an extended period of time? Do they prefer or avoid particular species of lettuces? How do they specifically react to rain? Are they able to find gaps underneath the barrier fences to access fields? Are they able to easily trespass the barrier fences? Do treefrogs found in PVC pipes use the pipes over a prolonged period? Do they leave the pipes to forage in the fields at night? To answer these questions we would need to radio track movements of frogs. This would entail outfitting frogs with transmitters and tracking their daily movements. Another beneficial research project would be to compare capture success of baited vs. unbaited Gee traps in order to maximize trap efficiently. It would also be beneficial to test different diameters and thickness of PVC pipes to determine which pipe type is most efficient at attracting frogs. These are labor intensive projects that would require adequate financial support. These projects could form the basis of a graduate student's project if supported by Veg Pro and TKM.

ACKNOWLEDGEMENTS

We would like to thank Veg Pro for funding this project and in particular Daniel Malenfant for executing the award to the University of Florida. We especially thank Carlos Restrepo for all his help in the fields and for working closely with us to conduct the project. And we appreciate the help of Veg Pro staff under the guidance of Carlos Restrepo for installing barrier fences and PVC pipes, and help maintaining this equipment.

Report Figures



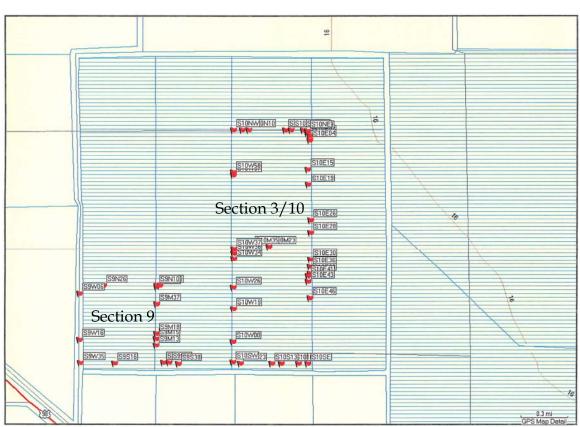


Figure 1. Barrier fence supported by rebar stake in the 2005-06 growing season. The guide wire is visible at the top of the barrier material.

Figure 2. Locations of PVC pipes where Green Treefrogs (*Hyla cinerea*) were captured in Season 2.

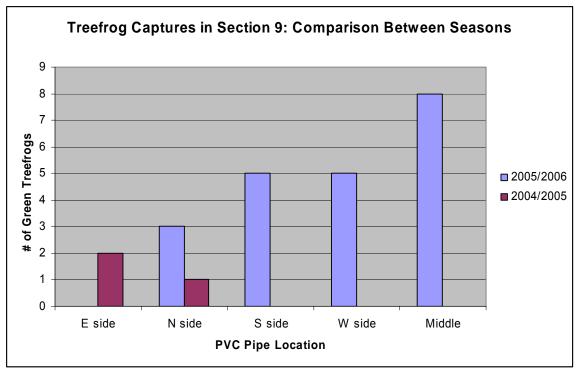


Figure 3. Total number of treefrog captures in Section 9 PVC Pipes from Seasons 1 (December 2004-May 2005) and 2 (December 2005-March 2006). In the 2004/2005 season, no PVC pipes were placed along the middle canal.

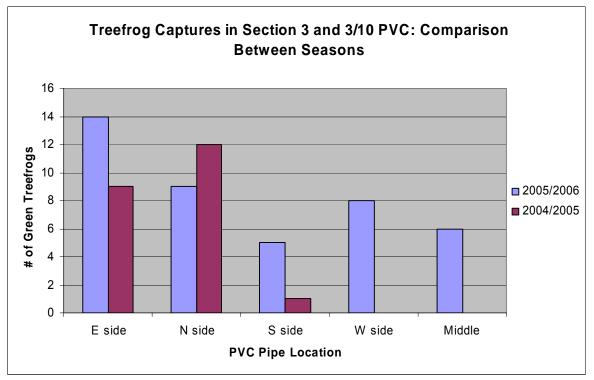


Figure 4. Total number of treefrog captures in Section 3/10 PVC Pipes from Seasons 1 (December 2004-May 2005) and 2 (December 2005-March 2006). In the 2004/2005 season no PVC pipes were placed along the middle canal.



Figure 5. Barrier fence material used in an attempt to cover a lateral canal.

Appendix Summaries of Fieldwork Conducted at Veg Pro Fields During the 2005-06 Growing Season

Trip 1: December 12-15 2005

General Summary

This was the first trip for the University of Florida crew in the 2005/06 growing season under a sponsored program agreement between UF and Veg Pro. Dr. Steve A. Johnson, Esther Langan, and Melissa Friedman met Carlos Restrepo in the fields on Monday, 12 Dec. Carlos gave us a tour of the fields to reacquaint us with the site and show us the barrier fences and PVC pipes that he and his crew had erected. Veg Pro had installed barrier fences around all of the main canals and the vast majority of the lateral canals. Carlos had also set two Gee traps in each lateral canal—one at each end. Prior to this visit he had not captured any tadpoles, but had encountered several (4-5) Green Treefrogs in PVC pipes in Section 3. These frogs were left in the pipes. During our visit we deployed 10 Gee traps in each canal on a rotating basis so that all but a few lateral canals were sampled during our trip (see below). We also checked all PVC pipes for Green Treefrogs and repositioned many of the pipes farther away from the fences to prevent possible trespass by frogs using the pipes to jump over the fences. We also dispersed 56 cover boards (2 at the ends of each lateral canal), in an effort to detect Greenhouse Frogs. Despite an ambitious trapping effort, we did not capture any tadpoles. However, we removed a total of 36 Green Treefrogs from the PVC pipes. Sampling recommendations for Veg Pro prior to our next visit, which is scheduled for January 17-21, are included at the end of this summary.

<u>12 December 2005:</u> It was relatively cool (mainly in the 60s). Carlos had two Gee traps located at the ends of each lateral canal. There were 56 Gee traps owned by Veg Pro that we wished to use for tadpole monitoring in the lateral canals. However, prior setting the traps we had to purchase missing trap clasps that secure the trap halves together. We also purchased flags and spray paint for use to mark Gee trap locations and to number cover board traps, respectively. Upon returning from Home Depot we placed eight additional traps in Section 9 lateral canals starting from canal 1/2 (i.e., 1A or 1B to 2A or 2B) to canal 7/8. Therefore, these seven canals each had ten traps total. Traps were positioned along the total length of each canal for a more comprehensive trapping effort per canal.

<u>13 December 2005:</u> Dr. Steve Johnson, Esther Langan, and Melissa Friedman met with Carlos Restrepo and Daniel Malenfant in the morning. Daniel Malenfant voiced concern that some PVC pipes were placed so close to the barrier fence as to facilitate treefrog access to Veg Pro fields. To address this concern Steve

Johnson agreed to either move pipes farther away from the barrier fence, if possible, or to move PVC pipes to the inside (roadside versus canal side) of the fences. After the meeting we checked all 70 Gee traps located in Section 9. We captured no tadpoles and very few fish (mainly mosquito fish, *Gambusia holbrooki*) and crayfish. We suspect the overall lack of animal captures in the Gee traps may be directly related to the fact that the canals were dredged twice since Hurricane Wilma. We then removed all traps except the two originally placed at each canal's end by Carlos. We relocated the 56 traps to canals 8/9 through 11/12 in Section 9 as well as canals 9/10 through 11/12 in Section 3/10.

We placed 56 cover boards total in both Sections 9 and 3/10. Cover boards are simply plywood pieces approximately 0.5 m² in size. When placed in close contact with the ground they serve as refugia to many vertebrates, including Greenhouse Frogs (*Eleutherodactylus planirostris*), Southern Toads, snakes, and lizards. Our target animal for the cover boards are Greenhouse Frogs since we captured three of these in funnel traps last year. Cover boards will serve as more effective monitoring devices for the presence of this species in the fields. We placed cover boards numbered 1-26 at Section 3/10 and cover boards numbered 27-56 at Section 9. We placed one cover board at the ends of each lateral canal as well as six cover boards at Section 9's ramp. Cover boards were numbered with black spray paint to provide exact locations of captured frogs that we will ultimately GPS.

In the afternoon we met with Carlos, Daniel, and a ReadyPak client to discuss and explain our methodologies for monitoring and reducing amphibian presence in the fields. After the meeting, Steve Johnson had to return to Plant City, Florida to attend a meeting. Esther Langan and Melissa Friedman then checked all PVC pipes in Section 3/10, moved pipes as described above, and gave each pipe a unique number for data collection purposes. For example, 10S13 refers to pipe 13 along the South side of Section 10 and 9M15 refers to pipe 15 along the middle canal of Section 9. Pipe numbers may not accurately reflect the number of pipes per side. This is because two persons numbered pipes if they were located on either side of a canal and because of the fact that disproportionate numbers of pipes were sometimes placed on either side of the field. Thus, pipes on one side will have even numbers and those on the other side will have odd numbers.

In Section 3/10 we captured eight Green Treefrogs, *Hyla cinerea*, along the East side; six Green Treefrogs along the North side; two Green Treefrogs along the South side; and eight Green Treefrogs along the West side of Section 3/10 for a total of 24 Green Treefrogs. Green Treefrogs in Section 3/10 ranged in snoutvent length (SVL) from 23-49 mm. Carlos Restrepo presented us with a Southern Toad (*Bufo terrestris*) that he captured from Section 9. All frogs were either adults or subadults.

<u>14 December 2005:</u> Esther Langan and Melissa Friedman with volunteer graduate student, Jason Martin, of the University of Florida's Everglades Research and Education Center (EREC) checked and moved necessary PVC pipes in Section 9. We captured two Green Treefrogs in the middle canal (located between fields 8 and 9); three Green Treefrogs along the North side; four Green Treefrogs along the South side; and three Green Treefrogs along the West side for a total of 12 Green Treefrogs in Section 9. The frogs ranged in size (SVL) from 35-47 mm. All frogs were either adults or subadults. We then checked aquatic traps in Sections 9 and 3/10 and again found no tadpoles and very few fish and crayfish. We relocated the 56 Gee traps to canals 2/3 through 8/9 in Section 3/10. Melissa Friedman encountered an old Southern Leopard Frog (*Rana sphenocephala*) carcass in Section 9.

<u>15 December 2005:</u> Esther Langan, Jason Martin, and Melissa Friedman checked and removed all aquatic traps from canals 2/3 to 8/9 in Section 3/10. We captured no tadpoles and very few fish or crayfish in these traps. Traps that Carlos Restrepo had placed on each end of all canals were removed from the water and placed on the outside of canal barrier fences for easy access if he decided to reset these traps in our absence. The 56 Gee traps that we removed from the above canals were taken apart and placed at Section 3/10's ramp.

24 H. cinerea

12 H. cinerea

PVC Pipes:

We captured many subadult or adult *H. cinerea* in Sections 9 and 3/10.

SECTION 3/10 PVC Pipes

13 December 2005:

- West Side: 8 *H. cinerea*
- South Side: 2 *H. cinerea*
- East Side: 8 *H. cinerea*
- North Side: 6 *H. cinerea*

SECTION 9 PVC Pipes

14 December 2005:

- West Side: 3 *H. cinerea*
- South Side: 4 *H. cinerea*
- North Side: 3 *H. cinerea*
- Middle Canal: 2 *H. cinerea*

A total of 36 Green Treefrogs was removed from the PVC pipes during this visit. Frogs were relocated to similar habitat at least five miles from the lettuce fields.

Aquatic Trapping:

During three days we sampled all but one lateral canal (canal 1/2 in Section 3/10) in both Sections for a total of 21 canals. We used ten Gee traps per canal.

Traps were placed in the canals in the afternoon and checked the following afternoon. We caught very few fish and crayfish and did not capture tadpoles during the three trapping days. This is likely due to the fact that the canals were dredged twice since Hurricane Wilma. It is also possible that Southern Leopard Frogs (*Rana sphenocephala*) have not begun breeding and/or the barrier fences are functioning to significantly reduce frog breeding access to the canals. It is also possible that tadpoles were present but they were too small (e.g., very young tadpoles) or there were too few of them for us to detect.

General Observations:

No live *R. sphenocephala* were encountered during this visit. We did, however, capture more Green Treefrogs in this one visit than during all of our visits last season. We captured 36 this visit compared to 25 total frogs captured last season.

Number of Green Treefrogs Captured:December 12-15Last SeasonSec 9123Sec 3/102422

Since Section 9 is half the size of Section 3/10, the same number of frogs per area were captured for both sections.

Recommendations:

Since the main species we encountered last season were Green Treefrogs and Southern Leopard Frogs, these are the two tadpole species we may encounter in Gee traps. Green Treefrog tadpoles generally take about 1 month while Southern Leopard Frogs generally take several months to reach metamorphosis. Also, we do not anticipate Green Treefrogs to breed until early spring—Leopard frogs, however, are winter breeders. Due to the relatively long tadpole stage of both species and the timing of Green Treefrog reproduction, at this point there is no need to trap lateral canals for four weeks. During our January visit we will again sample the lateral canals.

Cover boards should be checked sometime toward the end of December and at two week intervals thereafter. Greenhouse Frogs are small (1-4 cm long) brownish colored frogs (see images below) that will swiftly hop away when the board is lifted. Be careful when lifting coverboards because snakes occasionally hide under them. Be sure to instruct Veg Pro personnel not to kill snakes they may find—snakes eat frogs. Because of the large number of Green Treefrogs in the PVC pipes, we suggest that you check them once a week initially to gauge frog presence. The frequency of checking can then be altered based on the number of frogs captured. If high numbers are encountered then a more frequent monitoring regime should be implemented versus if few frogs are encountered then a less intensive monitoring schedule could be implemented.

Esther Langan sent datasheets for checking PVC pipes, cover boards, and Gee traps.

Greenhouse Frogs



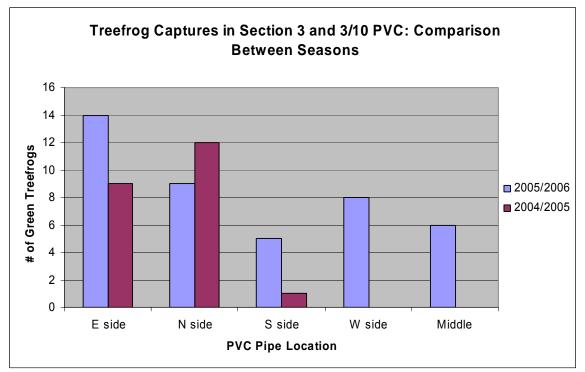


Figure 1. Total number of treefrog captures in Section 3 and 3/10 PVC Pipes from Seasons 1 (December 2004-May 2005) and 2 (December 2005-March 2006). In the 2004/2005 season, no PVC pipes were placed in the middle canal.

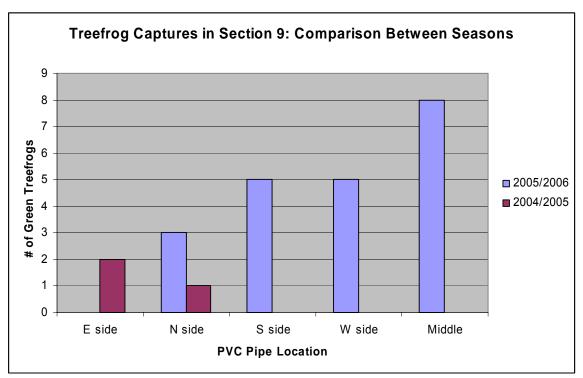


Figure 2. Total number of treefrog captures in Section 9 PVC Pipes from Seasons 1 (December 2004-May 2005) and 2 (December 2005-March 2006). In the 2004/2005 season, no PVC pipes were placed in the middle canal.

Trip 2: January 17-20 2006

General Summary

This was the second trip for the University of Florida crew in the 2005/06 growing season under a sponsored program agreement between UF and Veg Pro. Esther Langan and Melissa Friedman met Carlos Restrepo in the fields on Tuesday, 17 January. Since our last visit in mid December Carlos informed us that three Green Treefrogs had been found in the PVC pipes; three frog tadpoles (likely Southern Leopard Frogs) were found in canal 3/4 of Section 9; and one Southern Toad was found under cover board #20 in Section 3/10. To our knowledge Carlos did not remove these individuals. There was a lot of barrier fence damage throughout the fields. During this visit we again deployed 10 Gee traps in each canal on a rotating basis, sampling all lateral canals except the large canals at the ends of Section 3/10 and at one end of Section 9. We also checked all PVC pipes for Green Treefrogs and all (56) cover boards. We removed four Green Treefrogs from the PVC pipes, two frogs from each Section. We also removed ten Southern Leopard Frog tadpoles from Canal 3/4 in Section 9. Sampling recommendations for Veg Pro prior to our next visit, which is scheduled for March 13-17, are included at the end of this summary.

<u>17 January 2006:</u> It was relatively warm but very windy. We placed ten Gee traps each in canals 1/2 through 7/8 of Section 9 and also checked cover boards located at either end of these canals. We did not find Greenhouse Frogs or Southern Toads under these cover boards. About half of these cover boards had fire ant mounds beneath them. Fire ants may deter frogs from hiding under the cover boards, and thus reduce their effectiveness as a method for monitoring Greenhouse Frogs. In Section 9's 5/6 canal approximately 15m of fence was on the ground. We checked all PVC pipes in Section 9 and found two Green Treefrogs: one along the South side in pipe number 38 (SVL 37mm; weight 2.9g) and one along the middle canal in pipe number 18 (SVL 51mm; weight 7.7g). These two frogs were removed and released into similar habitat five miles from Veg Pro's fields.

<u>18 January 2006</u>: We first checked aquatic traps in the seven canals of Section 9 (canals 1/2 through 7/8). We captured only ten Southern Leopard Frog tadpoles, all from canal 3/4, the same canal where Carlos had captured three tadpoles prior to this visit. Progressing north to south, each trap contained 1, 1, 3, 0, 2, 0, 0, 1, 2, and 0 tadpoles, respectively. There appeared to be no concentration of tadpoles at a particular location within this canal. We retrieved the Gee traps, leaving the permanent traps set up by Carlos on either end of each canal. The Gee traps were moved to canals 8/9 through 11/12 in Section 9 and canals 1/2 through 4/5 in Section 3/10. We deployed ten traps per canal with the exception of canal 8/9 in Section 9. Because only part of this canal

contained water we were able to deploy only two aquatic traps. After leaving the Veg Pro fields, we released the ten Southern Leopard Frog tadpoles in a canal located five miles from the fields.

We checked all PVC pipes in Section 3/10, removing two Green Treefrogs from the South side, one from pipe number 2 (SVL 41mm, weight 3.6g) and one from pipe number 6 (SVL 41mm, weight 4.25g). We released the Green Treefrogs at a canal five miles from the fields.

The barrier fences at some places were in need of maintenance. The bottom of the barrier fence was not secured all along Section 3/10's north side. There was also minor fence damage along the south side of Section 3/10 as well.

While checking the traps we also checked the cover boards located at the canal ends. We did not find any amphibians under the cover boards. Many cover boards in Section 9 had fire ants underneath them. Also, Melissa Friedman found a dead, adult Southern Leopard Frog on the outside of lateral canal 11/12's barrier fence.

<u>19 January 2006:</u> We checked and collected the aquatic traps in Section 9 (canals 8/9 through 11/12) and Section 3/10 (canals 1/2 through 4/5) and again checked associated cover boards a second time. No tadpoles were found in any of the traps and no amphibians were found underneath the cover boards. Ten Gee traps each were then deployed in canals 5/6 through 11/12 in Section 3/10. No fire ants were found underneath Section 3/10 cover boards. We moved all cover boards in Section 9 that had fire ants underneath them. Cover boards were either moved to the other side of the canal or at least five feet away from the original location.

<u>20 January 2006</u>: We retrieved aquatic traps from Section 3/10, canals 5/6 through 11/12. We did not capture tadpoles in any of the traps. We left the two Gee traps per canal (located at either end of the canal) that Carlos originally set up so that he may deploy them in our absence. All other Gee traps were replaced at their ramp location in Section 3/10.

PVC Pipes:

We captured four Green Treefrogs (Hyla cinerea) in Sections 9 and 3/10.

SECTION 3/10 PVC Pipes

Total Frogs

18 January 2006:

• South Side: 2 *H. cinerea*

2 H. cinerea

SECTION 9 PVC Pipes 17 January 2006:

- South Side: 1 *H. cinerea*
- Middle Canal: 1 *H. cinerea*

2 H. cinerea

A total of four Green Treefrogs was removed from the PVC pipes during this visit. Frogs were relocated to similar habitat at least five miles from the lettuce fields.

Aquatic Trapping:

During three days we sampled all lateral canals within the fields for a total of 22 canals. We used ten Gee traps per canal. Traps were placed in the canals in the afternoon and checked the following morning or afternoon. As in our last visit, we again caught very few fish and crayfish. However, we removed ten Southern Leopard Frog tadpoles from canal 3/4 in Section 9. This is the same canal that Carlos captured three Southern Leopard Frog tadpoles.

Recommendations:

Since we captured tadpoles in only one canal in Section 9 (canal 3/4), we recommend that Veg Pro continue to regularly monitor this canal until our next visit, which is scheduled for mid-March. We recommend that at least 10 traps be deployed in this canal overnight (or several consecutive nights) at weekly intervals until no or very few tadpoles are captured. It is also advisable to conduct a complete trapping effort in all canals, setting traps on a rotational basis as described above and in the December report, in mid to late February. If tadpoles are captured in certain canals, those canals should then receive increased trapping effort until no or very few tadpoles are caught. And it is a good idea to continue to regularly monitor the 'permanent' traps at the ends of all lateral canals.

We also suggest that the PVC pipes be checked at weekly to bi-weekly intervals. Any frogs should be released well off site.

The cover boards should also be checked on a weekly to bi-weekly basis, and any amphibians be released off site. We suspect that the fire ants found in about half of the cover boards in Section 9 will be a recurring and unavoidable problem. If Veg Pro personnel repeatedly observe fire ants underneath cover boards in our absence it is recommended that the boards be removed and placed at locations next to canals within Section 3/10 where we have not yet encountered fire ants. Alternatively, it would be fine to treat the ant mounds with some acceptable bait/poison to reduce the fire ant problem.

The barrier fences need to be inspected and repaired when they are pushed over and where there are gaps under the fences. Lastly, it is very important that we be kept updated on frogs and tadpoles captured during Veg Pro's monitoring/trapping in our absence. We also urge Veg Pro personnel to use the data sheet we sent to record your effort and the number and location of captures.

Trip 3: March 13-17 2006

General Summary

This was the third trip for the University of Florida crew in the 2005/06 growing season under a sponsored program agreement between UF and Veg Pro. Esther Langan and Jason Martin (volunteer) met Carlos Restrepo in the fields on Monday afternoon, March 13. Seven Green Treefrogs (*Hyla cinerea*) had been captured since our last visit in January, 2006. Four of these were from Section 9, all along the middle canal. The remaining three Green Treefrogs were found in Section 10, two from the middle canal and one from the south side. These treefrogs were not removed. A Yellow Ratsnake (*Elaphe obsoleta quadrivittata*) was also found in a pipe along the middle canal of Section 9. We received no reports of tadpole captures between this visit and the one in January.

Veg Pro personnel had checked most of the pipes on the morning of March 13, (just prior to our arrival) and found four Green Treefrogs. We checked the remaining pipes and removed nine Green Treefrogs from Section 3/10. We deployed ten Gee traps per lateral canal except in canal 8/9 (i.e., between fields 8 and 9) of Section 9 because it was completely dry. We removed a total of ten Southern Leopard Frog tadpoles: seven in canal 3/4 of Section 9 and three in canal 2/3 of Section 3/10. During trap deployment and removal we checked all 56 cover boards twice and found one Southern Toad (*Bufo terrestris*) under the board at canal 2/3B in Section 3/10 and a Yellow Ratsnake (*Elaphe obsoleta quadrivittata*) under the board at canal 9/10A in Section 9.

<u>13 March 2006</u>: We met with Carlos Restrepo in the afternoon to collect PVC capture data from him. We were informed that Veg Pro personnel had already checked PVC pipes in about half of Section 3/10 and all of Section 9. They found four Green Treefrogs: three were along the north side of Section 3/10 and one was along the west side of Section 9. We spent the rest of the afternoon replacing string and clips on many of the Gee minnow traps (the majority of traps had a thin string that tangles easily). We then deployed ten traps into canal 1/2 of Section 3/10 and checked the cover boards at either end of this canal. No amphibians were found under the boards.

<u>14 March 2006:</u> We first checked the aquatic traps in canal 1/2 of Section 3/10 and found no tadpoles. We retrieved the Gee traps, leaving only the permanent traps set up by Carlos at either end of the canal. In Section 3/10 we deployed ten traps each into canals 2/3 through 8/9. We checked the cover boards at both

ends of these canals and found an adult Southern Toad under cover board #10 at canal 2/3B (west side). The toad was removed and later released at least five miles from the Veg Pro fields.

<u>15 March 2006</u>: We checked Gee traps in canals 2/3 through 8/9 in Section 3/10 and found three Southern Toad tadpoles in canal 2/3. This is the first direct evidence we have of breeding by this species at Veg Pro's fields. In Section 3/10, Gee traps were numbered 1-10, east to west. Trap 3 contained two tadpoles and trap 4 contained one tadpole. Tadpoles were removed and later released offsite at least five miles away. This is the first time that tadpoles have been captured in this canal. Thus far, we had captured tadpoles only in canal 3/4 of Section 9.

Next we deployed ten Gee traps each into the remaining three canals in Section 3/10: canals 9/10, 10/11, and 11/12. We deployed ten Gee traps each into canals 9/10, 10/11, and 11/12 of Section 9 as well. The majority of cover boards at canal ends of 2/3 through 11/12 in Section 3/10 and at canal ends of 9/10 through 11/12 of Section 9 had fire ants under them and no amphibians. The exception was cover board #29 at 9/10A (north side) of Section 9 which had a juvenile Yellow Ratsnake beneath it. We photographed but did not remove the snake since Veg Pro has not had any incidents of snakes in their product. Furthermore, these animals prey upon frogs and rodents and thus are beneficial to Veg Pro.

16 March 2006: We retrieved aquatic traps from Section 3/10 (canals 9/10 through 11/12) and from Section 9 (canals 9/10 through 11/12). We did not capture tadpoles in any of the traps. The juvenile Yellow Ratnsake was still present at the same location. We deployed Gee traps in the remaining canals in Section 9 except for canal 8/9 since it was completely dry. This canal was almost completely dry in January as well. Five of the 11 canals in Section 9 were partially dry or too shallow for the traps, causing us to concentrate traps either at one end of these canals or at both ends. We placed ten Gee traps in canal 5/6A, all on the north side, approximately five meters apart. We also placed ten Gee traps each on the north side of canals 1/2A and 2/3A with about three meters spacing between them. Ten Gee traps were highly concentrated in canal 6/7A, the water so shallow and the south side of the canal so dry that we were only able to place these traps ten meters into the canal from the road. Canal 4/5 had five traps deployed about 20 meters into the field from the road on both ends (north and south) of the canal. Finally, the water in canals 7/8 and 4/5 was just deep enough to allow the deployment of ten traps each along the total length of these canals. Cover boards did not have amphibians under them, but many again had fire ants.

<u>17 March 2006:</u> We checked the Gee traps in canals 1/2 through 7/8 in Section 9. Only canal 3/4 had tadpoles. Progressing north to south, each trap contained 0, 0, 1, 0, 0, 2, 3, 0, 1, and 0 tadpoles, respectively, for a total of seven Southern Leopard Frog tadpoles. Tadpoles were again released at least five miles from the fields.

We checked the remaining PVC pipes in Section 3/10. These included pipes on either side of the middle road, all pipes south of the middle road (i.e., the southern half of the section), and pipes north of the middle road on the east side of the section, opposite from Veg Pro fields (i.e., adjacent to sugarcane field). We removed a total of nine adult Green Treefrogs from these pipes: six from the east side, next to sugarcane field; two from the south side; and one from the middle. We used a GPS unit to record the PVC pipe location of all frog captures for this season. With the GPS unit we also recorded the corner locations of Section 3/10.

PVC Pipes:

We captured 13 adult Green Treefrogs (*Hyla cinerea*) during this visit: 12 in Section 3/10 and one in Section 9 (west side). Six of the 12 frogs from Section 3/10 were along the east side on the road opposite from Veg Pro (i.e., adjacent to sugarcane fields). The vegetation along this canal was much thicker and taller than anywhere else and might explain why the majority of treefrogs was captured here. Two of the 12 treefrogs from Section 3/10 were found on the south side, one was found along the middle road, and three were found along the north side. We did not observe loose skin on the throats of any Green Treefrogs, indicating that they likely are not yet breeding. Loose skin appears on males during the breeding season as a result of calling activity.

SECTION 3/10 PVC Pipes

13 March 2006:

- North Side: 3 *H. cinerea* 12 *H. cinerea*
- 17 March 2006:
 - East Side: 6 *H. cinerea*
 - South Side: 2 H. cinerea
 - Middle Canal: 1 *H. cinerea*

SECTION 9 PVC Pipes

14 March 2006:

• West Side: 1 *H. cinerea*

1 H. cinerea

A total of 13 Green Treefrogs was removed from the PVC pipes during this visit. Frogs were relocated to similar habitat at least five miles from the lettuce fields.

Cover Boards:

We captured one Southern Toad underneath cover board #10 located at the west end of canal 2/3 in Section 3/10. We also encountered a juvenile Yellow Ratsnake (*Elaphe obsoleta quadrivittata*) underneath board #29 located at the north end of canal 9/10. During this visit, roughly 80% of the cover boards were used by fire ant colonies.

Aquatic Trapping:

During four days we sampled all but one lateral canal within the fields for a total of 21 canals. Canal 8/9 in Section 9 was not sampled since it was completely dry. We used ten Gee traps per canal. Traps were placed in the canals in the morning or afternoon and checked the following morning or afternoon. We removed ten Southern Leopard Frog tadpoles from two canals. We captured seven tadpoles in canal 3/4 in Section 9 and three tadpoles in canal 2/3 of Section 3/10. To date, these are the only canals with tadpole captures and this visit was the first time tadpoles were captured in a canal other than 3/4 in Section 9. As with previous visits, compared to last season we caught very few fish and crayfish.

Recommendations:

Canal 3/4 in Section 9 and canal 2/3 in Section 3/10 should be trapped more intensively in our absence since tadpoles were captured in these canals. At least ten traps should be placed in each canal with equal spacing between traps. However, if time is a concern the traps can be concentrated at the ends of the canals to facilitate faster sampling by Veg Pro personnel. This latter option is still desirable over simply deploying one trap at each end of these canals where tadpoles have been consistently captured. Veg Pro personnel should continue sampling all other lateral canals on a regular basis. Since it has been a rather dry season thus far, we suggest that PVC pipes be checked every two weeks and immediately following rains.

Many parts of the barrier fencing are damaged and need to be repaired as soon as possible. We strongly suggest that Veg Pro establish a protocol of regularly monitoring the condition of the barrier fences (e.g., weekly at least) and making repairs in a timely manner. Cover boards are not being used by amphibians but may still be checked when aquatic traps are deployed since they are right next to each other.