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ECOLOGICAL STUDIES OF APPLE SNAILS

(*Pomacea paludosa,* SAY)

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EXECUTIVE SUMMARY

The Florida apple snail *(Pomacea paludosa*, Say) is a critical food web component in Florida wetlands. Water resource managers and the U.S. Fish & Wildlife Service have identified apple snail research as a high priority in central and south Florida wetland restoration efforts. The main impetus for apple snail ecological research is derived from interests in managing wetland and lake water levels to support Florida's population of endangered snail kites (*Rostramus sociabilis*). It is generally accepted that increased frequency and duration of dry downs beyond natural levels negatively influences snail kite populations. It is assumed that the negative impact manifests itself through depressed apple snail populations, although no data from controlled studies exist.

Dry downs are a natural process in the evolution and maintenance of the mosaic of plant communities within central and south Florida wetlands. The natural hydrologic regime of these wetlands has, however, been altered substantially due to installation of a network of canals and flood control structures. The overall goal of this report is to provide information critical to understanding the ramifications of water management practices on apple snail populations, and subsequently snail kites, as water resource managers endeavor to achieve the delicate balance of inundation and dry down in which Florida wetland communities evolved. The specific objectives of our research on apple snail ecology were to: 1) develop a reliable sampling technique for estimating snail density and/or relative abundance; 2) compare density and/or relative abundance of apple snails between a variety of common habitat types (sawgrass, cattail, wet prairie, and slough); 3) determine the behavioral

responses (migration or aestivation) of apple snails to drying conditions; and 4) estimate survival of apple snails during a drying event and evaluate the influence of hydrologic parameters (rate, extent, and duration of dry downs) on survival.

Many basic questions about the ecology or population dynamics of any organism require some measure of abundance, or at least relative abundance. Over the past two decades, several direct and indirect measures of apple snail abundance have been proposed, but none of these methods have been sufficiently evaluated to draw conclusions about their utility and reliability. In addressing objective 1, we compared three different methods (bar seine, dip net and suction dredge) for extracting snails from 1-m^2 throw traps. We also investigated the utility of two different trap systems, crayfish traps and trap arrays, to determine relative abundance. We used crayfish traps to explore the use of a mark-recapture technique to determine snail density. Finally, we examined the use of apple snail egg cluster counts as an index to population density.

We found that regardless of the method employed, obtaining reliable estimates of apple snail density will be time and labor intensive. The egg cluster index, highly desirable due to its ease and simplicity, was found to be of little value given its high degree of temporal and spatial variation. The bar seine was eliminated early in the throw trap investigation due to its low efficiency relative to other extraction methods. The suction dredge appeared somewhat less sensitive to habitat differences and tended to have slightly higher overall recovery probabilities than the dip net. However, the dip net required less effort and may require less initial investment. We encourage use of either the dip net or suction dredge if a throw trap method is to be employed. We found that it is imperative to

assess efficiency of extraction when using throw traps, especially when sampling across habitat types. Without information on extraction efficiencies, investigators risk misinterpreting site to site recovery variability as a real difference in snail density.

The use of crayfish traps or trap arrays as an index of relative abundance may be appropriate in some situations, but great care must be taken to control for time effects and site to site variation that might affect capture probabilities. If capture probability is not directly measured (e.g. a mark-recapture regime), then potential differences in capture probabilities may be inaccurately interpreted as a difference in snail abundance (as noted with throw trap recoveries). We believe that the use of crayfish traps within a mark-recapture sampling regime has the greatest potential to provide reliable estimates of apple snail densities. Mark-recapture data, obtained and analyzed as described in this report, provides not only population density information, but also information on survival, movements, and behavior of snails in the population. All of these parameters effect sampling protocols.

While testing throw trap and mark-recapture trapping techniques, we were able to draw some conclusions about the distribution of apple snails in graminoid marshes (Objective 2). Apple snails were found in all habitat types (sawgrass, prairie, slough, and cattail) encountered during our research. No consistent pattern of distribution among habitat types was observed, although it appears that higher densities of snails are more likely to be found in prairie or cattail habitats in some areas. Our egg cluster data, snail density data, and telemetry monitoring data revealed that snails utilize densely vegetated areas (e.g., interior sawgrass and cattail habitats). This contradicts earlier reports that concluded that snails have

difficulty penetrating dense stands of vegetation, and that habitat use is clearly skewed to less densely vegetated habitats. Our results do not dispute the importance of the prairie/sawgrass or slough/sawgrass ecotones as being critical for oviposition. We would simply add that the interior of sawgrass and cattail plant communities be recognized as important apple snail habitat. We agree with earlier reports that most favorable snail habitat would likely include a mosaic of densely vegetated and sparsely vegetated habitats within a wetland system. Based on our experience with site to site variability in the habitats and distributions of snails, we anticipate that a considerably greater effort will be required to make generalizations about snail distribution in different habitat types. Understanding snail use of different habitat types remains an important issue related to natural resource management practices which affect the plant community (i.e., hydroperiod, fire, aquatic weed control, nutrient loading), and most certainly in turn, apple snail populations.

Telemetry studies revealed that apple snails do not seek out deep water refuge during a dry down (Objective 3). Telemetry and crayfish trapping data indicate that apple snail reproductive ecology drives the movement patterns of snails more so than does hydrology. Water depth does, however, influence snail movements. An approximate depth of 10 cm appears to be a threshold level at which snail movements become impeded. At this point snails settle in one spot and, as residual water recedes, they become subjected to dry down conditions. They do not burrow, but they do conserve moisture through tight closure of their operculum.

Finally, we investigated snail survival during the dry season with one field and one laboratory study (Objective 4). Desiccation during the dry season is not necessarily a

predominate cause of mortality. The 1995 telemetry data, coupled with information gained through egg cluster counts and crayfish trap surveys in 1996, indicates that snails die within a few weeks after reproducing. In order to more closely examine the relationship between hydrology and adult snail survival, we conducted a laboratory study with controls and experimental tanks from which water was withdrawn to simulate a dry down. Laboratory studies confirmed that regardless of hydrologic conditions, post-reproductive adult size snails reach the end of their life span (estimated at 12 to 18 months) and die. Snails which did survive dry down conditions for 8 weeks (laboratory snails) and 12 weeks (observed in the field), tended to be juvenile size snails. We hypothesize that snail tolerance to desiccation is a function of snail size and/or reproductive status.

Further understanding of the relationship between dry down tolerance and snail physiological status will provide critically needed information about the impact of the timing and duration of dry downs under the control of water management districts. However, we already have information on the potential impacts of hydrologic regime on another critical factor regulating snail populations: recruitment. Our egg cluster surveys in the Blue Cypress Water Management Area in 1996, as well as earlier surveys done by other researchers on Lake Okeechobee and in Silver springs, reveal that peak egg cluster production by apple snails in central and south Florida consistently occurs between March and July; the majority of eggs are laid over a 4 to 12 week period. Dry downs which encompass the time period of peak reproduction may reduce or eliminate recruitment in the effected area. We do not suggest that dry downs be avoided, only that water management regimes consider their timing. Dry downs occurring later in the reproductive season (i.e.,

after peak reproduction) likely pose significantly less harm to snail populations. Our data indicate that young of the year snails can survive at least 2 to 3 months in dry down conditions. We believe rapid early growth enables snails hatched in March and April (typically a substantial portion of the total hatch) to reach sufficient size to survive a mid- to late spring dry down. The critical issue for snail populations may not be whether or not dry downs occur, but rather when they occur.

Our research has revealed the importance of understanding how snail population dynamics can affect interpretation of study results, and how the timing of hydrologic changes affects survival and recruitment in snail populations. Balancing the distribution of water for human use and for maintaining sustainable snail populations may narrow down to a few critical weeks or months late in the dry season.

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1.0 INTRODUCTION

The Florida apple snail *(Pomacea paludosa*, Say) is a critical food web component in Florida wetlands, one for which further research has been identified as a high priority in wetland restoration efforts in all south Florida wetlands including the Everglades (Science SubGroup, South Florida Restoration Task Force, 1996) and the Upper St. Johns River Basin (USFWS 1986, Turner 1994). The apple snail is the nearly exclusive food of the endangered snail kite (*Rostramus sociabilis*) (Snyder and Snyder 1969) and comprises over 75% of the diet of limpkins (*Aramus guarauna*) in central and south Florida (Cottam 1936, Synder and Snyder 1969). Other avian predators include white ibis (*Eudocimus albus*) (Kushlan 1974) and boat-tailed grackles (*Cassidix mexicanus*) (Snyder and Snyder 1969). In addition, alligators (*Alligator missippienisis*) (Fogarty and Albury 1968, Delaney and Abercombie 1986), redear sunfish (*Lepomis microlophus*) (Chable 1947), and soft-shelled turtles (*Trionyx ferox*) (Dalrymple 1977) prey on apple snails. Despite their long recognized importance in Florida wetlands, especially with regard to snail kites (Snyder and Snyder 1969, Perry 1974, Beissenger 1986, Bennetts and Kitchens 1997), surprisingly little is known regarding the life history, ecology, and population dynamics of *Pomacea paludosa*. Through our efforts described in this report, we have unraveled some of the mystery associated with snail ecology, particularly the impact of hydrology on apple snail population dynamics.

Seasonal fluctuations in rainfall occur in Florida, historically providing a hydrologic regime that supports wetland plant communities that benefit from cyclical drought and inundation (Gunderson 1994). Populations of *P. paludosa*, like other members of the gastropod family Pilidae, must contend with the annual and inter-annual hydrologic

fluctuations which shape the wetland habitats they occupy. The natural hydrologic regime of wetlands in Florida, however, has been altered substantially due to the installation of water control structures since the 1930's, particularly in South Florida (Light and Dineen 1994). Anthropogenic alterations to the natural hydrologic cycle to accommodate agricultural and urban needs now compromise the success of ecological strategies characteristic of some wetland species. For example, the untimely and unnaturally large influx of water from agricultural areas into the Everglades system has increased incidences of alligator nest flooding (Kushlan 1990). Conversely, diverting water to supply agricultural and urban needs has shortened hydroperiods in the southern Everglades (i.e., Everglades National Park), resulting in more frequent and longer dry downs which alter macrophyte community structure (Davis et al. 1994) and depress fish populations (Loftus and Eklund 1994).

Through their persistence and wide distribution in Florida, it is apparent that apple snail populations have adapted to the natural hydrologic dynamics of Florida wetland habitats. Undoubtedly changes in hydrology which deviate from the natural fluctuations will impact snails, but to what extent is unknown. Anecdotal evidence from observations of foraging limpkins suggests that snails become stranded during dry downs (Snyder and Snyder 1969). Some studies indicate that stranded snails are intolerant of dry downs as short as two weeks (Little 1968, Turner 1994). It is also apparent that snail populations respond to seasonal changes in hydrology, and that snail populations decline during extensive dry periods (Kushlan 1975). However, sampling efforts to determine snail abundance, and therefore population trends, have proceeded without knowledge of their efficacy.

The main impetus for apple snail ecological research is derived from interests in managing wetland and lake water levels to support Florida's population of endangered snail kites (USFWS 1986). It is generally accepted that increased frequency and duration of dry downs beyond natural levels negatively influences snail kite populations (Sykes 1983, Takekawa and Beissenger 1989, Bennetts and Kitchens 1997). It is assumed the negative impact manifests itself through depressed apple snail populations, although no data from controlled studies exist. Providing sufficiently long, and appropriately timed, wetland hydroperiods to support apple snails falls largely under the auspices of the South Florida and St. Johns River Water Management Districts. Bennetts and Kitchens (1997) point out that effective water management for kites does not mean excluding dry downs, which are a natural process in the evolution and maintenance of Florida's mosaic of wetland plant communities (Davis et al. 1994). The overall goal of this report is to provide information critical to understanding the ramifications of water management practices on apple snails, and subsequently snail kites, as the districts endeavor to achieve the delicate balance of inundation and dry down in which Florida wetland communities evolved.

The main goals of the research described herein were to evaluate apple snail sampling techniques and to investigate the extent of the impacts of drying events on snail populations. The specific objectives of our research on apple snail ecology are as follows:

1) Develop a reliable sampling technique for estimating snail density and/or relative abundance.

2) Compare density and/or relative abundance of apple snails between a variety of common habitat types (e.g., sawgrass, cattail, wet prairie, and slough).

3) Determine the behavioral responses of apple snails (e.g., migrate or aestivate) to drying conditions.

4) Estimate survival of apple snails during a drying event and evaluate the influence of hydrologic parameters (e.g., rate, extent, and duration of dry down) on survival.

The research was conducted in two phases in the period from March 1995- June 1997, in four different wetland systems (Water Conservation Areas 3A and 2B, Blue Cypress Water Management Area, and the Lake Kissimmee littoral zone) and in a laboratory setting. Data from both years and from more than one system were compiled to address each objective stated above. However, we report the results to reflect the objectives, not necessarily the chronological order in which the data were obtained. The following synopsis clarifies when and where the data were collected. The study sites are described in Chapter 2.

Phase I

Water Conservation Area 2B. A pilot study comparison of throw trap extraction techniques was conducted. A portion of the egg cluster index data was also

collected during this effort. Sampling was conducted from May 1995 - February 1996.

Blue Cypress Water Management Area. The first field study of snail movements and survival over the course of a marsh dry down was performed. Monitoring occurred from March - August 1995.

Lake Kissimmee. A study of snail movements and survival during an extreme draw down of the lake was conducted. Monitoring occurred from October 1995 - February 1996.

Phase II

Water Conservation Area 3A. A comparison of dip net and suction dredge extraction of throw traps was conducted in three sites in southwestern WCA3A. This data was collected from May 1996 through August 1996. The mark-recapture method was also tested in WCA3A, but in the eastern section. The mark-recapture data was collected from March 1997 through May 1997. During both the throw trap and mark-recapture data sampling periods, we also collected data on egg clusters as an abundance index, and compared snail densities in different vegetation types (sawgrass, cattail, and wet prairie).

Blue Cypress Water Management Area. A follow up study on snail movements was conducted to assess the relationship between snail breeding season and movements. This work was conducted from February 1996 through August 1996.

Laboratory Studies. A laboratory study was performed to compare the survival of snails exposed to dry down conditions to those under control (inundated) conditions. The study was started in May 1996 and completed in August 1996. We also performed temperature tolerance studies in October - November 1996.

In the process of achieving the project objectives, we also collected data on mating behavior, temporal and spatial variation in snail egg production, growth and senescence, and temperature tolerances. In addition we have developed novel techniques for conducting radio-telemetry studies and for collecting large numbers of apple snails for scientific study.

2.0 STUDY SITES

Our two year study on apple snail ecology included the three largest freshwater marsh systems in Florida: The Everglades, The Upper St. Johns Marsh, and the Kissimmee Marsh (Kushlan 1990). The hydrology of each of these areas has been altered by construction of levees and canals and installation of water control structures (Lowe 1983, Light and Dineen 1994, GFC 1995). The natural intra-annual and inter-annual variation in rainfall results in seasonal dry downs (typically in late Spring) which contribute to the maintenance of community structure of these and all central and south Florida wetlands (Kushlan 1990, Davis et al. 1994). Understanding water management impacts on apple snails in these particular wetland systems has been identified as important to recovery of the Florida snail kite (Bennetts and Kitchens 1997).

Apple snails inhabit many types of freshwater habitats in Florida, including forested swamps, rivers and streams, lakes, and agricultural canals and ponds (pers. obs.). Our study results and conclusions apply only to the graminoid wetlands and littoral zone habitats in which the research was performed. Important distinctions between graminoid wetlands and other aquatic systems, especially in terms of hydrology, may preclude extending our conclusions about snail ecology to non-graminoid aquatic habitat types.

2.1 Everglades Water Conservation Areas

The Everglades Ecosystem is part of a watershed that includes the Lake Kissimmee Chain of Lakes, Lake Okeechobee, the Everglades Water Conservation Areas, and

Everglades National Park. A history of the watershed and its management can be found in Light and Dineen (1994). Most of our work on apple snail survey techniques occurred in the Water Conservation Areas.

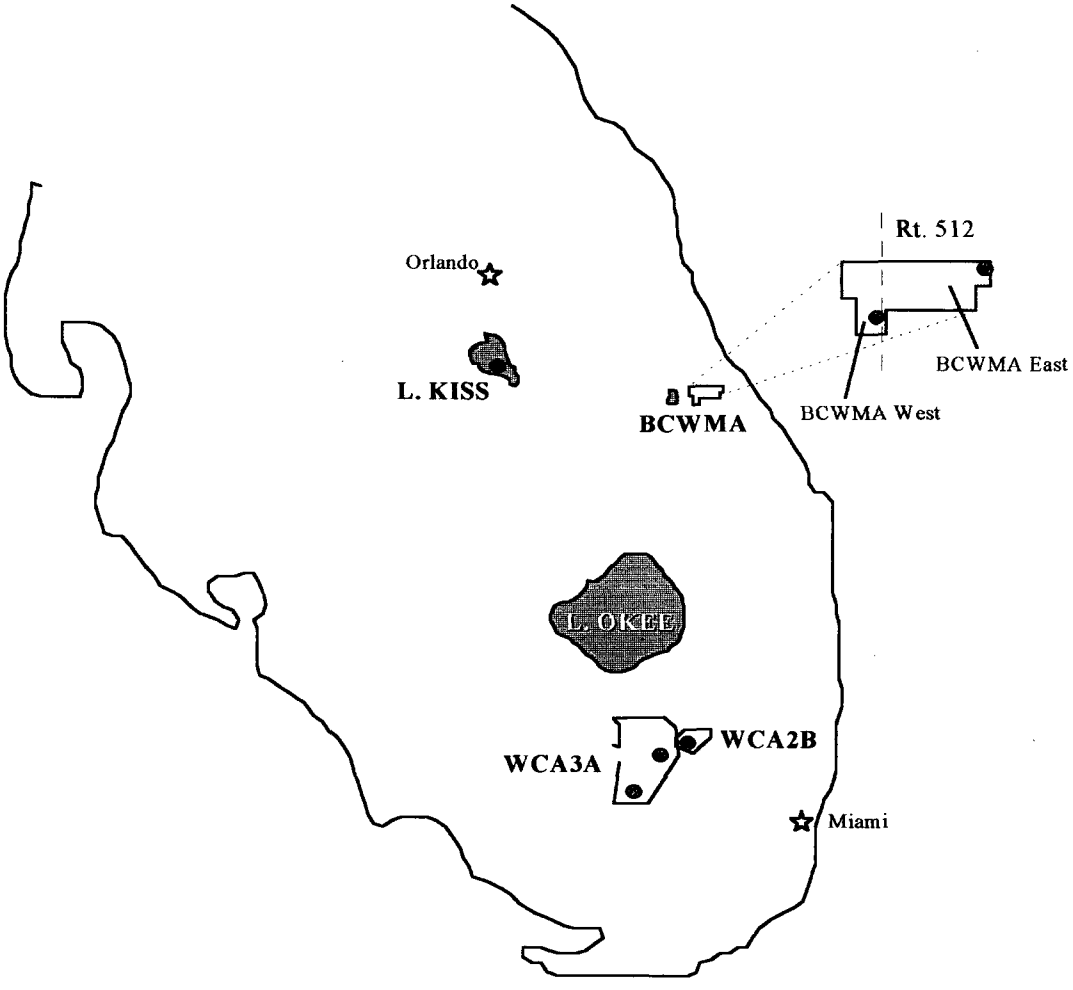
Water Conservation Area 3A (WCA3A)

WCA3A is a 237,000 ha wetland impoundment in Dade and Broward Counties (Figure 1). Historically, the area now confined within WCA3A was continuous with the Everglades system topographic gradient, and it conveyed the southerly flow of water into what is now the Everglades National Park. Water control structures have since diverted that flow toward the east, and now the eastern section of 3A, especially adjacent to Levee 67A, has deeper water and longer hydroperiods relative to western 3A. Because we sampled two distinct areas in WCA3A, we describe them separately.

South Western WCA3A

The plant community in south western WCA3A is a mosaic of sawgrass (*Cladium jamiacense*) marsh interspersed with wet prairie and slough, and dotted by tree islands of bay (*Persea palustris*), pond apple (*Annona glabra*) and/or willow (*Salix caroliniana*). Wet prairie habitats are characterized by an abundance of periphyton and the presence of emergent macrophytes such as spike rush (*Eleocharis cellulosa*), maidencane (*Panicum hemitomon*), beak rushes (*Rhynchospora* spp.), and arrowhead (*Sagittaria* spp.) Typical slough habitats support floating-leaved plants such as water lily (*Nymphaea odorata*) and submerged plants such as bladderwort (*Utricularia* spp.). We found gradations between wet

Figure 1. Map of south Florida showing the location of field study areas as described in Chapter 2 and referred to throughout the report (L. Kiss = Lake Kissimmee, BCWMA = Blue Cypress Water Management Area, WCA3A = Water Conservation Area 3A). Specific sampling sites noted by ●. Cities (☆) and Lake Okeechobee (L. Okee) included for reference.



prairie and slough difficult to distinguish within sites sampled, so we refer to these habitats collectively as prairie/slough. The marsh substrate consists of fibrous peat, but we did encounter some limestone rock in localized areas within sampling sites. Water depths at sampling sites ranged from 60 to 45 cm during our May through July 1996 sampling period.

Eastern WCA3A

We determined snail density in three distinct habitat types in eastern WCA3A; two sites each of cattail (*Typha* sp.), prairie/slough, and sawgrass. All sampling locations were within 2.0 km of the L-67A canal. The cattail sites were dominated by 2.5 to 3-m high cattail, with scattered stems of sawgrass and small pockets of water lily. Emergent vegetation in the prairie/slough habitat was dominated by spike rush. Sawgrass, approximately 2.0 to 2.5-m high, dominated the third habitat type. In general, sawgrass sites contained more open pockets of water lily and pickerelweed (*Pontederia cordata)* than the cattail sites. Water depths ranged from 89 to 72 cm in slough, 93 to 63 cm in cattail and 91 to 68 cm in sawgrass over the March - May 1997 sampling period. The underlying substrate in all sites was fibrous peat. Cattail sites maintained a flocculent organic debris layer over the peat; this flocculent debris layer was not observed in the prairie/slough or sawgrass sites. The slough sites supported a thick layer of periphyton. Periphyton was limited to small open patches in sawgrass sites, but was not discernible in cattail sites.

Water Conservation Area 2B (WCA2B)

WCA2B is an 11,300 ha impoundment in Broward County (Figure 1). The plant community is similar to that described for southwestern WCA3A, except the introduced Melaleuca tree (*Melaleuca quinquenervia*) is much more common. The substrate here is also a fibrous peat, but unlike WCA3A no limestone rock was encountered. Water levels in 1995 ranged from approximately 20 to 100 cm, but note that these measurements were

collected over 9 months. We moved to WCA3A in 1996 to complete the sampling study due to low water levels in WCA2B.

2.2 Blue Cypress Water Management Area

The Blue Cypress Water Management Area, a part of the Upper St. Johns River Basin, located in Indian River County, FL, is a large graminoid marsh system which, like the Everglades, is undergoing a substantial restoration effort. Interest in the apple snail population of the BCWMA grew substantially when the endangered snail kite returned to this area to nest and roost after years of infrequent kite sightings (Turner 1996). Our research on snails included two distinct areas within the Upper St. Johns Basin, BCWMA East and BCWMA West (Figure 1).

BCWMA East

The majority of our field investigations of snail movements and survival were conducted in the eastern-most quarter of BCWMA East. Work was conducted there in March through July 1995 and February through August 1996. The pilot study for the mark-recapture method was done in BCWMA East in 1996. Trap arrays and crayfish traps were also deployed in this area in 1996 to collect snails for laboratory studies.

The BCWMA East plant community consists of patches of sawgrass surrounded by mixed emergents (*Panicum* spp., *Eleocharis* spp., *Sagittaria* spp., *Pontederia cordata*, and in shallower areas, *Xyris* sp.). Periphyton forms thick layers over the sand substrate which characterizes BCWMA East. In 1995, portions of our study area dried to below ground level;

no dry down occurred in this area in 1996. During our 1995 study of dry season survival, water depths throughout the study site (excluding canals) ranged from 15 to 70 cm in March - April, and from 0 to 50 cm in May - July. In 1996, water depths throughout the study site ranged from 35 to 90 cm in February - April and from 20 to 70 cm in May-August.

BCWMA West

During the 1995 study on survival and movements, we also monitored snails in BCWMA West. The hydrology of this section of the Upper St. Johns marsh is distinct from that of the BCWMA East, reflected in part by the dominant vegetation and substrate. Sawgrass, water lily and cypress (*Taxodium* spp.) are the dominate vegetation. In contrast to the sand substrate of BCWMA East, the substrate of BCWMA West is a fibrous peat. During April - June 1995, water depths ranged from 40 to 80 cm.

2.3 Lake Kissimmee

Lake Kissimmee is an approximately 14,000 ha lake in Osceola County (Figure 1). The extensive littoral zone supports numerous species of macrophytes including maidencane, pickerelweed and cattail. Long term stabilization of water levels has resulted in an accumulation of muck in many localized areas of the lake. Muck sites (n=3) supported dense growths of spatterdock (*Nuphar luteum*). Three sites on the lake had a predominately sand substrate with a thin (< 15 cm), patchily distributed, flocculent organic layer, while the remaining two sites had a clean sand substrate. The 1995 trap array validation was conducted in a range of water depths (45 to 100 cm) depending upon how close we could get

to shore. Lake levels were stable during the trap validation study. During the 1995-1996 draw down, the snail movement study site initially had water depths of 50 to 200 cm (depending on distance from shore); by February 1996 most of the site had no standing water.

3.0 METHODS FOR DETERMINING SNAIL ABUNDANCE

Many basic questions about the ecology or population dynamics of any organism require some measure of abundance, or at least relative abundance. Indeed, one of the reasons for the paucity of information on snail ecology is a lack of reliable techniques for sampling apple snail populations. Over the past two decades, several direct and indirect measures of snail abundance have been proposed (Owre and Rich 1987, Bennetts et al. 1988), but none of these methods have been sufficiently evaluated to draw conclusions about their utility and reliability.

In this chapter, we compare several direct and indirect measures of snail abundance. We compare three different methods for extracting snails from 1-m^2 throw trap sampling (3.1). We also investigate the utility of two different trap systems to determine relative abundance (3.2), and we further explore one of these trap systems for estimating snail density using a mark-recapture approach (3.3). Finally, we examine the use of apple snail egg cluster counts as an index to population density (3.4). In each section we end with a discussion of the results for that particular method. The chapter concludes with an overall discussion of the relative utility of all the methods investigated.

3.1 Throw Trap Extraction Techniques to Determine Snail Density

Throw traps which encompass a small area (approximately 1 m^2 to 2 m^2) have been used for sampling fish and macro-invertebrates in the Everglades system (Kushlan 1981, Owre and Rich 1987, Chick et al. 1992, Jordan et al. 1996). A metal sided 1-m^2 throw trap

has been found most effective for sampling fish and invertebrates in vegetated habitats (Chick et al. 1992, Jordan et al. 1996). Extraction techniques for sampling fish and invertebrates from throw traps include a dip net (Kushlan 1981, Jacobsen and Kushlan 1987, Chick et al. 1992), bar seine (Rozas and Odum 1988, Chick et al. 1992), and suction dredge (Brook 1979, Owre and Rich 1987, Bennetts et al. 1988). Although each of these methods has also been used to sample apple snails (Owre and Rich 1987, Bennetts et al. 1988, F. Jordan, pers. comm.), no quantitative comparison has been made among these extraction techniques.

Methods

During 1995 in WCA2B, we conducted a pilot investigation of throw trap sampling to (1) refine our sampling protocols in preparation for a second season, (2) compare the proportion of marked animals that were recovered from throw traps using three extraction techniques, and (3) evaluate the effort required for each extraction method.

Sampling was accomplished using a throw trap, which quickly encloses a 1-m^2 area after being thrown into the marsh (Kushlan 1981, Chick et al. 1992). The throw trap was a 60 cm high by 100 cm x 100 cm box constructed of welded aluminum pipe enclosed with aluminum sheeting that lacked a top and bottom (Chick et al. 1992). A 40 cm extension was attached to the top of the trap, as necessary, to permit sampling in water depths up to 100 cm. The trap was hand thrown from a standing position in a randomly selected direction. The trap was immediately pushed into the substrate to prevent animals from escaping under the trap. All vegetation was then uprooted, rinsed vigorously, and examined for snails.

Three sites for throw trap sampling were selected in southwestern WCA3A based on signs of snail presence (i.e., egg clusters on emergent vegetation and/or catching a few snails in a preliminary trapping effort) and the presence of sawgrass stands adjacent to prairie/slough habitat. We selected juxtaposed habitat types to minimize marked substrate differences between habitats which might occur over large spatial scales. Since we selected sites based in part on snail presence, these sites may not have represented snail densities throughout the area. Because our purpose was to compare methods, rather than to estimate wild snail densities, having a sufficient sample of snails was of greater concern than having representative densities. All throw traps were placed at least 10 m from the ecotone defined by the two habitat types to avoid edge effects. Within each of these habitats, at least 50 throw trap samples were collected. Bennetts and Kitchens (1993) calculated coefficients of variation (CV) based on throwing up to 80 traps per site, and estimated that at least 50, and maybe up to 100, throw traps per site were required to obtain reasonable precision (CV of 20 to 30%). They suggested that obtaining substantially lower coefficients of variation would not have been logistically feasible given the labor intensity of the methods and variability in snail abundance.

During 1996 we compared the number of snails extracted from throw traps using either a dip net or a suction dredge. One of these two methods was randomly selected and used to clear the trap. The dip net was constructed of welded aluminum pipe, consisting of a 1.5 m handle centered on a 0.30m (h) x 0.66m (l) frame, which supported 1.3 cm mesh netting. It required two sweeps of the net to cover the entire trap. Once the vegetation was removed, the net was passed through the trap until 20 consecutive sweeps (10 in each half of

the trap) devoid of snails were obtained. The suction dredge consisted of a self-priming 2-cycle 5-hp pump, a Mays fluid transformer (Keene Engineering, Northridge, CA) to induce suction (Brook 1979), and a reinforced rubber intake hose (7.5 cm diameter) [Neither the State of Florida nor the Federal Government endorses commercial products mentioned in this text]. The hose was attached to a 7.5 cm diameter aluminum handle with a 15 cm x 15 cm box on the end. The dredge was operated until we had extracted the top 8-10 cm of the 1-m^2 area enclosed by the trap. All material extracted through the suction hose passed over a sorting tray of 1.3 cm wire screen and into a 1.3 cm mesh bag at the end of the sorting tray. Extracted material was then sorted and all snails were removed. During the 1995 pilot study we also explored the use of a bar seine for extracting snails from throw traps. The bar seine was a 1m x 1m aluminum frame with two handles extending 0.5 m from each side of the frame. The frame was covered with 1.3 cm mesh netting. The bar seine was swept through the trap until 10 consecutive sweeps devoid of snails were obtained (one sweep covers the entire trap).

For throw trap sampling to provide reliable estimates of snail density, it either must be assumed that all animals within each throw trap are counted or the proportion of animals counted must be estimated (Burnham 1981, Nichols 1992). We estimated the proportion of animals counted using marked snails, which were placed in some throw traps after deployment but prior to plant removal. The number of snails placed in the traps ranged from 0 to 5, which reflected the number of snails collected in 1-m^2 traps (Bennetts and Kitchens 1993). The proportion of animals recovered from throw traps was then estimated as the proportion of snails recovered to marked snails placed in the trap. This procedure was

intended to be "blind" (i.e., the person collecting the sample did not know if or how many snails were placed in each throw trap); however, we later realized that our "blind" protocol had not been strictly adhered to during our 1995 pilot study.

Effort was measured as the time required to clear a given throw trap at four sites during 1995 using each of the initial three extraction methods. Extraction time was the time from when the throw trap was positioned until it had been completely cleared; thus, it included the removal of vegetation. This procedure was only intended to compare extraction time among methods and did not reflect the total time required for sampling, which would have included additional time for transportation, setup, and equipment maintenance.

Data Analysis

A preliminary analysis of the throw trap data revealed that they were not normally distributed (Shapiro Wilk test, $P< 0.001$) (SAS Inc. 1988), but were reasonably well fitted by an unconstrained negative binomial distribution ($G=14.96$, 12 df, $P=0.244$) (White and Bennetts 1996). Consequently, we used the likelihood-ratio testing framework for a negative binomial distribution described by White and Bennetts (1996) to test for all main effects attributable to site, habitat, and extraction method. The negative binomial distribution has 2 parameters: m (the arithmetic mean) and k (a dispersion parameter) (Bliss and Fisher 1953). White and Bennetts' approach uses a combination of likelihood-ratio tests, Akaike's Information Criteria (AIC) (Akaike 1973, Shibata 1989), and goodness-of-fit tests to determine if m and/or k differ among treatment groups. A disadvantage of this approach is

that it is computationally difficult and limited software is available to analyze more complex designs (White and Bennetts 1996). Consequently, we used ANOVA to further explore the full suite of potential interaction effects. Although these data do not meet the assumptions of ANOVA, it has been shown that ANOVA is quite robust to violation of its assumptions when the data are distributed as negative binomial (Mitchell 1977), even when the variances are unequal (White and Bennetts 1996).

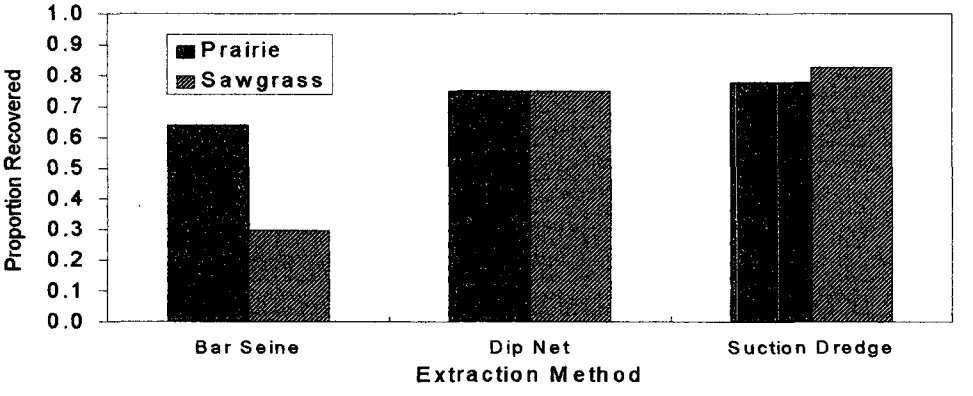
We began our analysis of effort by modeling the relationship between extraction time and the total number of marked or wild snails extracted using an ANOVA. Finding a snail in a trap inherently increased the time required for clearing the trap because our search criteria for both the dip net and bar seine were based on the number of sweeps without finding a snail (i.e., finding a snail would result in 10 and 20 additional sweeps for the bar seine and dip net, respectively). Consequently, we used the residuals from the first ANOVA as a dependent variable to examine the additional effects of extraction method, habitat, and site, having already accounted for increased time due to the number of snails extracted. We then examined the effects of site, habitat, and extraction method on the residual times. Because our "blind" protocol for estimating recovery of marked snails had not been adhered to during 1995, we included an additional effect in our analysis for whether or not marked snails had been placed in the throw trap. A significant interaction between this treatment effect and extraction method would have indicated if any bias was unequally distributed among treatments.

Results

During the pilot study of 1995, one to five marked snails were placed in 112 throw traps and extracted to examine sampling recovery efficiency. The proportion of marked snails recovered did not differ among extraction methods in prairie habitats (χ^2=0.47, 2 df, P=0.792), but was relatively poor using the bar seine in sawgrass habitats (χ^2=5.45, 2 df, P=0.065) (Figure 2). Based on this result, the bar seine was dropped as an extraction method for our 1996 effort in order to concentrate on the dip net and suction dredge.

In 1996 we extracted 610 throw traps to compare the performance of the dip net and suction dredge in assessing snail density. The most snails extracted from a single throw trap in 1996 was four, but most traps contained either one snail (94 traps) or no snails (492 traps). A negative binomial model in which dispersion (k) was constant (i.e. no variation due to habitat, site or method), but the mean number of snails per throw trap (m) differed among

Figure 2. The proportion of marked snails recovered from 112 throw traps in prairie/slough and sawgrass habitat using a bar seine, dip net, and suction dredge. Proportions were obtained by dividing the number of marked snails extracted by the number of marked snails put in the throw trap.



extraction methods, sites, and habitats was supported by our data (Table 1). This model had the lowest AIC score and was further supported by all likelihood-ratio tests (at P=0.05). We also had no evidence for lack-of-fit of this model (G=17.64, 23 df, P=0.777). An ANOVA also supported the conclusion of our negative binomial model indicating that mean number of snails per throw trap differed among all main effects (habitat, site, method), and further supported the inclusion of interaction effects (Table 2).

The number of snails/m^2 was substantially higher in prairie/slough habitat using the suction dredge at site 2 compared to other sites, habitats, or extraction methods (Figure 3).

Table 1. Description of negative binomial models and their corresponding Akaike Information Criteria (AIC) scores. Lower AIC scores indicate more parsimonious models. "None" refers to models not accounting for contributing effects of either Site, Habitat, or Method or any combination (i.e., potential effects are unknown or random). Also shown is the parameter structure (i.e., whether *m* [the arithmetic mean] and/or *k* [dispersion] differed among extraction methods, habitats, or sites).

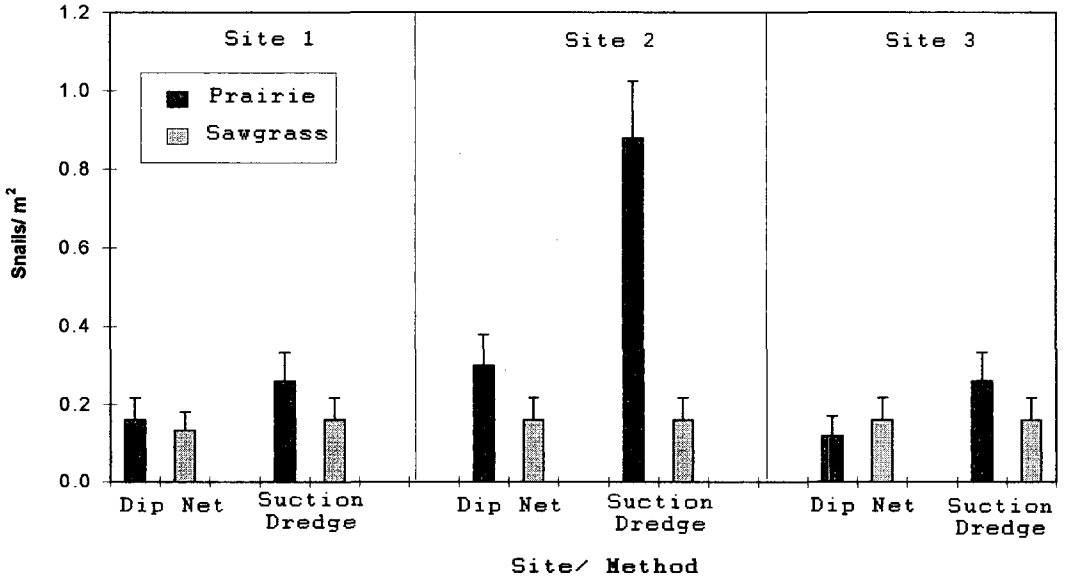
Model	Source(s) of Variation (*m*)	Source(s) of Variation (*k*)	No. Parameters	AIC
1	None	None	2	749.25
2[a]	Site, Habitat, Method	None	13	714.89
3	None	Site, Habitat,	13	767.89
4	Site, Habitat, Method	Site, Habitat,	24	734.21
5	Site	None	4	736.34
6	Habitat	None	3	734.93
7	Method	None	3	740.64
8	Habitat, Site	None	7	719.26
9	Method, Site	None	7	730.74
10	Habitat, Method	None	5	724.50

[a] Model selected based on AIC, likelihood-ratio tests and goodness-of-fit.

Table 2. Analysis of variance (ANOVA) table for fully saturated model of wild apple snail numbers in relation to habitat (HAB), site (SITE), and extraction method (METH) from throw traps. Sums of squares (SS) are type III partial SS, which are adjusted for all other terms in the model (SAS Inc. 1988).

Source	df	SS	MS	F	Source
HAB	1	4.629	4.629	17.57	<0.001
SITE	2	5.282	2.641	10.02	<0.001
METH	1	3.029	3.029	11.50	0.001
HAB*SITE	2	4.952	2.476	9.40	<0.001
HAB*METH	1	2.659	2.659	10.09	0.002
SITE*METH	2	1.677	0.838	3.18	0.042
SITE*HAB*MET	2	1.914	0.957	3.63	0.027
Error	598	157.553	0.263		

Figure 3. The mean (±SE) number of apple snails per m^2 in prairie and sawgrass habitats at each of 3 sites in WCA3A during 1996 using a dip net and suction dredge.



Higher numbers of snails tended to be extracted using the suction dredge in all prairie/slough habitats compared to sawgrass habitats (Figure 3). The suction dredge also extracted more snails than dip net in the prairie habitats of each site. Snail densities appeared similar among sites in sawgrass habitats using either extraction method (Figure 3).

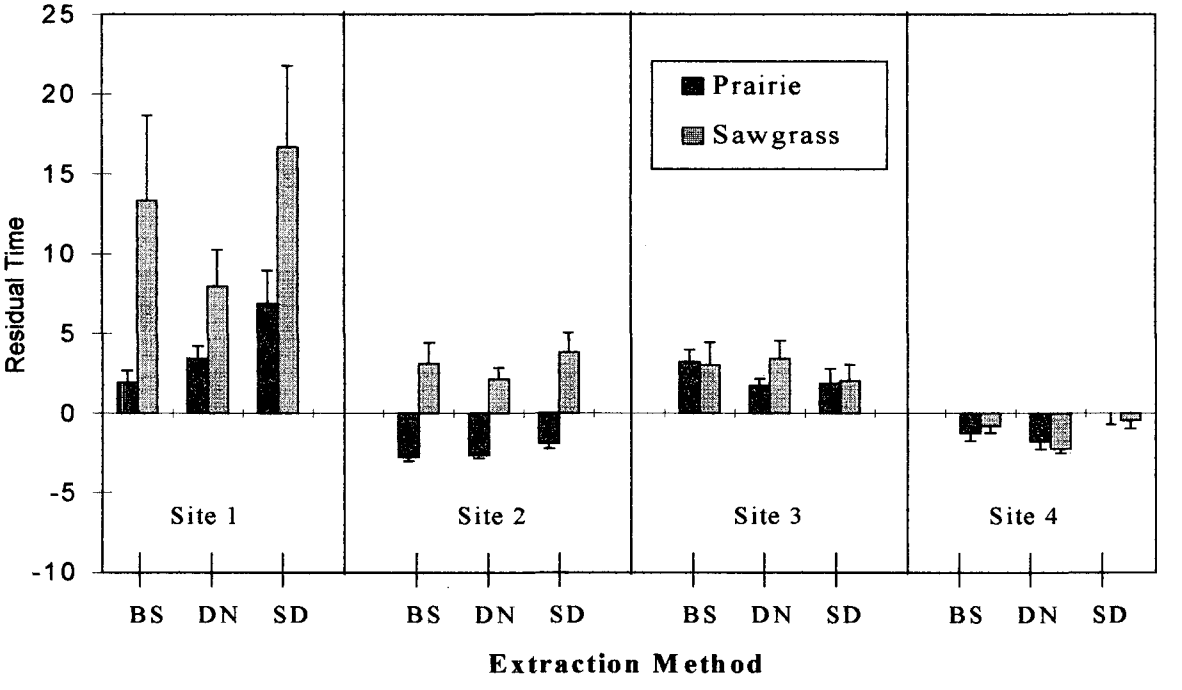
We evaluated extraction time (effort) from 955 throw traps during 1995. Our analysis indicated that after accounting for the number of snails (marked or unmarked), the time of extraction was influenced by site, habitat, extraction method, and whether or not marked snails had been placed in the throw trap (Table 3). Sawgrass habitats tended to take

Table 3. Analysis of variance (ANOVA) table for our final model of residual extraction time after having taken into account the time attributable to the number of snails extracted. Effects were habitat (HAB), site (SITE), extraction method (METH), and whether or not marked snails had been placed in the quadrat (MARK). Sums of squares (SS) are type III partial SS, which are adjusted for all other terms in the model (SAS Inc. 1988).

Source	df	SS	MS	F	Prob >F
HAB	1	1391.699	1391.699	54.43	<0.001
SITE	3	3246.463	1082.154	42.32	<0.001
METH	2	371.247	185.623	7.26	<0.001
MARK	1	116.109	116.109	4.54	0.033
SITE*HAB	3	2263.432	754.477	29.51	<0.001
MARK*SITE	2	304.762	152.381	5.96	<0.001
Error	942	34468.146			

more time than prairie/slough habitats, particularly at site 1 (Figure 4). Our final model also indicated a site*habitat interaction effect and an interaction between sites and whether or not marked snails had been placed in the throw trap. Overall, more time was expended on throw traps in which marked snails had been placed (residual = 2.70 minutes) compared to throw traps in which marked snails had not been placed (residual = -0.36 minutes). However, our data did not support the inclusion of an additional interaction between extraction method and whether or not marked snails had been placed in the throw trap ($F_{2,940}$=1.99, P=0.138).

Figure 4. The mean (±SE) residual extraction time in prairie and sawgrass habitats at each of 4 sites in WCA2B during 1995 using a bar seine (BS), dip net (DN), and suction dredge (SD) after having accounted for number of snails in the throw trap.



Discussion

Snail extraction using the suction dredge yielded the highest estimates of snail density. Because removal of snails precludes multiple counting of individuals, we also suggest that higher estimates probably were more accurate. This conclusion is further supported by our independent estimates of recovery probabilities. The suction dredge consistently had the highest recovery of marked individuals, regardless of habitat type.

The relative effectiveness of these extraction methods probably reflects how effective they are at removing snails from uneven substrates. Plant removal creates numerous small depressions and holes into which snails may fall, thereby avoiding collection by the dip net and bar seine. We have confirmed this by retrieving unrecovered marked snails by hand following attempted extraction. The use of marked snails to assess sampling efficiency, regardless of the extraction method employed, is strongly recommended when using throw traps.

Differences among habitats in extraction time from throw traps were not surprising. Sampling snails in sawgrass habitat, with its greater vegetation density and more rigid structure, takes longer regardless of the extraction method used. Differences in sites also were not surprising, given variability in substrates and vegetation density. In addition, site 1 was the first site of our pilot study and may have taken longer due to lack of experience working with the extraction techniques. Longer time for throw traps having marked snails was likely due to "observer expectancy bias" (Balph and Balph 1983). During the 1995 pilot, when extraction time was measured, observers were aware of whether or not marked snails were in the throw trap. When an observer knows that a snail is present in the throw

trap, whether intentional or not, effort may be increased to insure its recovery. We did not measure extraction time during 1996, but we have no reason expect this bias was again present, since observers were "blind" to whether or not marked snails were in the throw trap. The lack of an interaction between whether or not a marked snail was in the throw trap and the extraction method indicates that this bias was not differentially distributed among extraction methods. Consequently, we believe that our comparisons among capture probabilities of different methods during 1995 were reasonable. However, we strongly suggest that estimation of recovery probabilities always be "blind" as to whether or not marked snails are placed in a throw trap.

Logistical constraints precluded evaluation of some approaches that have been used to sample apple snails. For example, Donnay and Beissinger (1993) recently sampled *Pomacea doliodes* using a seine in conjunction with transects, rather than throw traps. Although using a seine without the constricting sides of a throw trap may improve its performance, it is likely to suffer from the same problems we encountered with missing snails in the substrate. Thus, estimation of recovery probabilities would likely be necessary for reliable results.

Despite its superior performance relative to the dip net and bar seine, the suction dredge does have limitations as a throw trap extraction technique. The pontoons supporting the dredge pump are cumbersome to maneuver through vegetation during sampling, especially in sawgrass. When sampling, the handle and hose are filled with water, which adds considerable weight (estimated at 10 kg) which the operator must move up and down to cover the 1-m^2 trap area. Also, the suction dredge cannot function adequately in less than 15

cm of water, a depth common to much of the apple snail's range, especially late in the dry season. The intake hose requires submersion in at least 15 cm of water. Another drawback with the suction dredge is the wire screen in the sorting tray; it had a tendency to damage the snails forced through the dredge. This may not be acceptable for a series of sampling efforts over time in the same site, for example. All the throw trap methods required vegetation removal, which alters the habitat and may influence subsequent sampling results.

An important consideration of any potential sampling method is the amount of effort involved in sampling. The throw trap we used to penetrate vegetated habitats to study snails weighs in excess of 18 kg. This, in combination with the effort required to uproot vegetation from the trap, makes this method very labor intensive regardless of the extraction technique employed. A large number of 1-m^2 throw trap samples also are needed to make comparisons of apple snail density (e.g. 492 of 610 traps yielded no snails) (Bennetts and Kitchens 1993). We deployed at least 50 traps per method per site to gain enough precision to compare the extraction methods in each habitat type.

Finally, the throw trap data provides some indication of the distribution of apple snails among two habitat types, sawgrass and prairie/slough. Owre and Rich (1987) and Turner (1994) hypothesized that apple snails do not use interior sawgrass marsh to any great extent. Their suggestion was based on egg cluster indices, which we have found are not reliable due to the high temporal and spatial variation (see Section 3.5 Egg Cluster Counts). Our data from the throw trap effort indicate that apple snails regularly occur within stands of sawgrass, although densities may be lower than in adjacent prairies or sloughs. Sawgrass represents a substantial proportion of South Florida's wetlands. For example, in WCA3A,

sawgrass covers more area than wet prairie (Davis et al. 1994). Thus, even at lower densities, the numbers of snails occurring in sawgrass may constitute a substantial portion of the overall apple snail population. Some additional data on habitat distribution are described in the context of our mark-recapture study (Section 3.3).

3.2 Movement Based Wire Traps

In addition to analyzing which extraction technique was most effective for determining snail densities from throw traps, we planned to investigate less labor and time intensive methods for determining relative abundance. For this purpose, we used throw traps as a direct measure of snail density to validate other potential methods of sampling snails. In this section, we present results of using wire traps as a tool for assessing relative snail abundance.

Owre and Rich (1987) had suggested using traps made from plastic containers in their treatise on snail sampling, but after limited success abandoned the idea. Mike Miltner (GFC, pers. comm.) at times captured nearly equal numbers of snails as crayfish while using wire crayfish traps baited with gizzard shad. Steve Miller (SJRWMD, pers. comm.) also found snails in baited crayfish traps in the BCWMA East. Tim Towles (GFC, pers. comm.) found dozens of snails in funnel traps used in wetland herpetological surveys. This anecdotal evidence indicated that the number of snails moving into wire traps warranted investigation as an indicator of snail abundance. In this section we describe two trapping systems which

can be used to collect snails, and we compare the snail catch in these traps to snail densities determined by throw traps extracted with a suction dredge.

Methods

Crayfish Traps

In a 1995 pilot study in BCWMA East we used 7 crayfish traps with and without dead fish as bait. After this pilot study and a concurrent telemetry study (Chapter 4), we realized bait was unnecessary and proceeded without bait with equal success. Upon ordering additional traps in 1996, we switched to a slightly modified version of a commercially available crayfish trap (Sam Lemmond Enterprises, Salt Springs, FL). These traps consist of three components: the funnel base, the stack, and a lid. The funnel base is constructed of vinyl coated wire with 19 x 24 mm hexagonal mesh. The base supports three funnel entrances which have an outer diameter of 15 cm and an inner diameter (i.e., inside the trap) of 5 cm. The mesh size and funnel openings limit our snail catch to snails with shell lengths of approximately 22 to 50 mm. [Mean snail ($\pm$ S.D.) size for all BCWMA snails collected in 1995 was 36.9 $\pm$ 3.6 mm]. The funnel base is 75 cm and tapers to 18 cm where it attaches to the stack. The stack is made from wire with a 25 x 13 mm rectangular mesh. The lid (not sold with the trap) is made from extruded polyethylene plastic with 13 mm mesh and is attached to the stack with nylon cable ties. The trap is secured in place by attaching a nylon cable tie through the stack wire and around the pvc pole driven into the substrate. The total trap height is 90 cm; use in water depths greater than 90 cm does not permit snails (or incidentally trapped animals) to breathe air.

Crayfish traps were deployed in BCWMA East and in southwestern WCA3A. These traps have been used in all wetland habitat types encountered, including wet prairie, slough, sawgrass and cattail. Deployment requires pushing aside only a 1 m diameter area of vegetation. Effort was made to place the trap as near to the substrate as possible, but successful use did not require that the trap rest firmly on the bottom (which would require labor intensive plant removal). We have observed snails in the field moving along vegetation and on periphyton suspended loosely above the substrate, and they likely can enter the funnel traps via plant stems, roots or a flocculent periphyton or organic layer.

BCWMA study- In 1996, we used crayfish traps in a study of snail movements in relation to reproductive activity in BCWMA East (see chapter 4). Data from this study are included here to illustrate the utility and limitations of using these traps to study snail populations. During the reproductive ecology study, we deployed 54 traps split into 3 groups of 18 traps (data from some traps were censored if they fell over). Traps were placed approximately 5 meters apart. We monitored traps each month from February through May, in late June/early July, and in August, for a total of six trap sessions and 622 trap checks. A trap session consisted of checking each trap on two occasions over a 7 to 12 day period. Traps were not moved during a trap session. We checked traps at 3 day intervals for 5 of the 12 occasions, 4 day intervals for 4 of 12 occasions, and for one occasion each a 5, 7, and 9 day interval. For each trapping session (n=6) we compared the total snail catch from the shortest time interval (i.e., 3 or 4 days) to the longer of the two time intervals (i.e., 4, 5, 7 or 9 day interval) to assess time effects within trap sessions using a one-way ANOVA (snail catch= trap interval + trap session + interactions). This was done using the General Linear

Model (GLM) procedure (PROC) in SAS (SAS Inc. 1988). Due to interaction effect between trap interval (short vs. long) and session, the short vs. long time interval within a session was compared using the "slice" option in PROC GLM in SAS (SAS Inc. 1992), wherein the interaction sums of squares were partitioned by the session sums of squares, and the denominator for the F-test (to compare trap interval within sessions) was the error term from the ANOVA. Because of the interaction effects, we could not perform a meaningful regression of snail catch to trap interval across sampling sessions. Based on our results of the effect of trap interval, we adjusted total snails per session to reflect a 7 day trap interval to give a snail catch per trap-week. The adjustment was made by dividing the number of snails by trap interval (days) and multiplying by 7 days/week.

We studied escape rates by placing marked snails (plastic i.d. numbers affixed to the shell with marine epoxy) in crayfish traps and checking traps 1 to 8 days later. We placed 21 marked snails in 16 traps for an 8 day check, 30 marked snails in 20 traps for a 2 day check, and 14 snails in 13 traps for a 1 day check.

WCA3A study- In southwestern WCA3A in 1996, we evaluated the use of crayfish traps as a method for determining relative snail abundance. Throw traps with suction dredge extraction was the standard for validation. The crayfish traps were deployed in two habitat types, sawgrass and wet prairie, at three different sites (i.e., six areas sampled) (sites described in section 3.2). Crayfish trapping was performed within one week of sampling using the throw trap and suction dredge. Two rows of 10 traps were placed at each area sampled; this (i.e., 20 traps) was considered one sampling unit. Rows were 10 m apart and

traps within rows were separated by 6 m. Traps were checked 3 days following initial placement, and then moved 10 meters to a new trap line. Traps were checked again after a 4 day interval, which concluded our 7 day trap effort within a habitat type. Since traps were moved after 3 days, we treated the second location as a replicate sample within the same habitat. We compared the mean snails captured per crayfish trap to the mean snail density/m^2 using linear regression (GLM procedure in SAS)(SAS Inc. 1988).

Trap Arrays

Trap arrays consist of a series of barriers which direct moving animals into funnel traps (Enge 1997). They are most frequently used for trapping amphibians and reptiles in uplands and wetland habitats (Enge 1997, Dodd 1991). Initially, we constructed a trap array with walls of silt fencing (woven polypropylene) and funnels of window screening as described by Enge (1997). After initial tests with these arrays, we decided to enhance their utility for trapping apple snails in wetland and lake habitats.

Our snail trap consists of three major components: 1) center funnel unit; 2) three single funnel units; and 3) three walls to divert animals into funnel units. The walls were configured in a Y-shaped array. At the end of each of the three walls was a funnel trap. We constructed the funnel traps to extend above water level (up to about 1 meter) in order to allow snails, or incidentally trapped animals, to breathe air. Plastic coated wire was used to construct the frame of the funnel units to increase stability. The funnel entrances were made from 13 mm netting, fabricated from extruded high density polyethylene (Nalle Plastics, Austin, TX). We also used the 13 mm plastic netting for construction of the 5-meter long

walls. Unlike plastic sheeting, the mesh stands up to wave action, which can be substantial due to boat traffic and winds. The extruded plastic material has excellent durability yet is sufficiently pliable to roll the walls and partially compress the funnel traps for transport. Four pvc poles, each two meters long, were attached to the walls using nylon cable ties, and these poles were driven into the substrate to hold up the walls and funnels during sampling.

For practical use, these traps are generally limited to wet prairie and slough habitats (e.g., areas with rushes, maidencane, and floating-leaved macrophytes in moderate to low densities). Proper deployment requires that the three 5-meter long barrier walls and funnel traps rest on the bottom, which requires uprooting or cutting down relatively large macrophytes such as sawgrass, cattail and pickerelweed. Engaging in this labor intensive process was counterproductive to our goal of developing a sampling method less labor intensive than throw traps, so we restricted trap array use to wet prairies and sloughs. Practical use of trap arrays is also limited to water depths of less than one meter, because deployment requires workers to handle trap components at the substrate level.

We compared snail densities, as determined by suction dredge extraction of 1 m^2 throw traps, to the number of snails captured in trap arrays at eight different sites on Lake Kissimmee. Sampling was conducted for 33 days from 9 October through 10 November 1995. For each of the eight site comparisons, 50 randomly placed throw trap samples were taken. Only snails larger than 13 mm in length were sampled due to the mesh size of our traps. We placed 13 trap arrays in the 8 sites. Vegetation in the immediate vicinity of the trap array walls and funnels was removed with a hoe, as necessary, in order to ensure that the trap system rested on the substrate. Five traps were moved within a site (to obtain a more

representative sample), and 5 traps were checked twice in one location for a total of 23 trap checks. A single trap array check was considered a sample unit, so n varied from 1 to 4 per site. The one site with n =1, we realized after a very difficult trap deployment, was extremely difficult to work in so we elected to limit this site to one check after a 23 day trap interval. Trap interval ranged from 7 to 23 d among the 23 checks. Six trap units were also deployed in BCWMA East in 1996 for collecting snails; data from this effort are included to illustrate general trap utility. The relationship between trap array snail catch and throw trap snail density was analyzed by linear regression (GLM procedure in SAS) (SAS Inc. 1988).

Results

Crayfish Traps

Crayfish traps proved to be an effective way to capture snails with much less labor than throw traps. A set of 20 traps can be set up in sawgrass, cattail, slough or wet prairie habitats by one person in less than 30 minutes. Twenty traps can be checked in 10-15 minutes. Monthly trapping efforts in BCWMA in 1996 yielded 93 to 226 snails per 100 trap checks. We examined the effect of sample size on the precision of the abundance estimate by plotting CV as a function of the number of traps deployed. It appears that using approximately 30 traps per area results in a stable CV of approximately 20 to 15% (Figure 5).

Total catch from crayfish traps was weakly related to snail density as determined by throw traps extracted with a suction dredge (R^2= 0.31, n= 6, P= 0.051) (Figure 6). However, ten of the crayfish trap catches were between 0.18 and 0.3 snails/m^2. If the two replicate crayfish trap catches from WCA3A site 2-prairie are removed from the analyses, leaving only

Figure 5. Change in coefficient of variation of snails captured in crayfish traps in the BCWMA with increasing sample sizes. Each sample represents the number of crayfish traps, all having a 7 day trap interval.

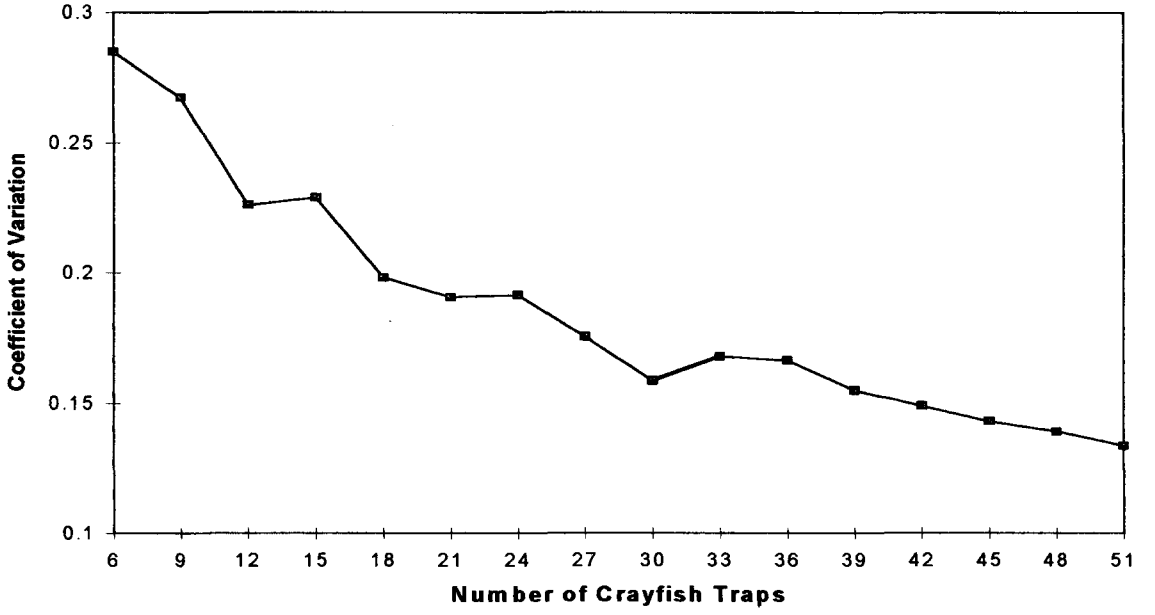
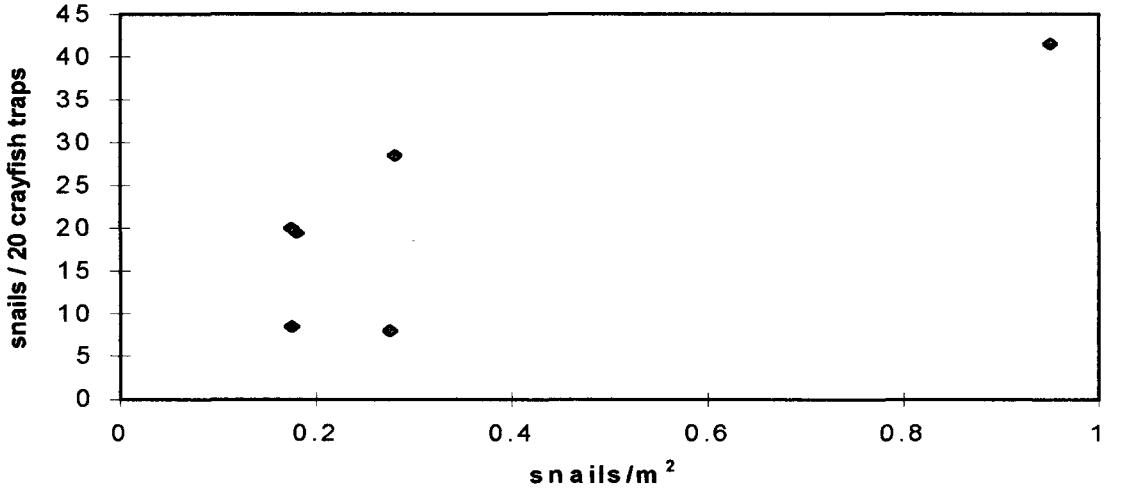


Figure 6. Snails caught in crayfish traps and estimated snail densities. Crayfish trap data are the total catch of snails per 20 traps placed in each site (n=6 sites). Snail density estimates were derived from throw trap sampling using a suction dredge in prairie/slough habitat.



the snail catch from sites with densities between 0.18 and 0.3 snails/m^2, the relationship falls apart (R^2=0.006, n=4, P=0.82).

In the BCWMA study, trap interval (number of days) did effect the number of snails captured in crayfish traps ($F_{1,15}$ = 3.86, P=0.0011) (Table 4), however there was an interaction between trap interval (short vs. long) and trap session (Table 4). The analysis between short and long trap interval within each session resulted in only 2 sessions (April and May) showing significant differences (Table 5). This did not affect the crayfish trap vs. throw trap regression, since trap interval (7 d) was constant for all sites. We decided to adjust all snail catches for the BCWMA study to reflect a one week trap interval, even though the difference in trap intervals was observed only in the April (3 versus 4-day trap interval) and May (3 versus 9-day interval). The adjustment was made by dividing the snail

Table 4. Analysis of variance (ANOVA) table for the model describing snail catch as related to trap interval (TI)(short vs. long) and trap session (SESS). Sums of squares are type III partial SS, which are adjusted for all other terms in the model (SAS Inc. 1988).

Source of Variation	df	SS	MS	F	Pr>F
TI	1	28.47	28.47	10.73	0.0011
SESS	5	155.33	31.07	11.71	<0.001
TI*SESS	5	65.25	13.05	4.92	<0.001
Error	600	1592.00	2.65		
Total	611	1841.06			

Table 5. Tests for differences between short and long interval within trap sessions. Comparisons were made using the results of ANOVA (Table 4) and partitioning the INT*SESS sums of squares by SESS sums of squares using the "slice" option in PROC GLM in SAS (1992). The denominator for the F-test is the error term from the ANOVA.

TRAP SESSION	F-Statistic	PR >F
FEB	0.85	0.36
MAR	0.44	0.51
APR	8.11	0.0045
MAY	16.99	0.0001
JUN/JUL	0.46	0.50
AUG	0.0012	0.97

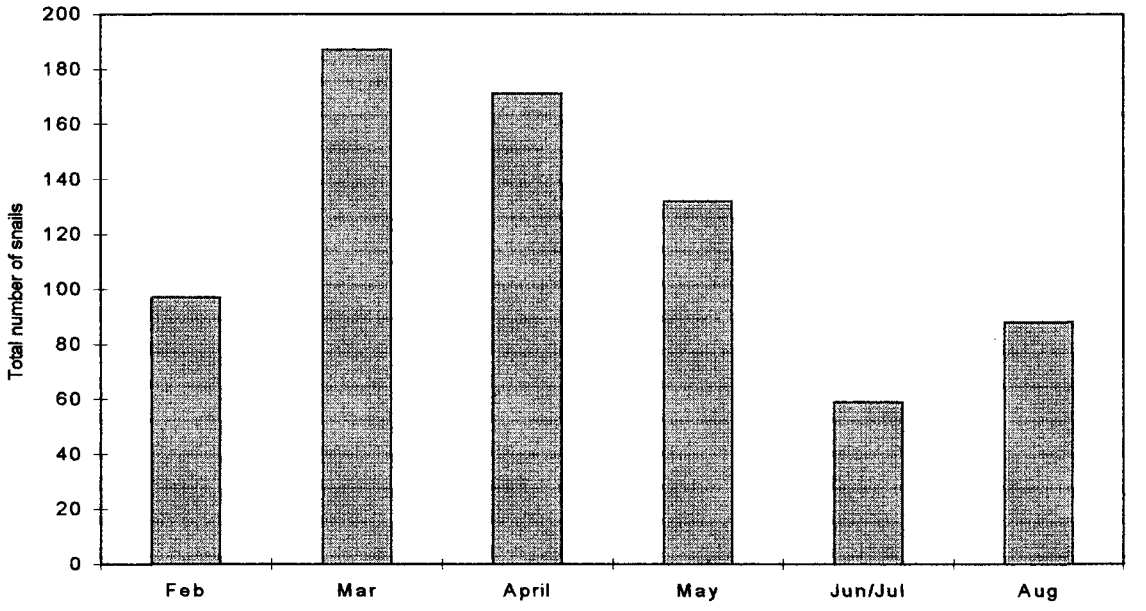
catch by trap interval (days) and multiplying by 7 days/week. Total catch/week did vary by trap session (Figure 7), and likely relates to changes in snail breeding activity, snail movements, and population turnover over the seven month period of sampling (see Chapter 5).

Escape rates from crayfish traps were 0 for both one and two-day trap intervals (n=14 and n=30, respectively). Mean 8-day escape rate was 28.6%.

Crayfish traps and trap arrays collect a variety of animals other than snails, some of which are air breathers. Out of 460 crayfish trap checks in BCWMA, we counted 127 incidental captures; these consisted of 15 gar, 88 centrarchid fish, 5 snakes (predominately green water snakes), 5 turtles (mud turtles), and 11 amphiumas. Snakes and gar, whose jaws

tended to get stuck in the mesh, suffered the greatest mortality (20% of snakes and 13% of gar captured).

Figure 7. Total catch of snails from crayfish traps (adjusted to reflect a 7 day trap interval) from six trapping sessions conducted February - August 1996 in BCWMA East. Data for each session are from either 52 or 53 traps.

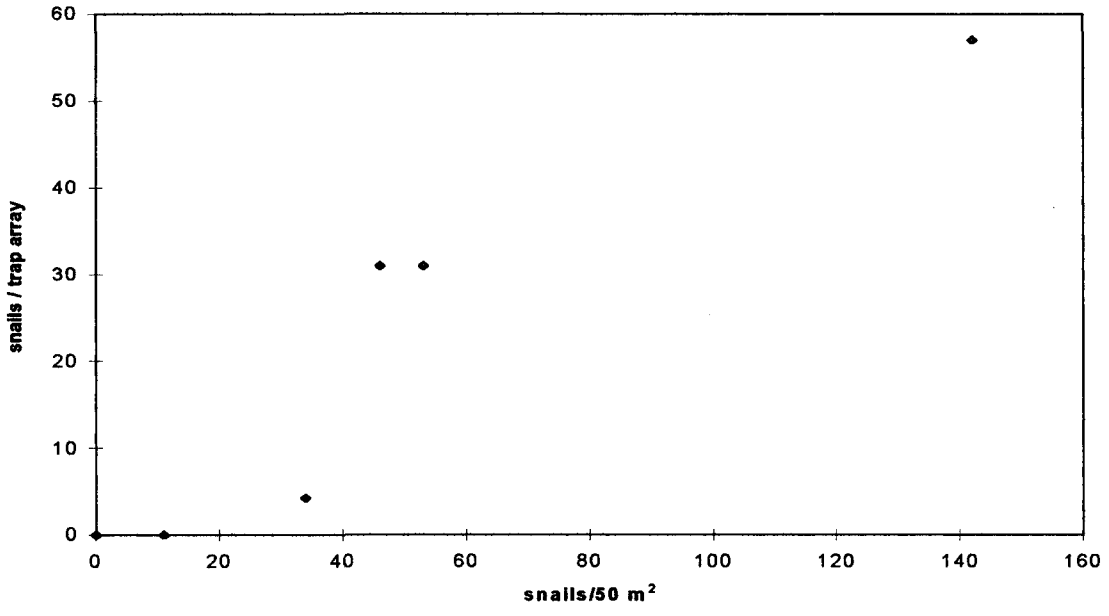


Trap Arrays

The trap arrays proved effective as a method for determining relative abundance of apple snails. Linear regression analysis of the data for snails collected using throw trap sampling versus trap arrays indicate a strong relationship (R^2=0.90, n=8, P <0.001) (Figure 8). Length of trap interval did not appear to affect trap catch, but we had a small set of samples from which to work (only 3 situations where traps in the same location were checked twice and that also had at least one snail captured). The reason for the longer

interval was poor weather (approximately October 15 through October 30), which may have caused the lower catch for those three particular occasions.

Figure 8. Snails caught in trap arrays and estimated snail densities on Lake Kissimmee. Trap array catch is the mean catch per trap for each site (n = 1-4). Snail density estimates were derived from throw trap sampling using a suction dredge (n = 50 throw traps per site). Eight sites were sampled (3 points overlap at 0 snails/m^2).



Out of 22 trap array checks (4 traps per array) in Lake Kissimmee, we counted 26 incidental captures. These captures consisted of 6 gar, 13 centrarchid fish, 1 green water snake, 2 turtles (one soft-shell), 1 frog, and one unharmed 0.5 m alligator. The deaths consisted of 4 gar (67%) and one centrarchid fish (7.7%).

Discussion

One of the main reasons the ecology of apple snails has remained a mystery is the difficulty associated with collecting snails from the graminoid marshes and littoral zones they inhabit. As a result of our effort to develop a trap-index of relative snail abundance, we have developed two effective trap systems for collecting large numbers of snails. Both systems require considerably less effort than dip nets, seines or a suction dredge. Both trap systems rely on snail movements and do not require bait to attract snails.

We believe that crayfish traps will be an effective tool for collecting apple snails in most habitats. They are easy to deploy, even in dense vegetation, and they require little, if any, permanent disturbance of the vegetation. Based on our use in areas where snails are known to exist, a capture rate of 1 to 3 snails per trap over a 3-5 day period is typical. Escape studies indicated that trap intervals should be limited to a few days as nearly 30% of snails escaped after eight days, but none escape after one or two days. Escape studies were not conducted with trap arrays.

Trap arrays are also effective in collecting snails. Trap arrays left for one week in eastern BCWMA collected 5 to 18 snails / trap check. On Lake Kissimmee a single trap set for one week collected from 12 to 91 snails per trap check. Unlike crayfish traps, trap arrays are not suitable for use in dense vegetation (e.g., sawgrass), and in general require more effort per snail captured than crayfish traps. Our trap array design proved to offer several advantages to other trap array designs. They can be set up quickly (typically less than 30 minutes, but up to one hour in muck sites). Compared to funnels made from fine screen and walls made from plastic sheeting, they collect much less drifting vegetation and debris and

stand up against boat and wave action. Snails and other air breathing animals collected in the traps (i.e., snakes, turtles) could breathe air in the trap arrays, whereas submerged screen funnels result in drowning. The impact of restricting snail access to aerial respiration is unknown; however, we do know that apple snails continue to breathe air in water with high dissolved oxygen concentrations (McClary 1964, pers. obs.).

The discrepancy between the two movement based methods (crayfish traps and trap arrays) and a direct measure of snail density (estimated with throw traps) may be explained by: 1) trap design and function; 2) the different seasons in which these comparisons were made; 3) different trap intervals; and/or 4) validation study design. The design of both crayfish trap and trap array sampling depends upon apple snails crawling into the traps. Neither trap requires bait, because, as will be discussed in chapter 4, snails are frequently on the move. However, the ways in which snails move into the funnels of the two trap systems differ. The array barrier walls (total linear surface of 30 meters if both sides of the three walls are considered) divert snails from their "intended" course of travel and "direct" them into the funnels. Snails enter crayfish trap funnels only if one of the three 0.012 m wide funnels lies in their course of travel (which includes horizontal directions of travel as well as vertical ascents to breathe air).

We have found that in a given area, a snail's tendency to crawl into the crayfish trap varies with the reproductive activity of the population (see Chapter 4). Since snails are not directed towards the traps, crayfish trap effectiveness depends more on snail behavior, which varies considerably over just a few weeks or months during spring and summer. This brings us to the issue of when the direct method validations were conducted for the trap arrays and

crayfish traps. Trap array validation was completed during Fall 1995. By fall, snail populations are 3 to 5 months past their peak reproductive activity (Odum 1957, Hanning 1979, this report Section 3.5) and egg production is minimal as populations approach the non-breeding winter months. In contrast, crayfish trap validation was spread out over June and July 1996, which typically includes the transitional period between peak reproductive activity and declining adult survival (see Chapters 4 and 5). Given these observations of survival and reproduction during spring/summer, the crayfish trap validation likely incorporated a high degree of temporal variation in snail movements and survival, and this may have contributed to a lack of a relationship to snail density.

We found considerable variation in snail catch within trap sessions (2 checks in 7 days), and between trap sessions (6 sessions over 7 month period). This variation may relate to changing weather, fluctuating water temperature, or biological factors affecting snail movements. Crayfish traps were checked at 3 or 4 day intervals for a total of 7 days. Arrays were checked at intervals of 7 to 23 day intervals over 21 to 28 days. The longer trap interval between array checks and the longer amount of total time sampling may negate or minimize the impact of short term fluctuations in snail movements.

Finally, validation study design may be an issue. In some cases, the crayfish traps used for validation were deployed in one site and then moved to another. Without some overlap between all sampling sites, time effects contribute to variation between sites. As mentioned earlier, considerable variation in crayfish trap snail catch occurs over as little as a few weeks, especially around peak breeding activity. In contrast, trap arrays were set out in eight sites with some overlap in sampling for all sites during the 21-28 days of sampling.

The simultaneous trapping among all eight sites controlled for time effects during the trap array validation.

Both crayfish traps and trap arrays are effective tools for collecting large numbers of apple snails, tools which already have contributed substantially to unraveling the mystery of apple snail ecology and life history. However, based on our assessment, crayfish trapping is not a suitable index of apple snail abundance. Trap arrays do appear to be reliable as an index, and we have gained important information on snail populations with their use (Darby et al. 1996). However, their practical use is limited to wet prairie and slough habitat, which does not permit studies of snail habitat use.

There are other issues which make trap arrays inconvenient to use. Trap arrays are not available commercially, so individual researchers must spend considerable time and effort to construct a trap (estimated 8 worker-hours per trap, estimated cost of supplies $200). Their bulk limits transport to two to three trap units in an airboat. Setting and checking trap arrays can be difficult, especially in water over 75 cm, because the workers placing the trap must be able to reach to the bottom of the funnels and barrier walls. Connecting the walls to the funnel units and lifting the center funnel unit is difficult without two workers. In contrast, crayfish traps are considerably more convenient to use. Crayfish traps are available commercially for $12 to $16 each. We frequently transported 30 to 35 traps in our airboat. Crayfish traps are easier to deploy (vegetation need not be cut or uprooted) and are easily checked by one person. Crayfish traps could conceivably be placed in any water depth, but if placed in water over 90 cm, drowning mortalities of incidentally trapped air breathers (turtles and snakes) will increase. We have not tested the rate of snail

mortality if access to aerial respiration is prevented. Crayfish traps are practical to use and effective in sawgrass and cattail as well as wet prairie and slough, but more data are needed to understand variations in their capture rates. Our understanding of capture probabilities with crayfish traps as part of mark-recapture studies is presented in the next section.

3.3 Mark-Recapture

Given their ease of use and efficacy in collecting apple snails, we decided to further explore the potential use of crayfish traps in determining snail density. Crayfish traps can be used to determine an actual density, rather than a relative abundance (as described in 3.2), if there is an associated estimate of capture probability. The method of mark-recapture (or capture-recapture) determines animal population or density based on such estimates of capture probability. Since the 1940's, the statistical methods for estimating population size using mark-recapture models have developed dramatically (Otis et al. 1978, White et al. 1982, Seber 1986, Nichols 1992). Mark-recapture methods have been used with a wide range of animals, including small mammals, large mammals and birds, and in more recent years with animals such as lobsters (Evans and Evans 1995) and snakes (Luiselli et al. 1996).

In general, mark-recapture studies proceed as follows. A number of traps are set up in the study area (e.g., 100 traps in a 10-trap by 10-trap grid). Using the traps, a sample (designated n_1) is taken from the population; the animals are marked and returned to the grid. After allowing time for the marked and unmarked animals to mix, a second sample (designated n_2) is taken. Of this second sample, the animals that are already marked (the recaptures) are designated as v_2. The proportion of the population that is marked is called

the "capture probability", and its estimate (p-hat) can be calculated by dividing the number recaptured by the total number caught in the second sample:

$$\text{p-hat} = v_2 / n_2$$

An estimate of the total population, N-hat, can then be obtained:

$$\text{N-hat} = n_1 / \text{p-hat}.$$

To improve the precision of the population estimate (N-hat), one would continue marking the unmarked animals caught, releasing them, and resampling. A density estimate can be determined by dividing the population estimate by the area of the grid. However, this is a 'naive' density estimate, because the effective area of trapping is greater than the actual area of the trapping grid due to what is known as edge effect (Otis et al. 1978). The computer program CAPTURE, which we initially used for our snail analyses, takes edge effect into account by using concentric trap grids to allow concurrent estimation of density and edge width (Otis et al. 1978, Rexstad and Burnham 1991). A more recently developed program, TMSURVIV (Jim Hines, USFWS, Patuxent Wildlife Research Center), handles some violations of closure (explained below), but does not account for edge effects; therefore, a naive estimate of density is obtained.

CAPTURE and TMSURVIV each include analyses of several model types that are evaluated for their applicability to a given data set (see Data Analysis, below). Both

CAPTURE and TMSURVIV compute goodness-of-fit statistics and between-model test statistics that are used to test model assumptions and help select the most appropriate model. The results from both CAPTURE and TMSURVIV are discussed because they both illustrate issues involved in applying mark-recapture studies to apple snails.

The closed mark-recapture models in CAPTURE have four general assumptions. These are: 1) the population is closed; 2) animals do not lose their marks during the study; 3) all marks are correctly noted and recorded at each trapping occasion; and 4) each animal has an equal and constant probability of capture on each trapping occasion (Otis et al. 1978). The assumption that the population is closed (#1) means that the population size and composition are constant over the course of the study; there is no birth or death (meeting demographic closure) or immigration or emigration (meeting geographic closure). If either of the two components of closure (demographic and geographic) are not met, the population is said to be open. In order to increase the likelihood that the closure assumption is met, the experiment should be conducted over as short a time period as possible and at a time that will avoid recruitment (e.g., juveniles becoming trappable) and losses (Otis et al. 1978, White et al. 1982). These closure issues were considered in the grid design and sampling regime of our snail mark-recapture studies (see Methods, below). However, we found closure to be an issue for all seven grids, and therefore TMSURVIV, which utilizes open population models, was an important tool for interpreting the results of our apple snail mark-recapture studies.

Methods

Mark-Recapture Technique for Apple Snails

Each sampling grid consisted of 10 rows of 10 traps, for a total of 100 traps. In the pilot study, traps were an average 2.75 meters apart, encompassing a grid of 610 m^2. For all other grids, we used measured pvc poles as a guide to place traps 2.5 meters apart; the resulting grid size was 510 m^2. Inter-trap distances were never systematically tested to optimize capture probabilities; they were based on our success in collecting snails with crayfish traps and information on snail movements gained using telemetry (Chapter 4). Each trap assembly consisted of a modified crayfish trap (described in 3.2) attached to a 1.5-m pvc pole. Trap poles were numbered to keep track of snail capture location and to ensure release of marked snails near their trap of origin.

Each sampling grid was checked seven times, allowing for six recapture occasions. Trap check intervals were three or four days (based on capture rates from preliminary crayfish trap work), so each grid ran for approximately 21 days. The same procedure was used on each of the seven trap check occasions. The entire grid was checked, row by row, and all newly caught snails were placed in plastic bags labeled with the appropriate trap number. Information for recaptured snails (i.e., date, snail number and trap number) was recorded on the spot and the snails were immediately returned to the grid approximately 1 meter behind the trap in which they were found.

Unmarked snails were taken to the boat for processing. For each animal, we recorded the trap number in which it was caught, its size, gender, and designated tag number. Shell size was determined to the nearest millimeter using a vernier caliper (S-T Industries, MN,

USA). Laminated, 3 mm x 5 mm plastic tags (Floy Tag & Manufacturing, Inc., Seattle, WA) were used to mark snails. Tags were attached to the shell adjacent to the apex, approximately 1 to 2 cm from the aperture, using a marine epoxy. Each shell was dried with a towel, allowed to air dry, and lightly sanded in the area where the tag was to be placed. Each snail was returned to the water approximately one meter from the trap in which it was caught.

Pilot Study

The first mark-recapture grid for apple snails was set up in the northeast quarter of BCWMA East. The sampling was conducted over a 23-day period in June 1996.

Mark-Recapture Studies in Three Habitat Types

This work was conducted in eastern WCA3A. We operated a total of six sampling grids, three in Site 1 (Lat.: 25 59 13 N, Long.: 080 32 05 W) and three in Site 2 (Lat.: 25 57 67 N, Long.: 080 33 07 W). Three habitat types, cattail, prairie/slough, and sawgrass, were sampled in each site. Sites were chosen in WCA3A by first scouting for areas which possessed expanses of the three habitat types large enough for the grid and a 10 m wide border on all sides. Once the size and vegetation requirements were met, we looked for indications of snail presence (i.e., snail egg clusters or snails captured in crayfish traps). Trapping grids were placed only in sites with an indication of snail presence. We selected sawgrass, prairie/slough, and cattail habitats that were as close together as possible within a site. The average distance (± SD) between habitats within Site 1 was 1305 m ± 661 m. The

average distance (± SD) between habitats in Site 2 was 1042 m ± 314 m. The shortest distance between the two sites was approximately 3177 m (cattail-1 to cattail-2).

The sampling was conducted over a three-month period, March-May in 1997. Except for the first and last week of the study, we ran two grids simultaneously. There was always some temporal overlap between two grids.

Analyses

Each of the seven sampling grids was first analyzed using the computer program CAPTURE (Otis et al. 1978, White et al. 1982, Rexstad and Burnham 1991). In addition to density estimation, CAPTURE performs a test for closure (defined on p. 46); the utility of this test, however, is questionable in situations where a true failure of closure can not be distinguished from behavioral and some time variations in capture probabilities (White et al. 1982). CAPTURE also performs a uniform density test. This test examines the number of snails captured across the grid according to the columns, rows, and rings of the grid. The uniform density test, particularly the ring test, assists in assessing whether or not geographic closure was met.

Our analyses using CAPTURE indicated that the important assumption of population closure may have been violated in all seven grids. To further explore this possibility, we turned to the open population models in TMSURVIV (Pradel et al. 1997). This computer program is a modification of SURVIV (White 1986). We used six different models which allow for different combinations of survival being constant or variable, capture probability being constant or variable, and the absence or presence of transient animals passing through

the sampling grid; transience can be constant or variable. We focused the analysis on this set of open models because a preliminary analysis of one grid indicated that transient animals were present. Transient animals are defined as those traveling through the grid, whereas residents are those that inhabit the grid area throughout the sampling period (Pradel et al. 1997).

Results

The mark-recapture approach was successful in determining snail densities in the 7 grids set up in BCWMA and WCA3A. We found no evidence that snail tags were lost, and the ability to read the tags was not compromised by staining or biotic fouling. The number of individual snails captured in the 7 trapping grids ranged from 102 in sawgrass (WCA3A, Site 1) to 561 snails in prairie/slough (WCA3A, Site 2) (Table 6).

As stated above, the CAPTURE analyses indicated that the closure assumption may have been violated in all of the grids. The TMSURVIV analyses confirmed these results. Therefore, the snail population and density estimates presented here were obtained from TMSURVIV for open populations. Note that three different capture-recapture models were used to estimate densities for the seven grids (Table 7). This is because various mark-recapture grids had unique data characteristics for which particular models were appropriate. We found that no one model can be expected to fit all mark-recapture data for apple snails. In each analysis, the most parsimonious model was selected based on a combination of likelihood-ratio tests (LRTs), Akaike's Information Criterion (AIC) (Akaike 1973, Shibata 1989), and goodness-of-fit tests.

Table 6. Sampling periods and snail captures for six mark-recapture grids in WCA3A (1997) and one grid in BCWMA (1996). No. of Snails Captured refers to unique individual snails. Total No. of Captures refers to all snails captured in traps, including captures of the same individual on more than one occassion.

Dates Sampled	Site	Habitat Type	No. of Snails Captured	Total No. of Captures
6-01 to 6-24	BCWMA-1	Prairie/Slough	291	569
3-12 to 4-03	WCA3A-1	Cattail	121	184
3-18 to 4-09	WCA3A-1	Prairie/Slough	117	153
4-04 to 4-27	WCA3A-1	Sawgrass	102	188
5-09 to 5-31	WCA3A-2	Cattail	392	559
4-28 to 5-22	WCA3A-2	Prairie/Slough	561	844
4-14 to 5-08	WCA3A-2	Sawgrass	127	178

Table 7. Survival and capture probability estimates from mark-recapture grids using TMSURVIV. PR refers to prairie/slough habitat, CAT to cattail, and SAW to sawgrass. Numbers following habitat designation are site numbers. Model refers to the most parsimonious model determined by likelihood- ratio tests, AIC and goodness-of-fit tests.

Grid		Model *	Survival	p-hat
Blue Cypress Water Mangement Area				
	PR-1	SP	0.84 (0.020)	0.39 (0.026)
Water Conservation Area 3A				
	CAT-1	SPG	0.82 (0.063)	0.43 (0.064)
	PR-1	SP	0.64 (0.075)	0.27 (0.065)
	SAW-1	SPT	0.77 (0.039)	0.44 (0.086)**
	CAT-2	SPG	0.73 (0.043)	0.39 (0.040)
	PR-2	SP	0.54 (0.023)	0.58 (0.037)
	SAW-2	SP	0.72 (0.060)	0.32 (0.058)

* In model SP, survival and capture probability are constant, and there are no transient animals.
In model SPG, survival and capture probability are constant, and there are transient animals.
In model SPT, survival is constant, capture probability varies, and there are no transient animals.

** This model (SPT) generates p-hats for each trap occasion. The number shown is the mean (±SE) of these individual occasion p-hats. For the other models TMSURVIV directly provides one p-hat applicable to all trap occasions.

Pilot Study, BCWMA East- The percentage of recaptured snails per occasion was 39.6 % on the second occasion and leveled out to a high of about 65 %. The closure test performed by CAPTURE (Otis et al. 1978) indicated a violation of this assumption ($z = -3.21$, $P < 0.001$). The uniform density test by rings failed to reject the null hypothesis ($\chi^2 = 5.25$, 4 df, $P = 0.263$), indicating that density was uniform; this test did not suggest a

geographic closure violation. The test further indicated that density was uniform among grid columns (χ^2 = 12.07, 9 df, P = 0.209), but not among grid rows (χ^2 = 28.45, 9 df, P<0.001).

Using TMSURVIV for open populations, the model selected for the pilot study was SP, the simplest model (Table 7). Under this model both survival and capture probability are constant over the sampling period and there are no transient animals. This model had the lowest AIC score of any of the models, the goodness-of-fit was high (G = 32.869, 34 df, P = 0.523), and the LRTs accepted SP over the more general models. Snail survival in this grid was less than 1.0 (Table 7). This analysis indicates that the population was not open geographically (there were no transients), but since survival was less than 1.0 between sampling periods, it was open demographically. The population estimate, N (±SE), was 204.56 snails (± 14.02). The density (± SE), calculated by dividing N by the grid size, was an estimated 0.335 (± 0.023) snails per m^2.

Eastern WCA3A Sites

Capture probabilities ranged in the 6 grids from 0.27 (±0.065) in prairie/slough-1 to 0.58 (±0.037) in prairie/slough-2. Capture probabilities did not differ noticeably between habitat types (cattail $_{AVG}$=0.41, prairie/slough $_{AVG}$=0.43, and sawgrass $_{AVG}$=0.38) or sites (i.e., Site 1_{AVG}=0.38 vs. Site 2_{AVG}= 0.43). The models selected and survival (an issue of closure) in each grid are summarized in Table 7, and discussed separately as follows.

Cattail-1 Over the course of sampling, the percentage of recaptured snails ranged from 22% to 48.6 % (Figure 9). The closure test indicated that closure was violated

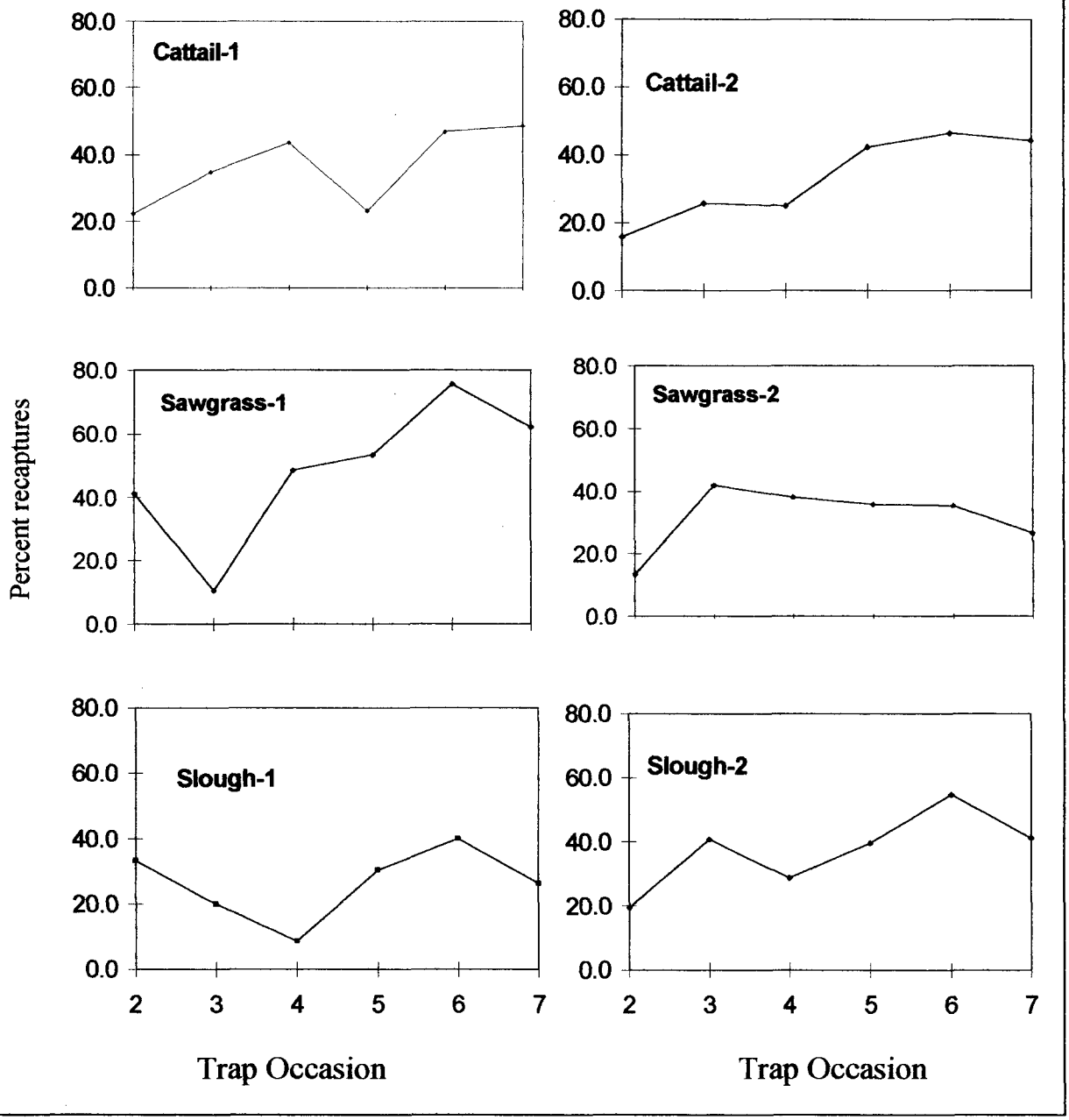
($z = -2.123$, $P = 0.017$). The uniform density test performed by CAPTURE indicated that density was uniform throughout the grid (rings: $\chi^2 = 8.23$, 4 df, $P = 0.084$; rows: $\chi^2 = 15.02$, 9 df, $P = 0.349$; and columns: $\chi^2 = 15.02$, 9 df, $P = 0.090$). The results of this test do not suggest a geographic closure violation

The model selected for this grid using TMSURVIV was SPG, in which survival, capture probability, and the proportion of transients are all constant. SPG had the lowest AIC score of any of the models, the goodness-of-fit was the highest ($G = 55.673$, 33 df, $P = 0.008$), and a LRT failed to accept the more reduced model. The survival of nontransient snails was less than 1.0 (Table 7), indicating that snails were being lost. The population was open both geographically (i.e., transients were present) and demographically (i.e., survival < 1.0). The proportion of residents (non-transients) among new (not previously marked) snails was 0.653 (± 0.120 SE). The population estimate, N (±SE), over the sampling period was 65.43 snails (± 9.55), and the density estimate was 0.129 snails per m^2 (± 0.019).

Prairie/slough-1 The percentage of recaptures per occasion fluctuated (Figure 9). According to the CAPTURE closure test, closure was violated ($z = -2.459$, $P = 0.007$). The uniform density test indicated that density was uniform among rings ($\chi^2 = 8.13$, 4 df, $P = 0.087$), rows ($\chi^2 = 10.33$, 9 df, $P = 0.324$), and columns ($\chi^2 = 7.20$, 9 df, $P = 0.617$).

Using TMSURVIV, the appropriate model for prairie/slough-1 was SP, in which survival and capture probability are constant and there are no transient snails. SP had the second lowest AIC score of any of the models, but it was different from the lowest-scored

Figure 9. Percent recaptures per trap occasion in mark-recapture grids in WCA3A.



model by less than 2.0; AIC scores within 2.0 or less are not considered statistically different (Sakamoto et al. 1986). Model SP also had a reasonable goodness-of-fit (G = 41.817, 34 df, P = 0.168), and the LRTs accepted SP over the more general models. The survival of snails was less than 1.0, indicating that snails were dying (Table 7). The population was open demographically but not geographically (there were no transient snails). The population estimate (± SE) over the sampling period was 81.64 snails (± 19.55). The estimated density (± SE) was 0.160 (± 0.04) snails per m^2.

Sawgrass-1 The percentage of recaptures fluctuated over time (Figure 9) and ranged from 10.5 % to 75.7 %. The closure test indicated a violation ($z = -5.080$, $P < 0.001$). The uniform density test by rings suggested a geographic closure problem ($\chi^2 = 10.89$, 4 df, $P = 0.028$); more animals were caught in the outer two rings than expected based on a uniform density. Also, density was not uniform among rows ($\chi^2 = 31.68$, 9 df, $P = 0.0002$) or columns ($\chi^2 = 17.43$, 9 df, $P = 0.043$).

The TMSURVIV analysis selected model SPT as the most appropriate model for sawgrass-1; with this model survival is constant, capture probability varies, and there are no transients. Model SPT had the lowest AIC score, a goodness-of-fit of G = 37.596, 29 df, P = 0.132, and LRTs failed to accept the more reduced model and more general models. Snail survival was again less than 1.0 (Table 7), indicating that the population was open demographically. The population estimate (±SE) for the sampling period was 81.62 snails (± 23.77). The estimated density was 0.160 (± 0.05) snails per m^2.

Cattail-2 The percentage of recaptures ranged from a low of 15.9 % to a high of 46.4 % (Figure 9). The closure test indicated that closure was violated ($z = -4.042$, $P < 0.001$). The uniform density test by rings indicated a non-uniform density ($\chi^2 = 17.64$, 4 df, $P = 0.0014$), which suggests that the population may be open geographically. The test further indicated that density was uniform among grid columns ($\chi^2 = 11.64$, 9 df, $P=0.234$), but not among rows ($\chi^2 = 18.62$, 9 df, $P = 0.029$).

Under TMSURVIV, the appropriate model was SPG; it had the lowest AIC score, an adequate goodness-of-fit ($G = 39.021$, d f= 33, $P = 0.217$), and LRTs indicated it was most appropriate. With SPG, survival, capture probability, and the proportion of nontransients among new snails are constant. The proportion of residents (or nontransients) among newly caught snails was 0.275 (± 0.095 SE); in other words, the proportion or probability that a newly caught snail was a transient was a high 0.725. Survival was again less than 1.0 (Table 7). According to the analysis, this population was open both demographically (survival < 1.0) and geographically (transients present). The estimated snail population (± SE) in cattail-2 was 210.76 snails (± 21.78). The snail density (±SE) was 0.413 (± 0.043) animals per m^2.

Prairie/slough-2 The percentage of recaptures per occasion ranged from 19.5 % to 55 % (Figure 9). Again the closure test indicated a closure violation ($z = -9.413$, $P < 0.001$). The uniform density test by rings was not significant ($\chi^2 = 3.95$, 4 df, $P = 0.412$) and did not suggest a violation of geographic closure. The three-part test also indicated that density was uniform among rows ($\chi^2 = 10.67$, 9 df, $P = 0.299$) and among columns ($\chi^2 = 15.31$, 9 df, $P=0.083$).

The appropriate TMSURVIV model for this grid was SP, in which survival and capture probability are constant over the course of the study and there are no transient snails. This model had the lowest AIC score, the goodness-of-fit was good (G = 28.928, 34 df, P = 0.714), and the LRTs accepted SP over the more general models. Survival was substantially less than 1.0 for this grid (i.e., 0.535) (Table 7), indicating that the population was not closed demographically. The capture probability (0.584) was the highest of any of the grids. The estimated population (±SE) was 218.15 snails (± 13.47), and the density (±SE) was 0.428 (± 0.026) snails per m^2.

Sawgrass-2 The percentage of recaptures peaked on the third occasion at 42 % (Figure 9). The closure test indicated a violation of the closure assumption (z= -3.339, P < 0.001). The test of uniform density by rings (χ^2 =6.97, 4 df, P =0.137) did not suggest a violation of geographic closure. Snail density was uniform among rows (χ^2 = 14.81, 9 df, P= 0.096), but not columns (χ^2 = 25.37, 9 df, P=0.003).

Model SP (survival and capture probability constant and no transients) was the appropriate model under TMSURVIV. It had the lowest AIC score of any of the models, the goodness-of-fit tests indicated it was best (G = 48.24, 34 df, P = 0.054), and the LRTs accepted SP over the more general models. Survival was less than 1.0 (Table 7), indicating that the population was open demographically. For the sampling period, the population size (±SE) was 82.30 snails (± 15.01). The estimated density was 0.161 (± 0.029) snails per m^2.

Discussion

We have demonstrated that the mark-recapture method can be applied to apple snails to determine snail density. We found that interpreting the results of crayfish trapping studies requires understanding the considerable variability in capture probabilities, and this can only be achieved with a mark-recapture technique. It is apparent from our mark-recapture data that snail population behavior is sufficiently complicated to preclude simple generalizations about snail density or demography among habitat types or during certain phases of the snail life cycle (e.g. early versus late in the breeding season).

We recommend that initial mark-recapture data analysis be based on the closed population models contained in CAPTURE. They initially were chosen as a first step in the analyses for several reasons. First, they tend to require less data than open models due to less rigorous assumptions and fewer parameters (Otis et al. 1978). Secondly, the closed models contained in CAPTURE provide information about sources of variation in the capture probabilities (e.g., time, behavioral response, and heterogeneity among individuals). CAPTURE also includes some tests of closure, which may or may not direct further analyses to open models. If closure is not a problem, the results from CAPTURE include a density estimate that takes into account edge effect. Closure may not be a problem for future apple snail studies if investigators take into account some of the information we have collected with regards to snail life history (see Chapter 6).

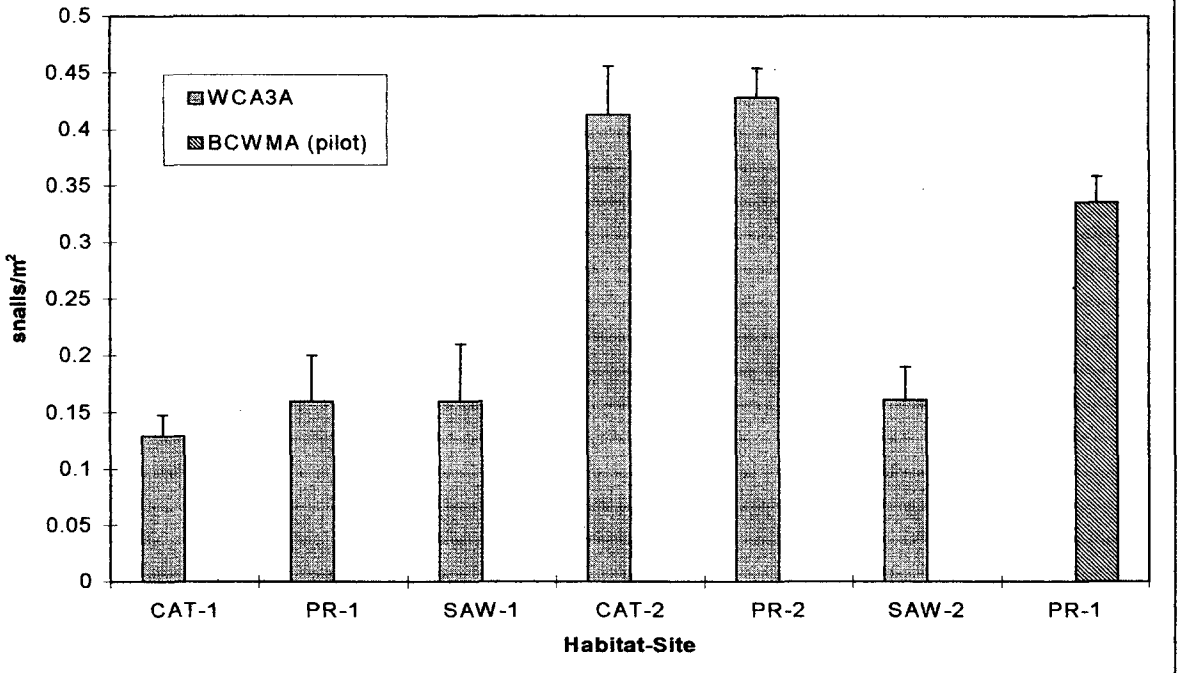
Despite efforts in this study to avoid expected periods of heavy adult mortality (see Chapters 4 and 5 regarding survival), demographic closure was violated in all 7 grids due to survival being less than 100%. Geographic closure violations, caused by snail movements in

and out of the grid, were also an issue in two of the grids. Closure violations resulted in our use of open models (TMSURVIV) to generate estimates of snail densities. TMSURVIV output indicated that the simplest open model, SP (survival and capture probability constant and no transients), was appropriate for four of the seven sampling grids. Note that constant survival does not refer to an absence of mortalities, but that mortalities occurred at a constant rate. Model SPT (survival constant, capture probability variable, and no transients) fit only sawgrass-1 in WCA3A. Transient snails (modeled in SPG, where survival and capture probability are constant) were identified in only two of the grids, cattail-1 and cattail-2 of WCA3A. We have no hypothesis as to why transient snails were only indicated for cattail habitat. Cattail-1 was the first site sampled in WCA3A (initiated 12 March) and cattail-2 was sampled last (initiated 9 May), so time effects would not explain why transients were only indicated for cattail. In addition, transients were not indicated for other sites sampled where temporal overlap with the cattail sites did occur (prairie-1 in the case of cattail-1, and prairie-2 for cattail-2) . We question whether habitat structure would contribute to this phenomenon, at least in terms of the ability of transient snails to penetrate denser habitat structure. Our sawgrass sites appeared sufficiently similar in both stem density and submerged vegetation abundance (e.g. periphyton or *Utricularia* sp., or both), at least within a range likely to relate to snail movements; however, transients were not identified in sawgrass sites. Some other aspect of the cattail sites, for example proximity to the canal, may, in some unknown manner, be related to the transient issue.

Our capture-recapture studies provided some additional information (i.e., supplementary to the throw trap data) on habitat distribution among sawgrass and

prairie/slough, as well as cattail habitats. In Site 1, the highest snail densities were found in prairie/slough and sawgrass, followed by cattail (Figure 10), but the differences were slight.

Figure 10. Snail densities (±S.E.) in six locations in WCA3A in 1997 and one location in BCWMA in 1996 obtained from mark-recapture grids. Densities were calculated using population estimates from TMSURVIV and dividing them by the grid area. CAT = cattail habitats, PR= prairie/slough habitats, and SAW = sawgrass habitats. Numbers refer to site number.



In Site 2, the highest snail densities were found in prairie/slough, but just slightly higher than cattail, and sawgrass had the lowest density. These densities and differences between habitat types are consistent with that of the throw trap; prairie sites appear more likely to have a higher density of snails than sawgrass, but this relationship varies from site to site. Cattail does not necessarily, as suggested in a previous study of gastropods in general (Davis 1994),

suppress apple snail populations. We observed densities in cattail similar to those in prairie habitats within both WCA3A sites. The uniform density tests from CAPTURE indicated that snails were uniformly distributed (based on concentric ring, row and column tests) within three of the grids, but not in 4 others. [These tests are not confounded by violations of closure.] Our capture-recapture results confirm that snails are patchily distributed over large scales even with similar vegetation types and hydroperiods, as evident from marked site to site variations in snail density (Figures 4 and 10). Generalizations about vegetation type and corresponding snail densities cannot be reliably inferred from our limited amount of data. However, it is apparent from the site to site variability that considerably more effort will be required to understand how vegetation type affects snail densities.

When comparing density values reported from our mark-recapture studies to other published results, recall that TMSURVIV does not account for an edge width, which increases the effective trapping area beyond the boundary defined by the traps. Therefore, the densities reported here may be slightly higher than if edge effect were factored in (Otis et al. 1978).

3.4 Egg Cluster Counts

Prior to this research, our knowledge of apple snail ecology from field studies was based largely on observations of egg clusters (Odum 1957, Perry 1974, Hanning 1979, Turner 1996). Unlike the cryptically colored submerged snails, egg clusters are conspicuous in wetlands; female snails attach clusters of 20 to 30 white eggs to stems of emergent macrophytes 2 to 200 cm above the water line (Hanning 1979, Turner 1996). Egg clusters

are easy to count along a transect (Hanning 1979) or within a throw trap unit which can be thrown into the marsh (Bennetts et al. 1988). These characteristics make sampling egg clusters as an index of snail abundance attractive. Counts of egg clusters have been explicitly suggested (Perry 1974, Owre and Rich 1987, Bennetts et al. 1988) or implied via loose application (Takekawa and Beissinger 1989) as an indicator of relative snail abundance. We used snail densities determined by throw trap and mark-recapture grids to evaluate the reliability of egg cluster counts as an index of apple snail abundance.

Methods

We examined the relationship between egg cluster counts and apple snail densities using data from four studies. First, we used data from Bennetts et al. (1988 unpubl. data), who counted egg clusters and estimated density at two sites in WCA3A during 1987. Their egg cluster counts were conducted such that each sample represented 15 m^2 using a 1m X 5 m polyvinylchloride (PVC) frame flipped end over end 3 times along the ecotonal edge (Bennetts et al. 1988). Sampling was conducted along the ecotone created by juxtaposed sawgrass and prairie/slough habitats. This is the habitat most frequently chosen by apple snails for oviposition in graminoid marshes (Bennetts et al. 1988, Turner 1996). Their estimates of apple snail density were derived using throw trap sampling with a suction dredge in the adjacent prairie/slough habitat. Second, we counted egg clusters at four sites each in WCA2B and western WCA3A during 1995 and 1996, respectively. Our counting technique in these areas was virtually identical to that of Bennetts *et. al.*, except that each 5 m^2 throw trap was one sample. Apple snail densities were estimated using throw traps

extracted by suction dredge. Third, during our BCWMA study of reproductive ecology, we repeated egg cluster counts at the same site six different times between February and August 1996. Again, we used 5 m^2 sampling units. The density estimate, however, came from the mark-recapture pilot study using the 100-trap trapping grid described in section 3.3. The fourth source of egg cluster and snail density data came from our work in eastern WCA3A in the spring of 1997. Again, egg clusters were sampled using 5 m^2 sampling units, and densities were obtained from the mark-recapture experiments.

For a comparison of egg cluster counts in relation to the sawgrass/prairie ecotone, we used data from Bennetts et al. (1988) in which egg clusters were counted along the ecotone, 7.5 m and 15 m from the ecotone into the interior sawgrass. This evaluation was repeated in this study in WCA3A in 1995, except that counts were conducted along the ecotone, and 5 m and 10 m from the ecotone into the interior sawgrass.

Results

We found no reliable relationship between egg cluster counts and estimated apple snail densities from a pooled sample from 5 separate studies (R^2 =0.005, n=14 P=0.81) (Figure 11). However, we found several potential sources of variation in egg cluster counts that could influence this result. Repeated sampling at the same site revealed a strong seasonal pattern in the number of egg clusters, with a peak occurring during April or May (Figure 12). A pattern also was observed in relation to the prairie/sawgrass ecotone. Marsh habitats throughout South Florida consist of a mosaic of open prairie or slough habitats interspersed among stands of sawgrass or cattail. Our data indicated higher numbers of egg

clusters along the ecotone compared to even just a few meters toward the sawgrass interior (Figure 13).

Figure 11. Egg cluster counts and estimated snail densities from a pooled sample from each of 5 studies. Snail density estimates were derived from throw trap sampling using a suction dredge in prairie/slough habitat (Bennetts et al. 1988, WCA3A 1996, WCA2B 1995) and from mark-recapture grids (BCWMA 1996, WCA3A 1997).

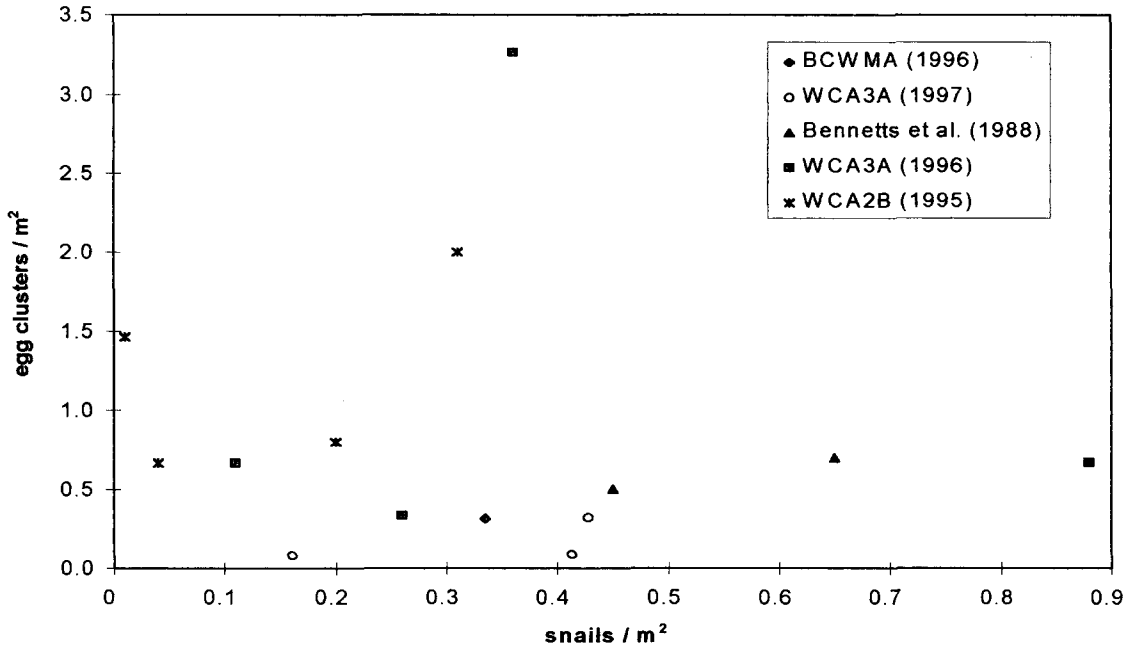


Figure 12. Mean (±SE) number of egg clusters/ 5-m^2 sampled at one site in BCWMA 6 times during 1996.

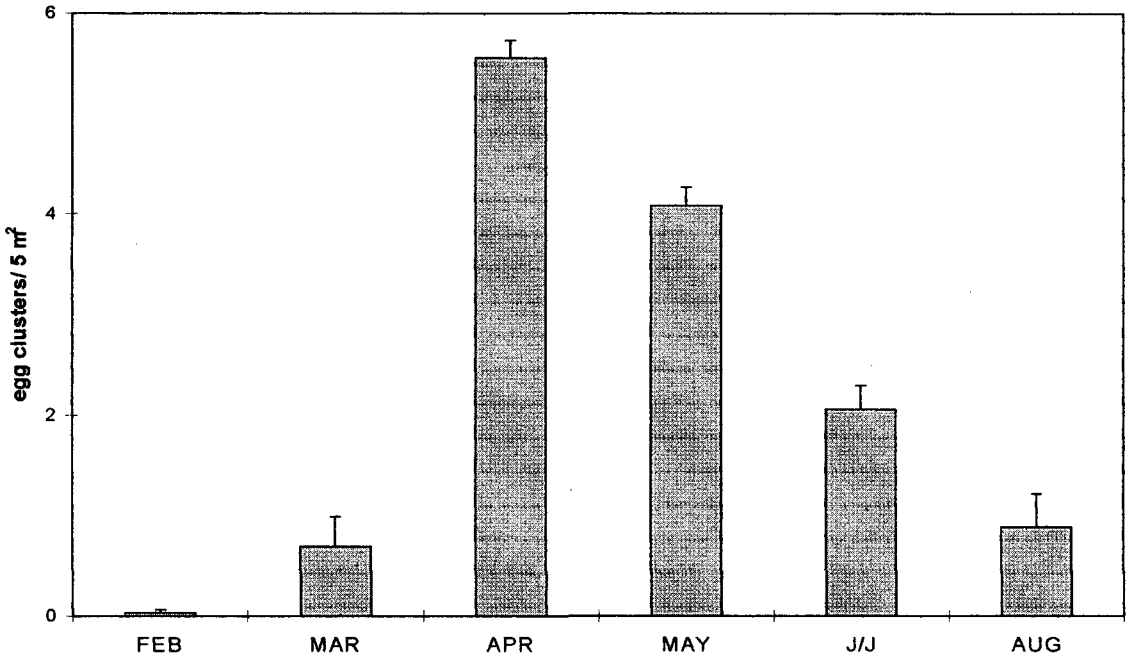
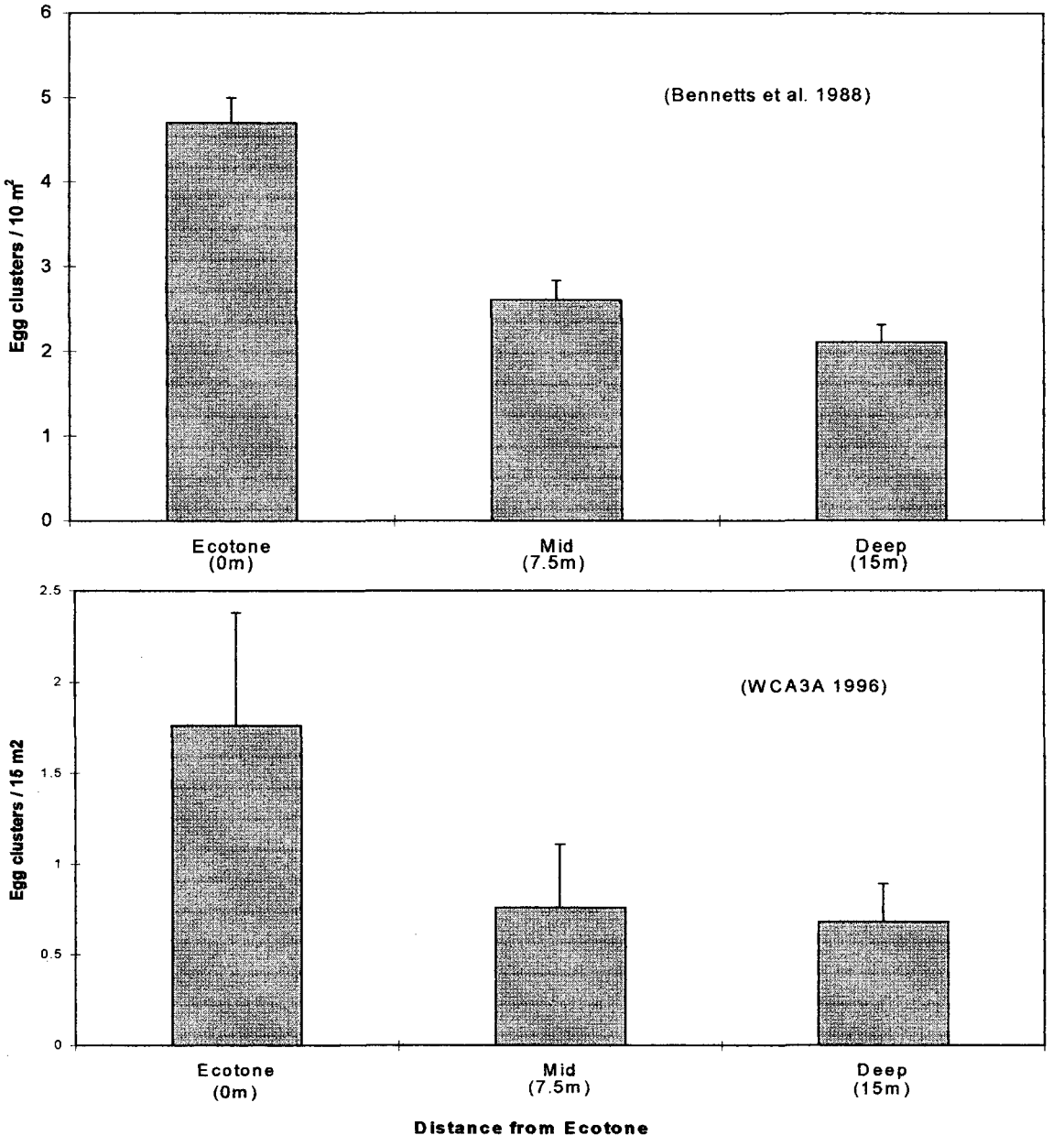
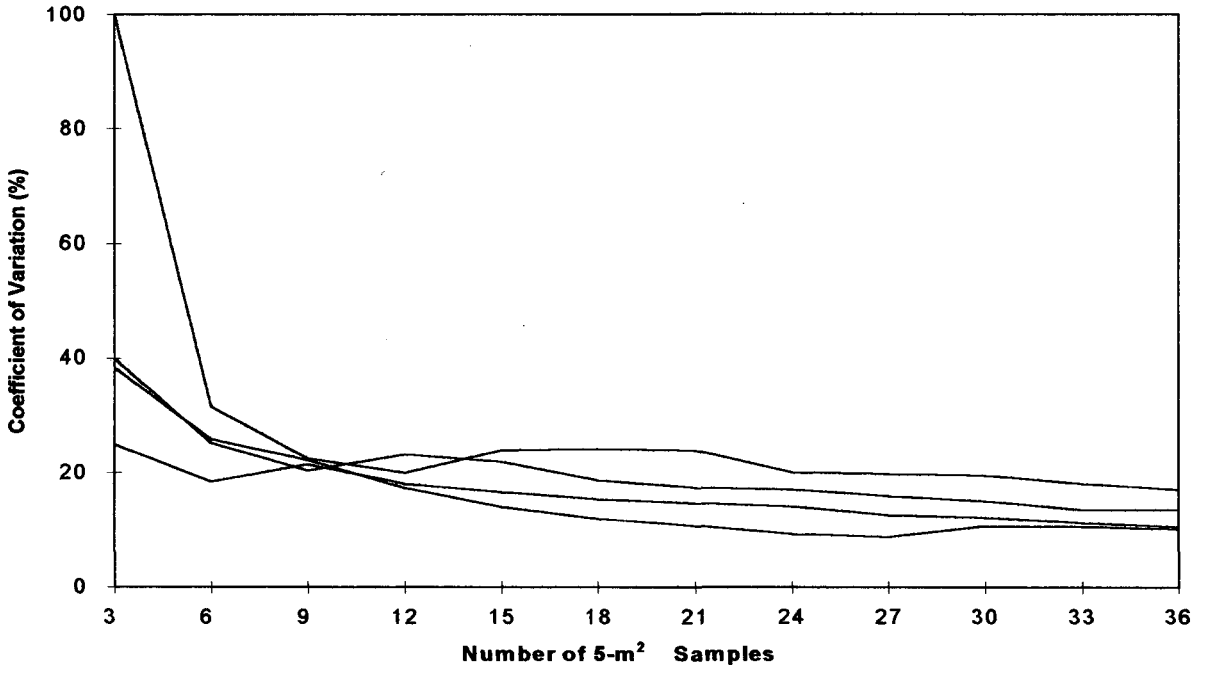


Figure 13. Mean (±SE) number of egg clusters sampled at 3 distances relative to the sawgrass/prairie ecotone. Samples from Bennetts et al. (1988) (n=206 for each distance class) were conducted during 1987 and samples from WCA3A (this study) (n=71 for each distance class) were conducted in 1996.



We examined the precision of our egg cluster estimates from the March through July clusters counts from BCWMA East in 1996. This effort indicted a relatively stable coefficient of variation of 10-20% after sample sizes (i.e., number of throw traps) reached 9 (Figure 14). Most of the data used in our analysis had sample sizes above this threshold. The two sites from Bennetts et al. (1988) had a sample size of 26 and 28. In our study from BCWMA East, the sample size was 12 throw traps. The data used from our mark-recapture study in eastern WCA3A in 1997 consisted of 10 throw traps. However, the data from western WCA3A in 1996 had sample sizes of only 3 per site.

Figure 14. Change in coefficient of variation of egg cluster counts with increasing sample sizes. Each sample represents the number of egg clusters counted within a 1 m x 5 m PVC frame. Data from surveys in March, April, May and June/July in BCWMA in 1996.



Discussion

Our counts of ecotone egg clusters did not correlate to snail densities in adjacent prairies. Bennetts et al. (1988) also found no relationship between counts of egg clusters and capture rates of foraging snail kites. We believe that these results are due to high variability and factors affecting oviposition of apple snails. Apple snail oviposition is influenced by many factors including temperature and vegetation (Hanning 1979, Turner 1996). Our results also indicated that egg laying is quite seasonal and the majority of eggs are deposited over a period of 8 to 12 weeks (Figure 12), which is consistent with reports from Odum (1957) and Hanning (1979). However, even sampling two sites simultaneously may not eliminate the problem. Hanning (1979) found spatial variation in peak egg laying among 6 transects in the southwestern littoral zone of Lake Okeechobee. We would also expect differences to occur along a latitudinal gradient due to the effects of temperature (Hanning 1979). We also found variation attributable to where in relation to the ecotone egg clusters are sampled, which concurs with observations of Turner (1996) and Bennetts et al. (1988). Thus, although it is possible that egg cluster counts could provide meaningful results for studies in which these sources of variation are carefully controlled, our results do not support the use of egg cluster counts as a reliable method of quantifying snail abundance.

3.5 Conclusions

Obtaining reliable estimates of apple snail density, regardless of the method, will be time and labor intensive. The Florida apple snail, although the largest aquatic gastropod in

North America, is a relatively small, inconspicuous animal that occurs in relatively low densities (compared to other invertebrates) in densely vegetated wetlands.

We evaluated one method of assessing snail abundance, egg cluster counts, which did not require extracting snails from their environment. This is the least labor intensive and least time consuming method we evaluated; for this reason it has great appeal as an index of snail abundance. However, we found no support for use of egg cluster counts as a reliable index of apple snail abundance.

Of the throw trap-based methods, the dip net and suction dredge were similar in performance. The suction dredge appeared a little less sensitive to habitat differences and tended to have slightly higher overall recovery probabilities. However, the dip net required less effort and may require less initial investment. In contrast to these two extraction methods, the bar seine had a lower overall capture probability and was substantially more affected by habitat type. Consequently, if a throw trap based method is to be used, we encourage use of either the dip net or suction dredge. The data for recovery of marked snails indicates that it is imperative to assess efficiency of extraction when using throw traps. We agree with previous authors (e.g., Burnham 1981, Nichols 1992) that counts of animals, whether they be from a throw trap or otherwise, are of questionable value without having an estimate of the proportion of animals being counted. Recovery studies add some effort to an already labor-intensive approach to sampling, but without information on extraction efficiencies, comparisons between sampling areas can not be performed with reliability.

The use of crayfish traps or trap arrays as an index of relative abundance may be appropriate in some situations, but great care must be taken to control for time effects and

site to site variation that might affect capture probabilities. For example, changes in weather pattern (e.g., dropping temperature, especially in shallow water) or mating behavior may affect snail movements, and therefore the likelihood of capture. Site variation may include the amount of submerged vegetation (e.g., *Utricularia* spp.) or flocculent substrate which may hinder movements. If capture probability is not directly measured (e.g., a mark-recapture regime), then these differences in capture probabilities may be inaccurately interpreted as a difference in snail abundance. If issues such as changing weather or habitat structure cannot be controlled for (e.g., by sampling all sites simultaneously, and by sampling in similar vegetation types), then an estimate of relative abundance using crayfish traps may not be reliable.

Trap arrays performed more reliably than crayfish traps with respect to their validation with throw trap data, but trap arrays are not available commercially and are not suitable for use in densely vegetated habitats. Our analysis of the relationship between crayfish traps and snail densities derived from throw traps involved a rather low sample size over a narrow range of snail densities. Given the relative ease and utility of crayfish traps in a variety of habitat types (e.g., sawgrass, wet prairie, cattail), additional effort to validate their use as an index of snail abundance is warranted.

We believe that the use of crayfish traps within a mark-recapture sampling regime has the greatest potential to provide reliable estimates of apple snail densities. Even though we found that closure could not be assumed for any of the mark-recapture populations, more recently developed software packages (e.g. TMSURVIV) permit survival and population estimates to be obtained for open populations. Mark-recapture data provides not only

population density information, but also information on survival, movements, and behavior of snails in the population. Our mark-recapture studies also revealed that capture probabilities may vary markedly over relatively short time intervals and from grid to grid within the same wetland system, thereby providing our justification for exercising caution with regards to crayfish traps as a tool for determining relative abundance.

We recognize that each sampling regime, throw trapping for snail density, movement-based traps for relative abundance, and capture-recapture for density, has its unique advantages and limitations. Amount of effort and expense is a limiting factor in any monitoring program or research effort. In this case, capital costs may counterbalance labor costs (i.e., a set of 100 crayfish traps are more expensive, approximately $1600, but require less overall labor than throw traps). Amount of effort also varies depending on the snail densities encountered. For example, in areas with high snail densities, fewer throw traps are necessary to obtain good precision for the snail density estimate. In contrast, higher snail densities may (depending on capture probabilities) result in more snails captured in trap grids, and marking and releasing hundreds of snails in one occasion is time consuming (estimated at 4 hours per 100 snails marked by two workers). Regardless of the investigators choice of sampling method, it is critical that effects of capture probability on survey results (or recovery as applied to throw traps) be understood and explicitly stated in the interpretation.

4.0 FIELD STUDIES OF MOVEMENTS AND SURVIVAL

Seasonal variations in rainfall leading to drying events are common among the tropical and subtropical wetland habitats occupied by *Pilidae* species, including the Florida apple snail (Little 1968, Burky et al. 1972, Kushlan 1975, Haniffa 1978a). However, the natural hydrology in which the Florida apple snail adapted has been altered considerably following the installation of canals and water control structures; this includes the three largest graminoid marsh systems, the Kissimmee Marsh, Upper St. Johns Marsh, and the Everglades (Lowe 1983, Light and Dineen 1994). Successful restoration of these areas, currently underway, requires implementation of a hydrologic regime in support of the biota which historically flourished in them. As increased water demands for agricultural, industrial, and residential areas have diverted water from their natural course, marsh dry downs have become more frequent and longer in duration. Earlier research indicated that these dry downs suppress apple snail populations (Kushlan 1975), and that this may contribute substantially to snail kite population declines (Takewawa and Beissenger 1989, Beissenger 1995). Balancing the needs of the human population with that of wetland biota requires understanding how marsh dry downs affect wetland inhabitants, including the apple snail, a critical component of wetland food webs in Florida (see Introduction). Understanding the snail's response to the duration and timing of dry downs will contribute substantially to the data base from which decisions on the effective allocation of water by the water management districts can be derived.

Any subtropical or tropical aquatic snail challenged with declining water levels must contend with higher temperatures and declining dissolved oxygen (DO) in residual water supplies, followed by desiccation and overheating once water levels drop to ground level (Haniffa 1978a, Aldridge 1983). Survival strategies involve either burrowing into the substrate or remaining in residual pools with tolerable conditions (Burky et al. 1972, Haniffa 1978a). *Pomacea* and *Pila* snails can aestivate from several months to over a year in dry conditions (Little 1968, Burky et al. 1972, Haniffa 1978a, Haniffa 1978b). We have not found any study which documents Pilid snails moving to deep water refugia during a drying event.

The results presented in this section reveal the movement patterns and survival rates of *Pomacea paludosa* as a function of declining water levels in two contexts. First, we studied snail movements and survival during a drying event in a graminoid marsh late in the dry season. Second, we studied snail movements and survival during a lake restoration dry down which occurred in winter. We also were able to reveal a relationship between movements and survival as a function of the ebb and flow of seasonal snail reproductive activity, which spans the hydrologic transition from dry season to wet season.

4.1 Methods

Telemetry Technique

Making inferences about apple snail responses to declining water levels required monitoring snails frequently enough to keep pace with changing hydrology (e.g., weekly or

biweekly). We selected miniature radio transmitters as the tool for monitoring apple snails in the field. Radio-telemetry was used to examine patterns of movements correlated with habitat conditions, to locate stranded snails in dry marsh, and to find snails in deep water (tested up to 2 meters deep). Telemetry permitted repeated snail location without labor-intensive sampling and with the greatest reliability of any other method available. The habitat was not disturbed in the process, except for gaining access to the snail by boat or on foot. Before releasing snails into the field, we made preliminary observations on nine snails, weighing approximately 14-28 grams each, to assess behavior while wearing a transmitter. Snails with transmitters were observed in an aquarium in each of the following behaviors: crawling on the bottom, climbing on aquarium sides or vegetation, feeding, breathing air, burrowing (near the substrate surface) in loose sand and peat, mating, laying eggs, and floating freely on the water surface. We saw no evidence that snail behavior was compromised by the transmitter.

We attached 1.6 gram transmitters (ATS, Inc., Isanti, MN) to the outside of the snail shell using the minimum amount of marine epoxy required for a firm hold. The area for attachment was towel dried and lightly sanded prior to epoxy application. We placed the transmitter 1-2 cm up from the aperture. Placement at the apex allows the snail to remain in an upright position when withdrawn in its shell. Approximately one-half of the transmitters were equipped with a 12-cm whip antenna positioned to trail behind the snail as it crawled. The other transmitters had an antenna coiled and encapsulated in the same protective resin which coats the circuitry and battery. We received signals up to 200 meters from

transmitters with whip antennae (100 meters with encapsulated antennae) submerged in graminoid marsh.

The transmitter can readily be located within an approximate 2 to 3- meter diameter area, but quantifying snail response to habitat conditions (e.g., D.O. or water depth) necessitated obtaining a more precise location. We also anticipated needing to locate snails in thick vegetation or buried in substrate. We found that a magnet can be used to locate transmitters precisely. When a magnet (10 cm x 3 cm x 2 cm, 25-kg pull) touched or came within 13 cm of the transmitter body or antennae, the pulsing signal was turned off or interrupted, or the pitch of the signal changed. The magnet altered the signal under water, when the transmitter was buried in sand, or when buried in sediment under water. Through use of the magnet probe, we reduced transmitter retrieval time from one to several hours (our experience without a probe) to typically 10-15 minutes.

Tracking snails during the marsh dry down and lake management draw down required monitoring for several months. The maximum battery life of the 1.6 gram transmitters used in this study was 60 days. Therefore, apple snail movements for the course of the dry season were documented by releasing transmitters in a staggered fashion. Functioning transmitters from dead snails were transferred to newly captured snails to increase sample size.

Movements During Drying Events

Blue Cypress Water Management Area

We selected our study site in the eastern-most portion of the BCWMA East (Figure 1), since it had the highest ground elevation and was therefore most likely to dry out. We also monitored snails in BCWMA West to compare and contrast potential differences in movements and survival based on hydrology, substrate and vegetation type.

We used an airboat to access the study areas. Apple snails were collected opportunistically during daytime searches in clear water or by spotlight at night. We also collected snails which were found mating with snails bearing transmitters. We had just begun deploying crayfish traps (see Chapter 3), so only a few snails were obtained via trapping.

Once collected, snails were sexed (based on shell morphology; Hanning 1979), weighed (using a spring scale), and their length measured (using a vernier caliper) prior to transmitter placement. Each snail was returned precisely to the spot from which it was taken. These and subsequent snail locations were marked with a pvc pole or flag bearing the snail's unique identification number.

At the time of collection and subsequent relocation, the following parameters and habitat conditions were measured:

1) Distance and direction from previous location
2) Water depth at current and previous location
3) Water temperature at current and previous location (mercury thermometer)
4) Dissolved oxygen (D.O.) at current and previous location (YSI 57 D.O. meter)

5) Substrate and plant composition

6) Depth of aperture of shell, if buried

7) Temperature of the sediment (if exposed)

Apple snail movements were examined on three temporal scales. Most movements were documented at approximately 5-8 day intervals (n= 98) until the snail or the transmitter battery died. For daily movements, an individual snail's location was documented two times over a 24 hr interval (e.g., 5 a.m. and 5 p.m.) (n= 48) . These data exclude initial snail locations and locations of snails found dead. We also monitored snail position over a 12 hour interval, including one location at night, to see if snail position was affected by the diurnal cycle (n= 20).

Lake Kissimmee Draw Down

We monitored apple snail movements in response to draw down of Lake Kissimmee which occurred over 18 weeks beginning in November 1995. The draw down, part of a lake restoration project to improve fisheries habitat (GFC 1995), resulted in an approximately 1.7 meter water level drop (from a high water benchmark of 16.7 meter MSL). The telemetry procedure used to monitor snail movements and survival was similar to that previously described for BCWMA. We decided not to measure DO, a decision based on the lack of impact of DO levels on movements observed during the BCWMA study.

Apple snail movements were monitored until the transmitter battery failed or until the snail was found dead. Throughout the 18 week study period, snails were checked at 7 to 11

day intervals in most cases (8 of 11 site visits); the remaining three intervals were 14, 17, and 17 days.

We selected our study site based on substrate and vegetation type, water level, and boat traffic. We wanted to avoid muck (i.e., flocculent, not fibrous) substrates, floating islands, and areas of high boat traffic (e.g., the vicinity of boat ramps). We chose the north end of Brahma Island as our telemetry study site. An airboat was used to access the area. We collected snails by wading and by using trap arrays. Snails were used only from trap arrays which were in water over 40 cm (the height of the funnel entrances, which tapered down to 6 cm) at the time of snail collection. At the time of collection and subsequent relocation, distance and direction from previous location, and water depth at current and previous locations were measured. Water temperature was monitored at seven stations marked by pvc poles, which included the entire range of depths for all snail locations. As the dry down proceeded, the stations closest to shore could not be used for water temperatures, but they were used to monitor the exposed substrate temperature. Movements and gradient data were derived as described for BCWMA.

Survival

Telemetry data from BCWMA and Lake Kissimmee were used to calculate survival for each study population. Each time a snail was located we checked for mortality. If no signs of activity were observed with an intact snail, we gently pushed on the operculum to see if there was resistance. For dead snails this often resulted in breaking the operculum seal and exposing dead flesh. If we were unsure of the snail's status we carefully inserted a knife

(8 mm blade width) between the operculum and the shell and gently pried it open in order to see the snail flesh. This was not done with excessive force, since we did not want to damage the operculum. If we could not pry the operculum open, we left the snail for the next week. Our experience shows that the operculum, accompanied by snail flesh, is very easily removed from a dead snail. If an empty shell was found, evidence of predation was noted (Snyder and Snyder 1969). The two primary signs of predation were 1) location in an obvious snail kite or limpkin shell pile, and 2) finding that a previously stranded snail had been extracted (by a predator) from the substrate .

Trapping Study and Egg Cluster Survey in BCWMA East

We supplemented our telemetry work with a snail trapping study to enhance our understanding of apple snail movements and survival in relation not only to drying events, but also to coincidentally occurring snail reproductive activity. The trap study was conducted in the BCWMA East in the same area that we monitored snail movements with transmitters during spring 1995. The crayfish trapping study was conducted winter through summer, 1996.

Preliminary observations lead us to two important points regarding the use of crayfish traps. First, bait is not needed to lure snails into the traps; snails apparently enter the trap funnels during horizontal movements and/or vertical ascent to breathe air or lay eggs (section 3.2). Second, sex ratios of captured snails suggested that males were lured by females that had crawled into the traps; whenever we found a female, one to six males were also in the

trap. We hypothesized that as reproductive activity increases, the male to female ratio of snails captured in traps would also increase. Since the snails must move to the traps, and no bait attractant is provided, increased movements (whether driven by mating, temperature, hydrology, etc.) should result in increased captures. If movements are based on males tracking females, we would expect the M:F ratio to be affected by variation in mating behavior. If the sex ratio of captured snails varied with some indicator of reproductive activity, then we would conclude that at least during certain times of the year, movement patterns change as a function of changing reproductive activity.

The basic trap unit was the crayfish trap (section 3.2). For this study we also wanted to test whether or not baiting traps with a live conspecific would attract more snails. We constructed an 8-cm diameter cylindrical enclosure which fit inside the crayfish trap and permitted the bait (snail) to reach the surface to breathe air. We used from 51 to 54 traps throughout our 7-month study. A cylindrical bait enclosure was installed in all traps. Approximately one-third of the traps were baited with adult females (F), a second third of the traps with adult males (M), and the remaining traps were not baited to serve as controls (O). Traps were numbered, and the same type of bait was used for each trap each time we set traps; this was done in case a chemical left by the bait attracted other snails. Six pvc poles were placed in the study site to monitor temperature and depth throughout the course of our study. Depth was measured using a one meter rule placed within 10 cm of the pole. These markers were left in place for the entire seven months of the study.

We conducted six trapping sessions from 30 January to 19 August 1996. A trapping session was initiated by placing 51 to 54 traps on pvc poles, approximately 5 meters apart,

throughout our study site. On the first occasion, the traps were randomly assigned locations in order to randomize the distribution of type of bait (M, F, or O). The pvc poles which support the traps were distributed at the same locations for each trapping session (we made a map using temperature/depth monitoring stations and vegetation as landmarks). However, at the initiation of each of the six trapping sessions, individual traps (and therefore bait type) were randomly distributed among those pvc poles to avoid any potential interactions between bait type and trap location.

For each trapping session, traps were checked on two occasions. Traps were not moved between checks. During 12 trap occasions (2 occasions for each of six sessions) we checked traps at 3-day intervals five times and 4-day intervals four times; the remaining occasions were at intervals of 5, 8 and 9 days. Snails found in traps were released approximately 2 meters from the trap (half the distance between traps). During each occasion, the shell lengths of at least 20 males and 20 females were measured using vernier calipers.

Egg cluster production was used as our index of reproductive activity over time. We deployed a 1 x 5 meter pvc quadrat 36 times during each trap session. We sampled the same transects during each session to control for variation due to sampling location and possibly due to individual female egg production.

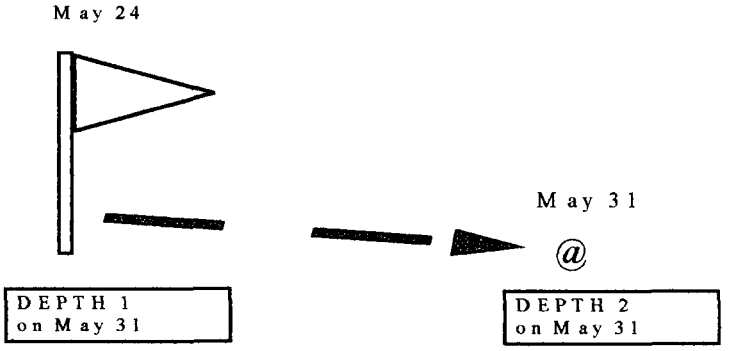
Analyses

Movements During Drying Events

Weekly apple snail movements were examined as a function of sex, time, and water depth and temperature. We grouped data by biweekly intervals to increase our sample size for the analyses. If an individual snail's movements were measured twice within a class interval, the mean value for the distances traveled was used for that individual. Distances traveled (in meters) were transformed using the function $\log_{10}$ (meters +1) in order to meet assumptions of normality for the analyses of variance. We used a mixed model ANOVA (snails monitored = random effect; week monitored = fixed effect) for all movement analyses (movements as a function of time and as a function of depth). Since some individuals were monitored across several biweekly intervals, each interval included different sets of individuals. We performed a repeated measures ANOVA (Crowder and Hand 1990, SAS Inc. 1992) to account for repeated measure of some individuals across intervals (model logmeter= temperature + sex + time + interactions). The analysis produces F-statistics that are not a ratio of sums of squares, but are instead from a Wald-test. Sums of squares (SS) and mean squares (MS) are therefore not reported for those analyses. For more information on this type of F-statistic see Searle et al. (1992). The same data were analyzed for distance traveled as a function of depth (depth in which the snail was last found) (model logmeter = sex + depth + interactions), to test if snails make larger movements to avoid being stranded when the water level reaches some critical depth. For this and subsequent analyses (see below), depth was divided into categories of 10 cm intervals.

Movements along gradients of depth, dissolved oxygen and temperature were analyzed differently. Gradients were calculated based on measurements taken simultaneously at two consecutive snail locations (Figure 15). Note that gradients refer to differences between two consecutive snail locations, so that the scale along which the gradient occurred depends on how far the snail traveled and where the snail was located in

Figure 15. An example of how gradient data were derived from telemetry data. A snail is located on May 24 and its location marked with a pvc pole. On May 31, we found the snail at its new location. We measured depth 1 and depth 2 simultaneously on May 31.



GRADIENT = DEPTH 2 - DEPTH 1

the marsh. Considerable topographic variation occurred within our study area (i.e., wet prairies adjacent to canals, and areas that eventually went dry adjacent to inundated sloughs). A mosaic of juxtaposed vegetation types (i.e., sawgrass, *Eleocharis* sloughs, *Panicum* wet prairies) also ensured availability of temperature and dissolved oxygen gradients within the areas in which snails moved. We were primarily interested in whether or not a snail moved

along a gradient (i.e., towards deeper water), not the actual value of the gradient. Water depth gradient values ranged from -90 cm to + 76 cm. If we had calculated the mean gradient experienced by a group of snails within some interval, one large change in water depth for one snail, for example -50 cm, would negate the weight of five snails which moved along a + 10 cm depth gradient. We therefore tested whether or not snails move along a positive depth gradient by scoring each individual movement as positive (P) or nonpositive (NP) (which included zero and negative gradients). The number of P and NP for each class interval were added, and a frequency table of the proportions of P and NP was generated. The association between gradients and either time or depth was tested using the Mantel-Haenszel chi-square statistic (SAS Inc. 1988, Mantel and Haenszel 1959). We analyzed the depth gradient both as a function of time (biweekly interval) and previous depth. Due to the low range of values for temperature and dissolved oxygen, we could not divide the data into classes of temperature or DO. Temperature and DO gradients were analyzed only as a function of time as described for depth gradients. For temperature, we analyzed the negative gradients relative to non-negative gradients (zero + positive) as well as P vs. NP, since we were also interested in seeing if snails moved to cooler water as water temperature increased.

Survival

Survival of BCWMA and Lake Kissimmee snails with transmitters was estimated at weekly intervals. The Kaplan-Meier procedure to accommodate staggered release of transmitters was used for the survival analyses (Pollock et al. 1989). Chi-square tests as

described by Pollock et al. (1989) were used to compare rates of survival between males and females.

Movements and Population Dynamics Related to Snail Reproductive Activity

We examined the effect of bait type (M, F or 0) using two separate analyses. In the first, we looked only at the number of males captured as a function of bait type (males= bait + session + session*bait). In the second analysis we looked only at females captured (females= bait + session + bait*session). We defined the number of snails captured per trap (male or female) as the cumulative data from both trap checks within each trapping session. We also analyzed snail size as a function of trapping session using ANOVA (size= sess + sex + sess*sx).

Male to female ratios were calculated for each individual trap check. We were only interested in whether males were lured into the traps by females that had crawled into the traps. Therefore, traps with no captured snails were not included in the M:F ratio data set. The resultant data also excluded trap checks which contained no females (which would be division by zero in M:F ratio). The sample size (number of M:F ratios) obtained in this way varied from 33 to 54 among the six trapping sessions. We analyzed M:F as a function of time using ANOVA (M:F = trap session). The relationship between M:F ratio and reproductive activity, as measured by egg clusters, was evaluated using a linear regression (M:F = eggs).

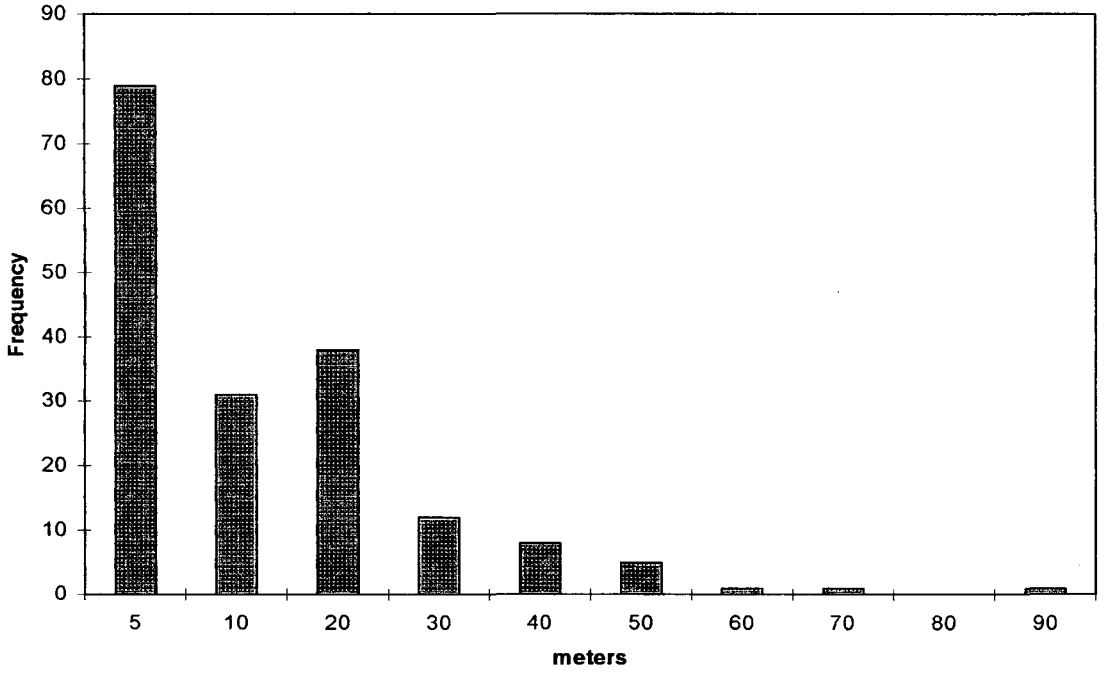
4.2 Results

Movements During Drying Events

Blue Cypress Water Management Area

We monitored 51 snails with transmitters in BCWMA East and 7 snails with transmitters in BCWMA West. One of the first major findings during our telemetry surveys were the weekly distances traveled by snails. Figure 16 presents a histogram of the weekly distances traveled by snails in BCWMA 1995 (we also included Lake Kissimmee data, which were similar to those obtained in BCWMA). Thirty-eight percent of the 176 weekly movements documented were more than 10 meters. The greatest distance measured for one week of travel was 82.5 meters.

Figure 16. Distances traveled by snails in BCWMA (Spring 1995) and on Lake Kissimmee (Winter 1995-1996). Data only for snails in water depths > 10 cm.



Distance traveled was proportional to the time interval between monitoring (Figure 17) (Table 8). In other words, it does not appear that snails seek out favorable habitat and then remain there for an extended time period. Males and females routinely moved in and

Figure 17. Distances traveled by apple snails over 12 h, 24 h, and 7 d (868 h) intervals in BCWMA in 1995. Data only for snails in water depths > 10 cm. Error bars are standard errors.

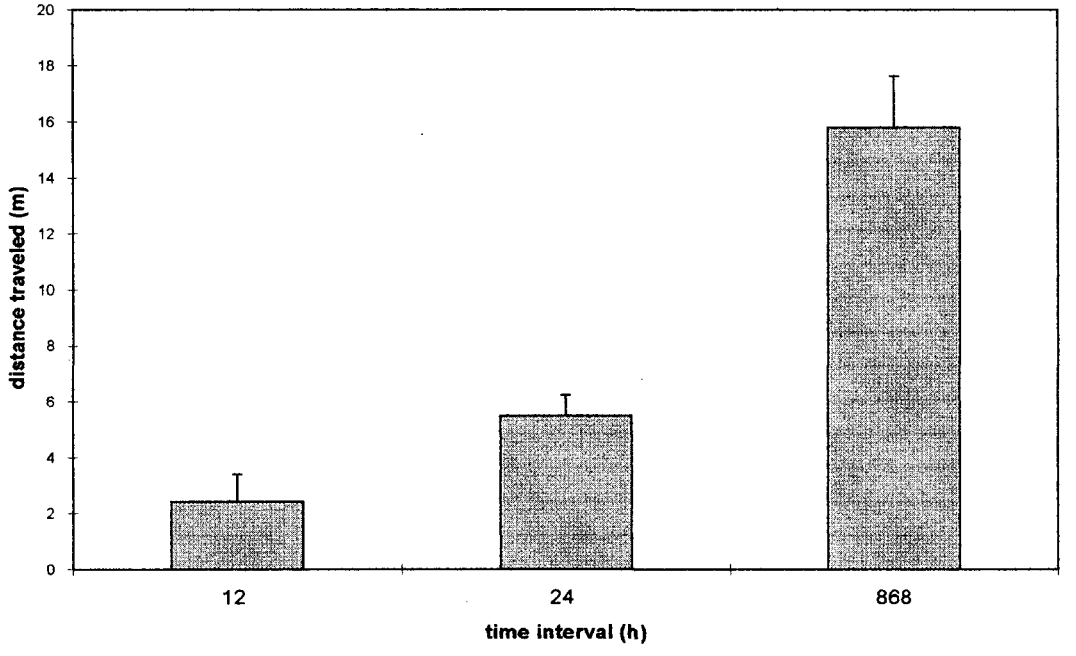


Table 8. Analysis of Variance table for snail distances traveled as a function of time interval between location checks (12 h, 24 h, or 7 day). Sources of variation were interval (INT), sex (SEX), and biweekly interval (WEEK). The F-statistic is based on the Wald test, so no sums of squares are produced (see 4.1 Methods: Analyses). Data is for snails in BCWMA from April 7 through June 16, 1995.

Source	df	F	Prob>F
INT	2	30.5	<0.001
SEX	1	0.65	0.422
INT*SEX	2	0.17	0.847
WEEK	3	2.29	0.085
INT*WEEK	6	0.36	0.899
SEX*WEEK	3	2.89	0.042
INT*SEX*WEEK	5	1.15	0.0343
error	67		

out of sawgrass and other thick vegetation over each of the time periods monitored. Based on the 20 different day to night observations, we observed no pattern of females using the sawgrass by night (for oviposition) and then moving into open slough by day (e.g., to feed). Some females remained among sawgrass for the entire life of their transmitter, while others moved day to night, day to day, and week to week in and out of different vegetation types and densities.

The effects of decreasing water depth were examined in several ways. The hypothesis that snails move along a depth gradient was not supported by our data for the 18 week period of investigation in 1995 (Table 9) (MH $\chi 2 = 1.26$, $p=0.261$).

The analysis of depth gradients as a function of depth indicates that snails do not begin to seek deep water refuge when some critical depth is reached (Table 10) (MH $\chi 2 = 2.843$, $p=0.092$). Although no overall significant difference was found, most snails at depths of 11-20 cm moved along a depth gradient (Table 10). We took a closer look at these depth

Table 9. Frequency tables for movements along depth gradients as a function of time. **N** referes to the number of movements along a negative (from deeper to shallower water) and zero (no difference in depth in consecutive locations) gradient. P refers to the number of movements along a positive depth gradient (from shallower to deeper water). Data from BCWMA East 1995.

Time Interval	N	P
March 11 - March 24	2	1
March 25 - April 7	6	5
April 8 - April 21	8	8
April 22 - May 5	7	12
May 6 - May 19	7	9
May 20 - June 2	6	5
June 3 - June 16	11	5
June 17 - June 30	6	3
July 1 - July 14	3	2

Table 10. Frequency tables for movements along depth gradients as a function of previous depth in which a snail was found. N referes to the number of movements along a negative (from deeper to shallower water) and zero (no difference in depth in consecutive locations) gradient. P refers to the number of movements along a positive depth gradient (from shallower to deeper water). Data from BCWMA East 1995.

Depth Class (cm)	N	P
5	32*	0
10	2	1
20	5	13
30	10	11
40	16	14
50	16	12
60	4	0
70	5	0

* snails had become stranded

ranges and associated movements to address the possibility that these snails gained some advantage by moving towards deeper water. In the 11-20 cm depth category, ten snails moved 13 times to deeper water. The fate of these snails suggests that little or no advantage to their survival was provided. Within 7 days of moving to deeper water, two of ten snails moved out of the deep water and became stranded in their new, shallower location. These locations were within one meter of deep water refuge (deep, shaded, cooler water). Two more of the ten snails eventually became stranded in the dry marsh. Two other snails died (a non-predatory and an unknown cause of death) within a week of moving along a positive

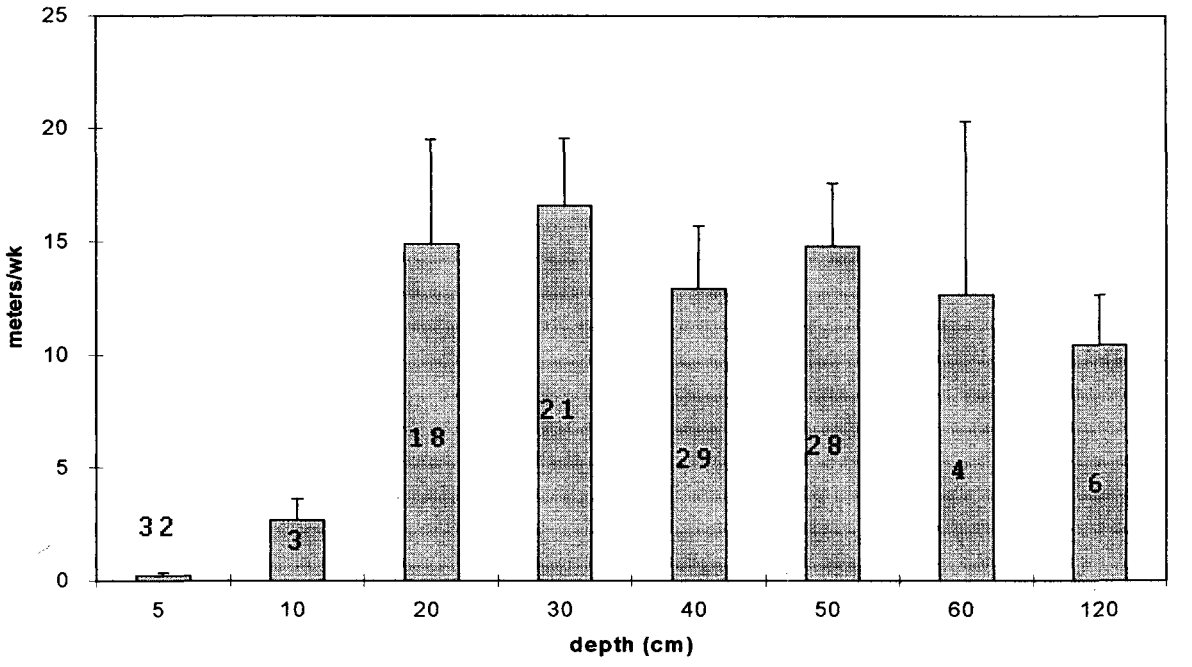
depth gradient. Even if snails were able to identify a depth gradient along which they moved to find refuge, the strategy did not enhance survival: 40% became stranded in dry marsh, and 20% died before the marsh dried down.

Table 11. Analysis of Variance (ANOVA) table for snail distances traveled as a function of depth in which snail was previously located. Source of variation was depth (DEPTH) which consisted of 4 depth categories; 0 to 10, 10 to 30, 30 to 50, and over 50. These were the smallest class intervals we could obtain estimates for in our repeated measures ANOVA. The F-statistic is based on the Wald test, so no sums of squares are produced (see 4.1 Methods: Analyses). We used the mean distance traveled for individual snails within a depth class in order to obtain an F-statistic. Data is from snails in BCWMA East, March through July 1995.

Source	df	F	Prob>F
depth	3	30.5	<0.001
error	48		

Although snails do not appear to move along a depth gradient or seek deep water refuge, we did find that snail movements are influenced by depth. The distances traveled by snails which were in 1- 10 cm of water were reduced relative to all other depths (Figure 18) (Table 11). Movements would certainly be curtailed at this point due to emergent and submerged or floating vegetation (e.g., *Utricularia* spp.) which settles out and forms an obstruction to movement.

Figure 18. Distances traveled by snails as a function of depth in BCWMA East in 1995. Data are from snails in water depths > 10 cm. Error bars are standard errors. Numbers above or inside bars are sample sizes.



Movements along temperature and dissolved oxygen gradients were also investigated. We observed no tendency for snails to move into areas with higher dissolved oxygen (Table 12, (MH $\chi 2 = 1.094$, $p=0.296$). The dissolved oxygen between locations in BCWMA East usually differed by less than 2 ppm. A similar small range in values was also observed for temperature; only eleven out of 100 temperature gradients were more than ±1 °C. Snails showed no tendency to move towards warmer water (Table 13) (MH $\chi 2 = 0.629$, $p=0.428$) or towards cooler water (Table 14) (MH $\chi 2 = 2.192$, $p=0.139$).

Table 12. Frequency tables for movements along dissolved oxygen (D.O.) gradients as a function of time. **N** referes to the number of movements along a negative (from high D.O. to low D.O.) and zero (no difference in D.O. between consecutive locations) gradient. **P** refers to the number of movements along a positive depth gradient (from low D.O. to high D.O. water). Data from BCWMA East 1995.

Time Interval	N	P
March 11 - March 24	2	0
March 25 - April 7	8	3
April 8 - April 21	8	8
April 22 - May 5	15	4
May 6 - May 19	10	8
May 20 - June 2	3	7
June 3 - June 16	13	2
June 17 - June 30	7	0
July 1 - July 14	1	0

Table 13. Frequency tables for movements along temperature gradients (cooler to warmer water) as a function of time. **N** referes to the number of movements along a negative (from high temperature to low temperature) and zero (no difference in temperature between consecutive locations) gradient. **P** refers to the number of movements along a positive temperature gradient (from low temperature to high temperature water). Data from BCWMA East 1995.

Time Interval	N	P
March 11 - March 24	2	0
March 25 - April 7	11	2
April 8 - April 21	9	7
April 22 - May 5	16	3
May 6 - May 19	10	7
May 20 - June 2	8	3
June 3 - June 16	12	4
June 17 - June 30	8	0
July 1 - July 14	4	1

Table 14. Frequency tables for movements along temperature gradients as a function of time (warm to cooler water). Note that N and P have different meanings from those designations in Table 12. **N** refers to the number of movements along a negative (from high temperature to low temperature). **P** refers to the number of movements along a positive temperature gradient (from low temperature to high temperature water) and zero (no difference in temperature between consecutive locations) gradient. Data from BCWMA East 1995.

Time Interval	N	P
March 11 - March 24	0	2
March 25 - April 7	4	9
April 8 - April 21	5	11
April 22 - May 5	4	15
May 6 - May 19	6	11
May 20 - June 2	1	10
June 3 - June 16	4	12
June 17 - June 30	4	4
July 1 - July 14	3	2

Lake Kissimmee Draw Down

We monitored 31 snails with transmitters at Lake Kissimmee. The results from monitoring these snails during the draw down were similar to those found in BCWMA.

Snails did not exhibit a tendency to move along a depth gradient as a function of previous depth (Table 15) (MH $\chi 2 = 1.168$, $p=0.280$). For these analyses we had 38 movements in depths greater than 10 cm from which to calculate movements in relation to

Table 15. Frequency table for movements along depth gradients as a function of previous depth in which a snail was found. N referes to the number of movements along a negative (from deeper to shallower water) and zero (no difference in depth in consecutive locations) gradient. P refers to the number of movements along a positive depth gradient (from shallower to deeper water). Data from Lake Kissimmee drawdown 1995-1996.

Depth Class (cm)	N	P
10	no data	no data
20	2	2
30	6	3
40	5	7
50	7	2
60	2	0

depth gradients. A closer examination of the 10 snails which moved along a positive depth gradient 14 times revealed little advantage to these snails; eight of ten snails later became stranded, one died just before it would have become stranded, and only one made it to deeper water by the end of our study. Snail movements in less than 10 cm were again greatly reduced (Figure 19) (Table 16).

Twenty-two of the 31 snails were released in over 20 cm of water; the mean (± SD) depth was 40.7 cm (± 9.6 cm). Ten of these snails traveled greater than 15 meters per week at least once during the draw down. Based on an average water level drop of 13 cm per week (determined from our water depth measurements) and distances traveled by Kissimmee snails, we believe that snails released in greater than 20 cm depths had the opportunity to stay ahead of the receding water and avoid being stranded. However, 15 of the 22 snails

(70%) became stranded. Of the remaining 30%, only 5% (1 in 22 snails) moved into an area which did not become dry; the remaining 25% died in shallow water just before they would have become stranded within the settling periphyton and vegetation.

Figure 19. Distances traveled by snails as a function of depth at Lake Kissimmee during the 1995-1996 draw down. Data are from snails in water depths > 10 cm. Error bars are standard errors. Numbers above or inside bars are sample sizes.

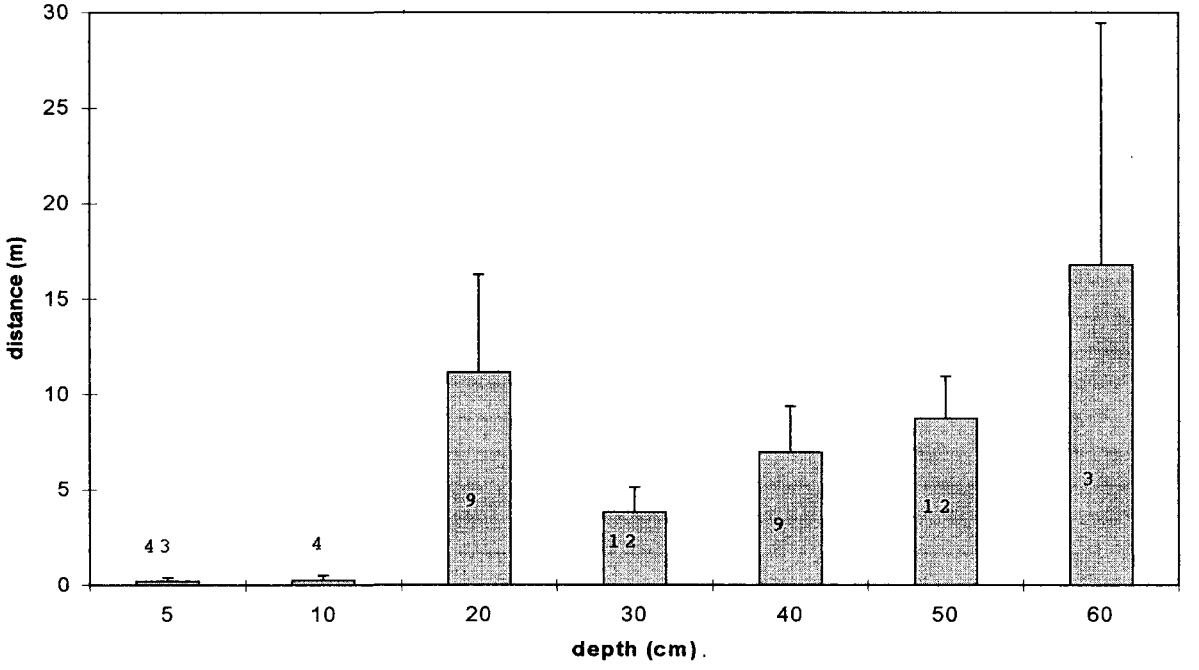
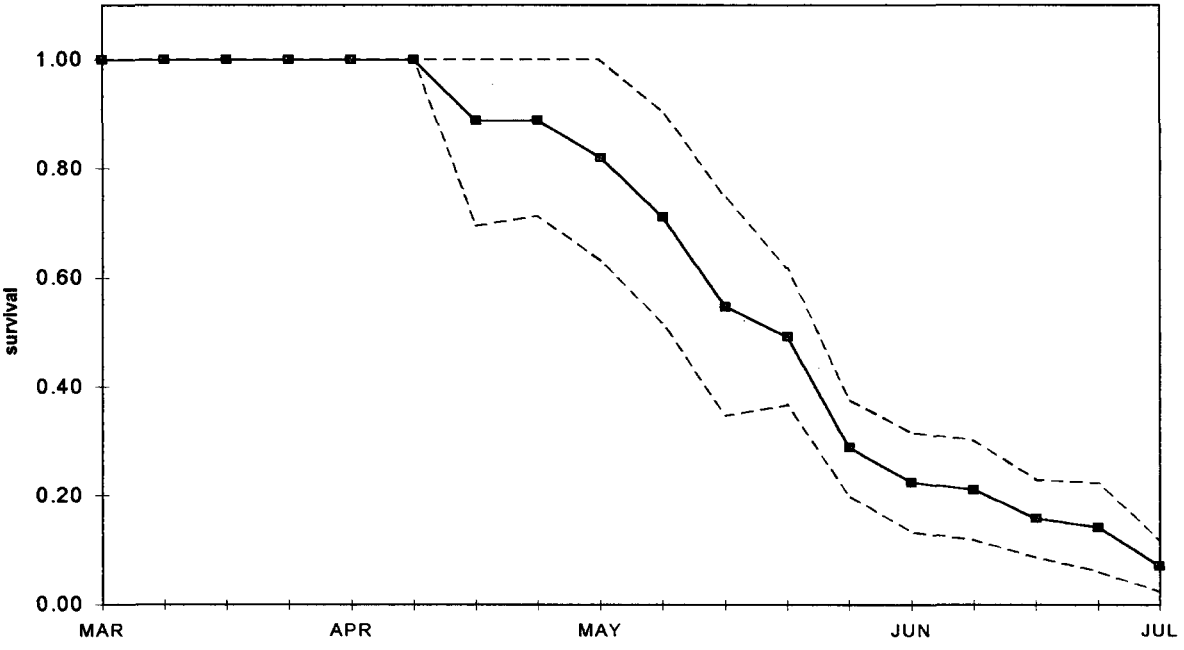


Table 16. Analysis of Variance (ANOVA) table for snail distances traveled as a function of depth in which snail was previously located. Source of variation was depth (DEPTH) which consisted of 6 depth classes: 10, 20, 30, 40, 50 cm, and over 50 cm. These were the smallest class intervals for which we could obtain estimates in our repeated measures ANOVA. The F-statistic is based on the Wald test, so no sums of squares are produced (see 4.1 Methods: Analyses). We used the mean distance traveled for individual snails within a depth class in order to obtain an F-statistic. Data is for snails at Lake Kissimmee, November 1995 through February 1996.

Source	df	F	Prob>F
depth	5	6.04	<0.001
error	40		

Figure 20. Survival of 51 snails in BCWMA East in 1995. 95% confidence intervals shown.

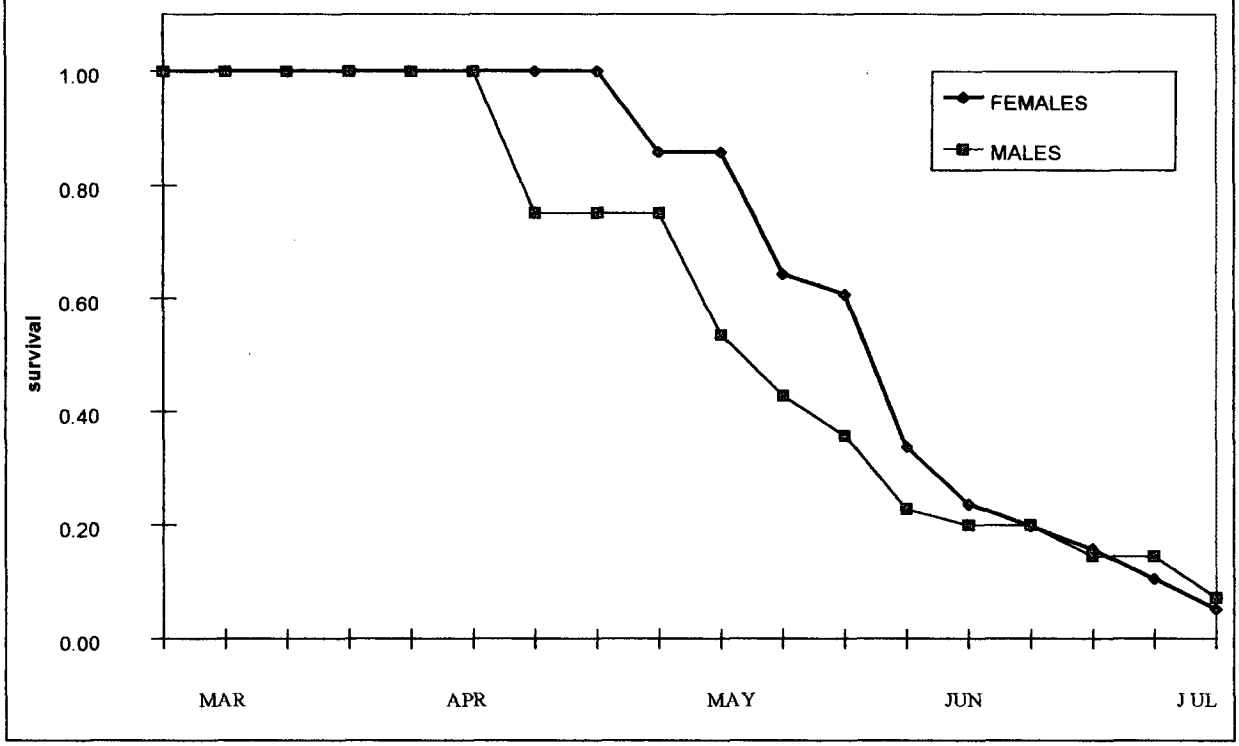


Survival

Blue Cypress Water Management Area

No snails were found dead during the first six weeks of the study (Figure 20). Only male snails were found dead in weeks 7 and 8; both male and female snails were found dead in each of the remaining weeks of the study (Figure 21). The male and female survival

Figure 21. Survival of male and female snails in BCWMA East in 1995.



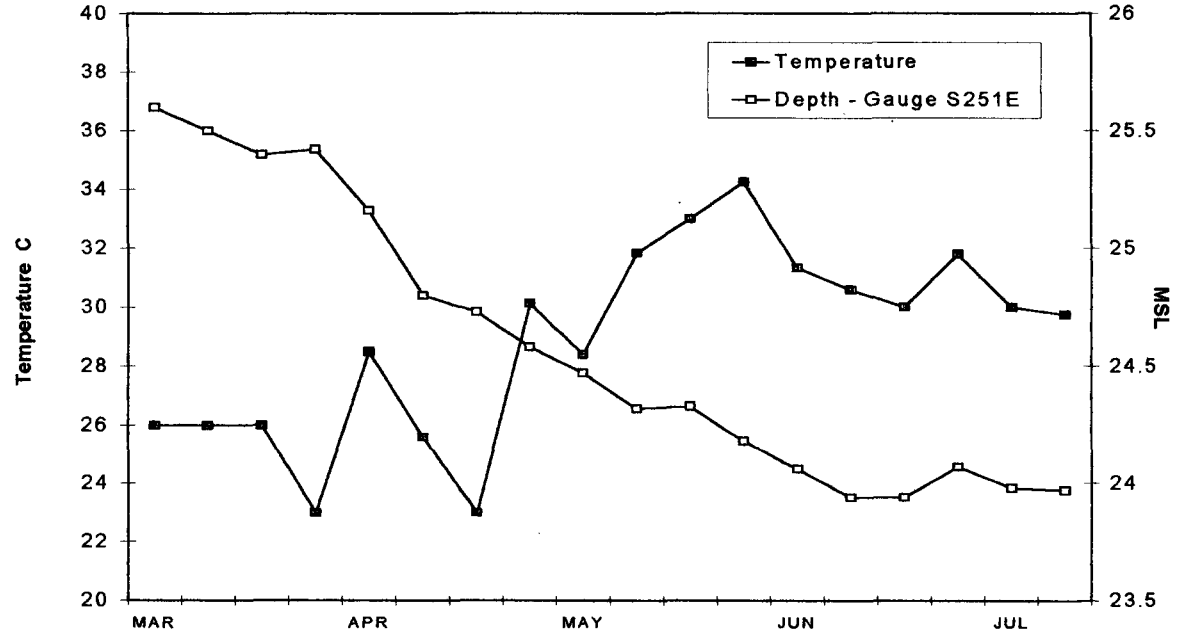
curves were not statistically different ($\chi^2 = 0.057$, df = 17, $p > 0.90$), even when comparing only data from April 22 through June 16 ($\chi^2 = 0.048$, df = 7, $p > 0.50$). These survival curves

include only 8 snails stranded in the dry down area; therefore, desiccation does not account for the large decline in survival.

Recall that transmitters were released in a staggered pattern. This means that some snails in week 13, for example, had worn a transmitter for 1 week, others for 3 weeks, and others for 9 weeks. Snails dying in a particular week had, therefore, carried a transmitter for a range of different times. Survival was not a function of time bearing a transmitter.

Temperature and depth data are presented in Figure 22. Survival drops steadily as water depths decrease and as water temperatures increase (in general) over the 18 week study. The steepest continuous drop in survival occurs during weeks 9 through 14 of the study period (Figure 20). During this same period we saw mean temperatures (between

Figure 22. BCWMA East water temperatures and water depths in Spring 1995. Temperatures are means from data taken between 11:30 a.m. and 1:30 p.m. (daylight-savings time).



11:30 a.m. to 1:30 p.m.) consistently exceed 30 °C, and for weeks 12 and 13 we observed late afternoon temperatures of 38 °C. During week 13 we found the greatest number of snail deaths relative to any other week in the 18 week study. For week 13, all snails found dead in the water (n=6) were from the open slough area, which experienced the highest water temperatures. We believe the high water temperature may have contributed to stress and the eventual mortality of these snails. Despite high temperatures in the marsh, D.O. was quite high due to the abundance of periphyton.

Snails challenged with receding marsh waters in BCWMA East became stranded in the dry marsh. Table 17 summarizes the fate of the 8 snails which we found stranded in dry

Table 17. Fate of stranded snails in BCWMA 1995.

Fate of Stranded Snail	No. of Snails
Non-predation death	3
Predation death	1
Unknown fate	2
Snail never died[1]	2

[1] Water level rose and snails revived and crawled away.

marsh. Three of these stranded snails did not have transmitters (we found them searching by hand), demonstrating that snails without transmitters became stranded. The mean survival time in dry down conditions was 3.9 ± 2.2 weeks.

All stranded snails were near the substrate surface with the aperture 1 - 3 cm from the surface. Some snails rested on top of the substrate. Even in patches of accumulated organic material, where for example a mud turtle was found completely buried, snails were near the surface. We saw no evidence that snails burrow to avoid dry down conditions, at least in the predominately sand substrate characteristic of BCWMA East.

All stranded snails were found under the protection of some kind of standing or bent over vegetation. We measured afternoon substrate temperatures at snail locations and at adjacent patches of substrate with no vegetation. We also measured water temperatures in the open slough area at the same time. Shaded substrate temperatures ranged from 27 to 33 °C, and were an average 2.8 °C cooler than substrates devoid of vegetation (unshaded). Stranded snails, whether under vegetation or not, experienced, on average, temperatures 4.6 °C cooler than snails which were still in inundated areas of the marsh.

Lake Kissimmee Draw Down

The survival rate of the 31 snails monitored at Lake Kissimmee is shown in Figure 23. Again, snail survival was not dependent on the length of time bearing a transmitter since at any time snails in the study population had worn transmitters for 1 to over 8 weeks. The first deaths did not occur until snails became stranded between weeks 6 and 8. However, intolerance to dry down conditions was not the direct cause of death in the majority of cases (Table 18).

Figure 23. Survival of 31 snails during the Lake Kissimmee 1995-1996 draw down. 95% confidence intervals shown.

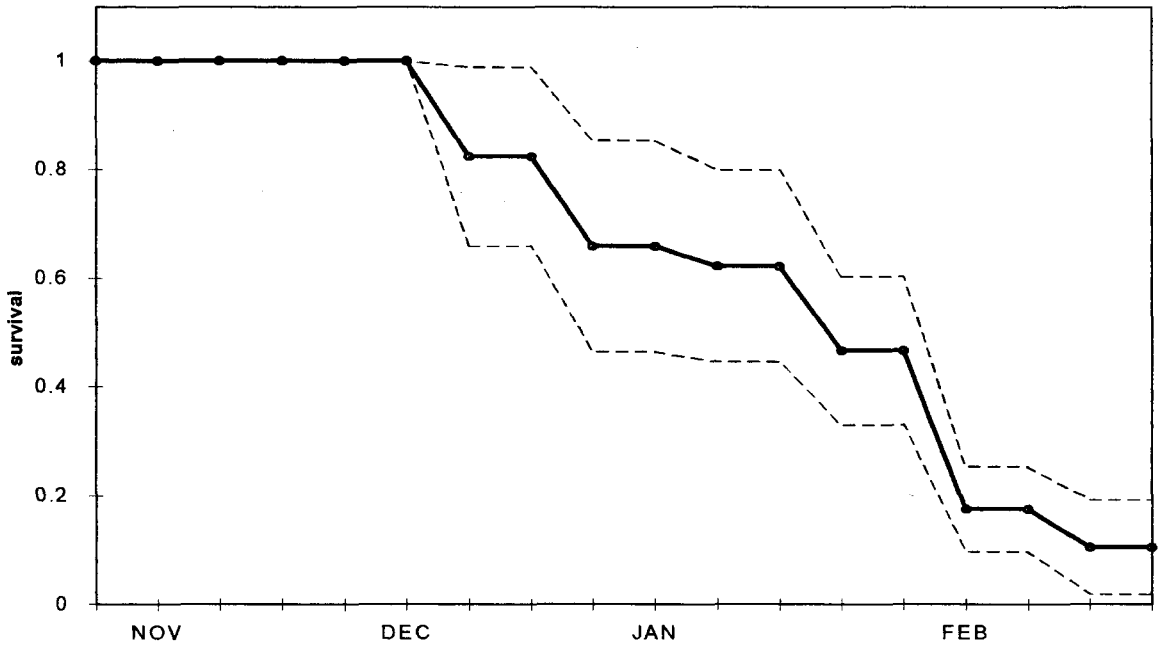


Table 18. Fate of stranded snails at Lake Kissimmee during the 1995-1996 draw down.

Fate of Stranded Snail	No. of Snails
Non-predation death	7
Predation death	15
Snail never died*	1

*Ended study. Snail revived in an aquarium.

We monitored the survival of 23 snails stranded in the dry marsh (2 of these had no transmitters and were found by hand searching). The mean (± SD) survival time for stranded

snails was 3.9 ± 3.1 weeks, approximately the same survival time found for snails stranded in BCWMA in 1995. However, unlike the BCWMA observations, the majority of deaths during the Lake Kissimmee draw down were a result of predation. Like BCWMA, temperature may be an issue, but at the opposite extreme. During the draw down central Florida experienced 5 freezes, 3 at 0°C, and two hard freezes (one at -2.2 °C and one at -3.3 °C); 11 of the 23 stranded snails survived one of these hard freezes. [Note that we did not measure the substrate temperatures where the snails were located during these late/night early morning hours; our discussion is based on air temperature data from National Weather Service for Orlando]. Each of these 11 snails survived from one to three nights at 0 °C. At the termination of the study, we collected the one remaining stranded snail, still alive after 8.3 weeks in the dry down area, and placed it in an aquarium with water. The snail revived within 24 hours and lived another 8 weeks. This snail had survived five nights at or below 0 °C. We also observed two snails revive in water which had survived at least 3 months in dry conditions. These snails, which were subadult in size, inadvertently had been left in the trap arrays that we stored on dry ground at our field station. They survived two nights below 0 °C and three nights at 0 °C (ambient air temperatures).

Movements and Population Dynamics Related to the Snail Breeding Season

Lack of evidence for snails seeking out deep water refuge leaves unanswered the question of what drives snails to move, on average, 10 to 20 meters per week. Telemetry data provided the first clues as to why snails move during the dry season.

We observed a gender difference in snail distances traveled in BCWMA in 1995, although sex effects were not significant in our repeated measures ANOVA ($p=0.287$). For most of the 18 week period females traveled an average of 10 to 20 meters per week, with no discernible difference over time (Figure 24). Male average weekly distances, however, did vary as a function of time, showing a peak during March 31 through May 5; this was followed by a gradual decline from May to the end of the study.

We also identified different movement patterns (distance and direction) for males and females. We limited our pattern assessment to snails traveling in water depths greater than 10 cm, and to patterns for which we monitored at least 3 consecutive movements. We did not include changes in location for snails found dead. Using these criteria, patterns were

Figure 24. Distances traveled by male and female snails in BCWMA East in 1995. Data are from snails in water depths >10 cm. Error bars are standard errors.

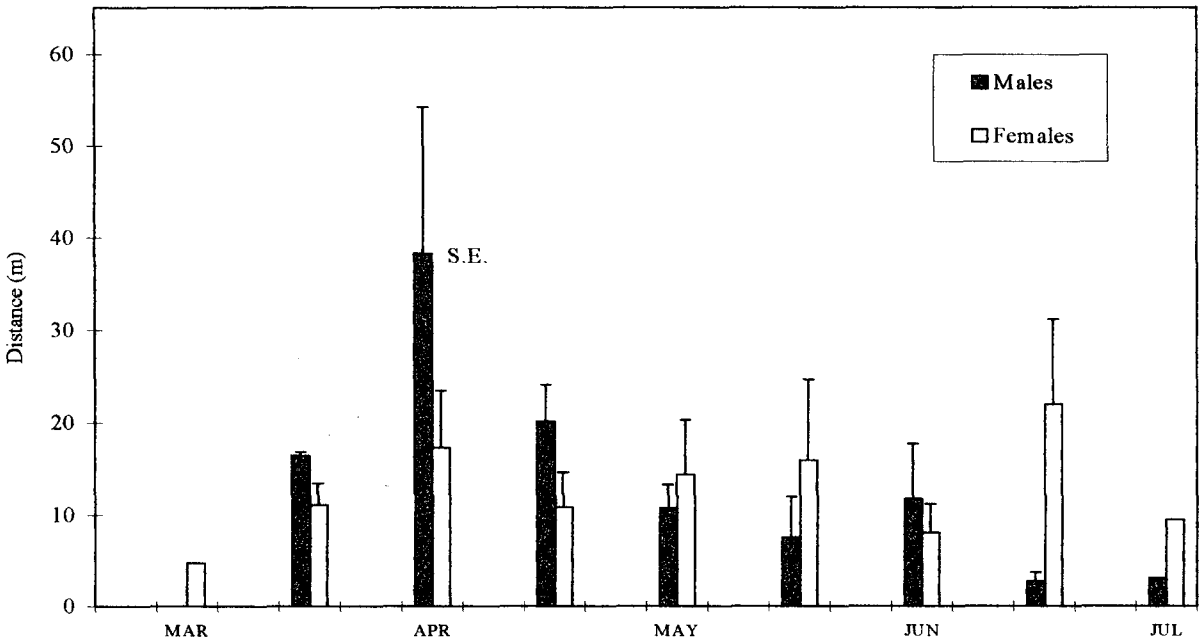
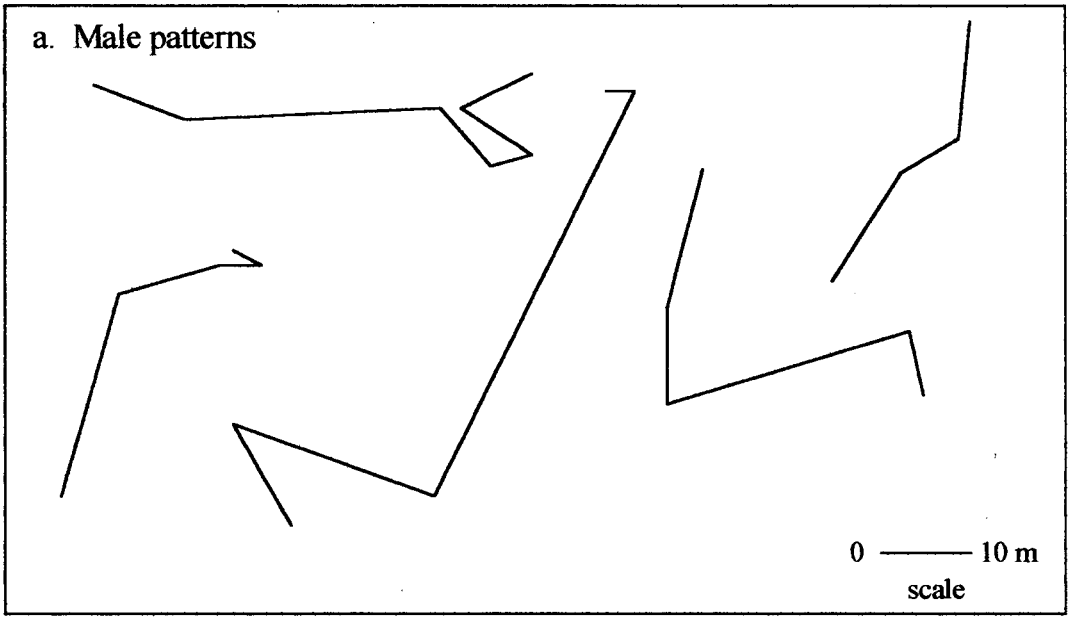
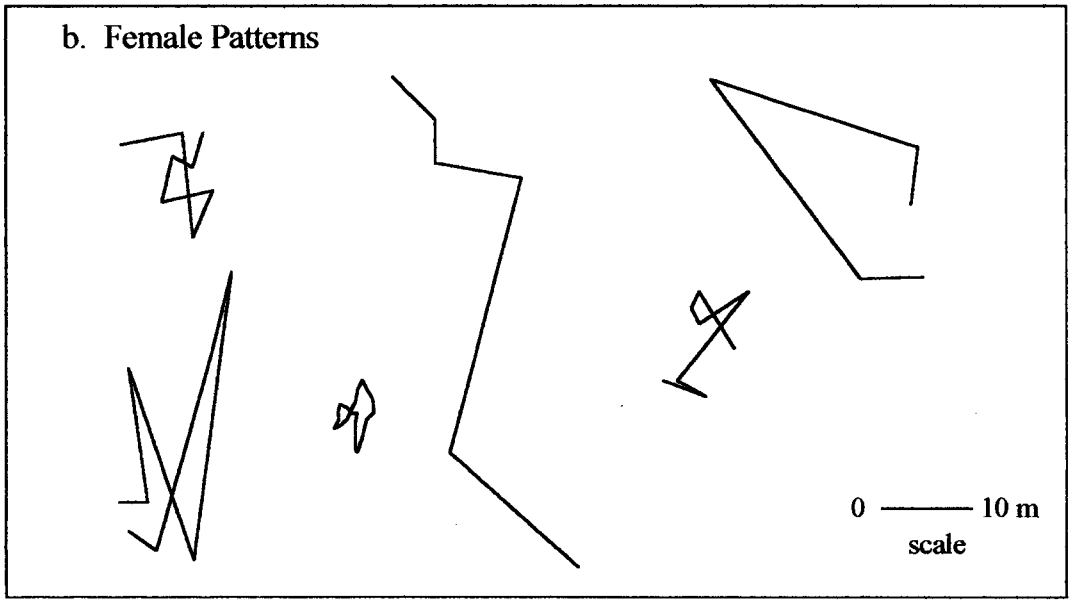


Figure 25. Movement patterns of male (a.) and female (b.) snails in BCWMA East in 1995. Only snails for which 3 or more movements were documented are included. Patterns are to scale.

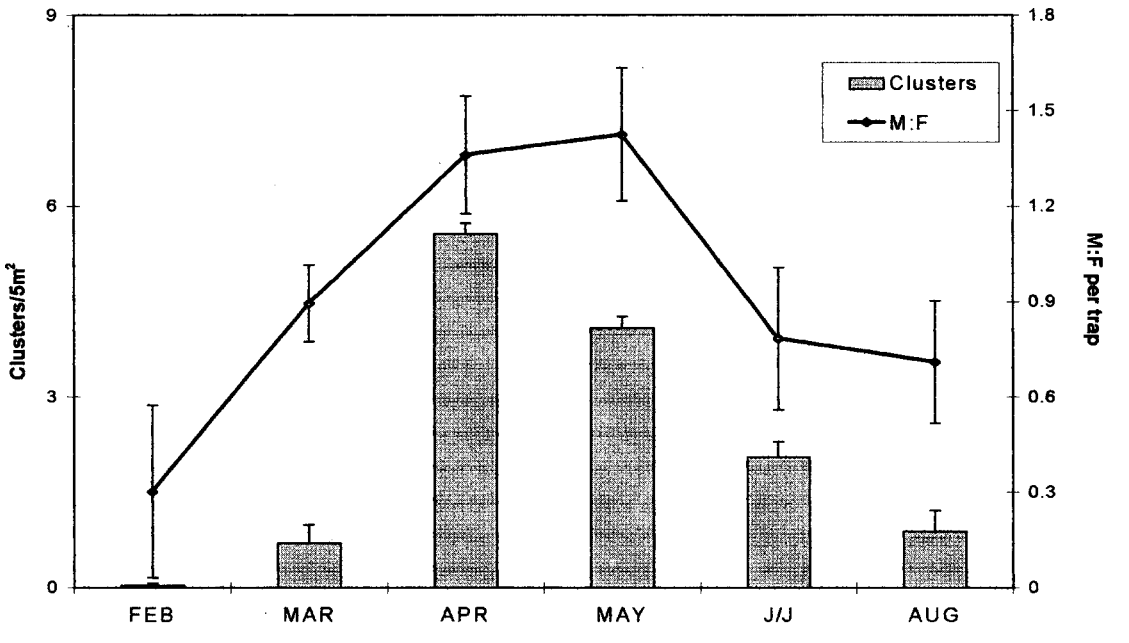




constructed for 11 snails, 6 females and 5 males. Males exhibited a tendency to move farther and more linearly (Figure 25a) relative to females, which moved within a more defined home range (Figure 25b). A gender difference suggested to us that variations in snail movements reflect reproductive activity.

Additional evidence that snail movement patterns reflect reproductive activity was provided by trapping data. Seven modified crayfish traps were distributed in BCWMA East in 1995 to supplement our collection efforts and to provide some preliminary information on their utility for snail research. We obtained data from 11 checks of traps with no bait. Five

Figure 26. Egg clusters and M:F ratios per trap in eastern BCWMA East in 1996. Error bars are standard errors.



of the 11 traps contained only males. The remaining six traps all contained one female; in five of these we found 1 male with each lone female, and in one case 5 males were trapped with the female. Females were never found alone in any of the traps. It appeared that females lured males into the traps. These results lead us to an expanded trapping effort in 1996 to examine changes in this pattern over the course of the snail mating season (Figure 26). Both M:F ratio and egg cluster production vary substantially over the 7 month sampling period (Table 19 and Table 20, respectively). Note that the M:F ratio does not reflect the actual population sex ratio, since we are not randomly sampling the population; rather, males are targeted during some periods since females apparently attract males into the traps. The ratio of males to females (calculated as mean ratio per trap check) is correlated to egg

Table 19. Analysis of Variance (ANOVA) table for M:F ratio per trap for snails captured in crayfish traps. We conducted six surveys in BCWMA East from February through August 1996. Source of variation is trapping session (SESS).

Source	df	SS	MS	F	Prob>F
SESS	5	3.11	0.623	8.17	0.0001
error	308	23.48			

Table 20. Analysis of Variance (ANOVA) table for egg clusters ratio per 5 m^2. We conducted six surveys in BCWMA East from February through August 1996 (same dates as M:F ratios in crayfish traps, Table 19). Source of variation is trapping session (SESS).

Source	df	SS	MS	F	Prob>F
SESS	5	851.48	170.3	45.46	0.0001
error	210	786.72	3.75		

cluster production (R^2=0.8, df =5, p=0.016). Temperature data collected during each session indicates that oviposition began once temperatures reached approximately 20 °C in March, and peaked around 28 °C in April. Although it appears that females lure males into the traps, baiting the traps with females (as compared to controls and traps baited with males) does not affect the number of males captured (p=0.609). The lack of bait effect is consistent with the hypothesis that males are lured into traps by a chemical trail left by females crawling in through the funnels. The bait (female snails) was placed directly into the traps, thus no chemical trail could lead males into the traps. The number of females captured also was not affected by bait (control, male, or female) (p=0.48).

The mean size of snails captured in the traps also varied over the 7 month crayfish trap sampling study (Figure 27) (Table 21). A session effect in snail size reflects both adult

Figure 27. Sizes of apple snails captured in crayfish traps in eastern BCWMA East in 1996.

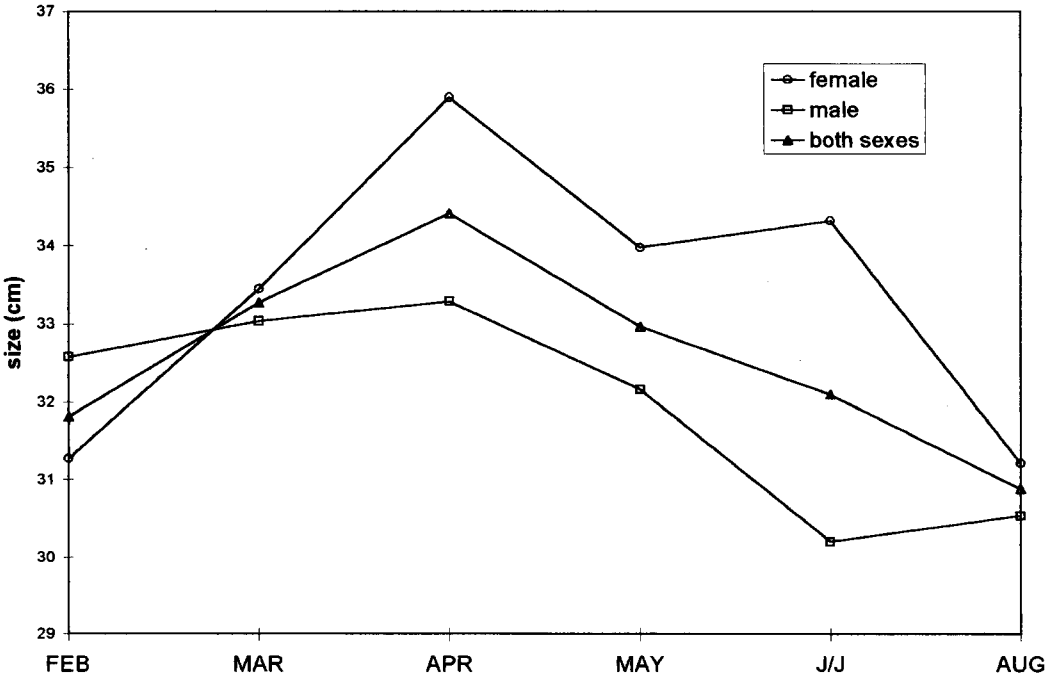


Table 21. Analysis of Variance (ANOVA) table for snail size (mm) for snails captured in crayfish traps. The snail size range that these traps can collect and hold is 20 to 40 mm. We conducted six surveys in BCWMA East from February through August 1996. Source of variation is trapping session (SESS).

Source	df	SS	MS	F	Prob>F
SESS	5	506.56	101.31	12.34	0.0001
SEX	1	154.96	154.96	18.87	0.001
SESS*SEX	5	215.31	43.06	5.24	0.001
error	356	2923.05	8.21		

snail mortality and snail recruitment in the last half of the study; this has also been documented by Hanning (1979). A sex effect would be expected, since female snails tend to be larger than males (Hanning 1979). The interaction between sex and session (Table 21) may reflect the change in M:F ratio captured over the sampling period, which peaked at the same time snail size peaked.

We now recognize that determining the gender of smaller snails may be more difficult than for mature snails. From our observations, snails from hatchling size to approximately 20 mm shell length most often appear to be female. Males may not acquire their characteristic flair and wider aperture until reaching adult size or reproductive status. The characteristic flair and wider aperture for males were observed for 46 of 82 snails between 25 and 30 mm, so at least in this size range it appears males can be identified. Unfortunately, we cannot determine reproductive status without extracting snails from their

shells. All snails captured in this study were at least 25 mm, but we can not quantify our error rate in sexing snails without further study. The M:F ratio in the last three trapping sessions (Figure 26) could be affected by inaccurate identification of males as females, due to young of the year snails which reached sufficient size to be caught in the traps. However, knowing the onset of egg cluster production (Figure 26), snail egg incubation time (Hanning 1979) and their growth rates (Hanning 1979), young of the year snails could not reach sufficient size to be captured in the traps in February, March or April.

4.3 Discussion

In considering the potential strategy for coping with seasonal dry-down, we monitored apple snail movement patterns over the course of the dry season. The first major finding derived from monitoring movements was the degree of mobility exhibited by snails, which affects how studies of apple snail ecology may be interpreted. For example, observations of changes in snail density based on fixed trap locations (e.g., as in Kushlan 1975) should be evaluated based on the fact that a fraction of the population may have emigrated (intentionally or not) as habitat conditions changed. We now recognize that immigration and emigration of snails may be important contributors to population dynamics, for example, in the recolonization of reflooded wetlands following a draw down.

Our data indicate that snails rarely reside in one specific location for extended periods. It appears that apple snails are routinely on the move, at least during the spring and early summer. Individual snails routinely move in and out, and through vegetation of different types (*Panicum sp.*, *Eleocharis sp.*, *Cladium jamaiciensis*) and densities.

Interpretations of habitat preferences and snail distribution data should consider the fact that individuals in the population frequently (on a daily to weekly basis) move in and out of many micro-habitat types within their range.

It is apparent that snails had the *capacity* to move sufficient distances to reach the deep water refuge which was available in our study area. However, we found no evidence that snail survival strategy includes seeking deep water refuge. Based on observations from a minimal (in area and duration) draw down in BCWMA in 1995 and an extensive draw down on Lake Kissimmee in 1995-1996, we suspect that the proportion of a snail population that becomes stranded would be proportional to the areal extent of the dry down. This trend has been documented for the Indian apple snail, *Pila globosa* (Hanniffa 1978a). *Pomacea paludosa* movements appeared to be incidental to changing hydrologic conditions.

Based on a lack of evidence that snails move along depth, temperature or D.O. gradients, and the identification of a gender difference in movement patterns (distances and direction), we conclude that apple snail reproductive ecology drives the movement patterns of snails more so than does hydrology, at least during the spring and early summer. Hanning (1979) alluded to observations that males seek out females for mating, but provided no data to support this conclusion. We found that M:F ratios of snails captured in crayfish traps were related to egg cluster production, confirming that movements are related to reproductive activity, and that males track females into the traps. Baiting the traps with female snails did not increase the catch of males; this suggests that males find females through chemicals in female mucous trails, rather than via a chemical gradient in the water column.

Chemoreception as a directional guide for movements has been documented for gastropods (Chase and Boulanger 1978, Cook 1979, Tomiyama 1992).

Snail movements are not driven by hydrologic conditions, but water depth does influence snail movements. An approximate depth of 10 cm appears to be a threshold level at which snail movements become impeded, probably a result of a concentration of settling and falling vegetation and suspended materials (organic debris and periphyton). At this point snails settle in one spot, and as residual water recedes they become subjected to dry down conditions. They do not burrow, but they do conserve moisture through tight closure of their operculum.

We observed survival rates of snails in dry down conditions that were higher than those reported for *P. paludosa* in laboratory experiments (Turner 1994). In Turner's three trials, snail survival rates after just 7 days exposed to air were 50%, 3%, and 2%. In a fourth trial, 33% had survived by day 29 of aerial exposure, a survival rate closer to our observations of snails in the field. Saturated sand provided little advantage to the snails in laboratory conditions, as Turner (1994) reported 13% survival after 7 days. Although we observed longer periods of survival in the field, our results, along with Turner (1994) and Little (1968), indicate that Florida apple snails have a limited capacity to survive dry down conditions relative to other Pilids, which can aestivate for several months (Burky et al. 1972, Coles 1968, Little 1968, Meenshaki 1964). However, the ability of Florida apple snails to survive mere weeks to a few months in dry down conditions may be ecologically significant given the rainfall patterns in central and southern Florida. In BCWMA East in 1995 for example, the majority of dry marsh was inundated by heavy rains after less than nine weeks

of dry conditions. Computer simulations of historical hydroperiods in the Everglades system suggest that dry downs were most often limited to 4 to 16 weeks (Fennema et al. 1994). Snails inadvertently left in traps stored dry for several months provide evidence that some snails (e.g., subadults) at certain times of year may have the capacity to survive 12 weeks or more in dry down conditions.

Hydrologic conditions undoubtedly influence survival of apple snails, but our research suggests that vulnerability to desiccation during the dry season is not necessarily a predominate cause of mortality. The survival curve for BCWMA snails in 1995 reflects survival of 51 individuals, only 8 of which were stranded in dry down conditions. The observed drop in survival in BCWMA East may, in part, be related to a general senescence of the population. Hanning (1979) observed an increase in floating dead adult snails during his summer surveys of Lake Okeechobee. The estimated life span for *Pomacea paludosa* is 15 to 20 months (Ferrer et al. 1990, Hanning 1979, pers. obs. in aquaria), and our BCWMA 1995 telemetry data are consistent with that estimate. We observed 38% of BCWMA snail deaths in 1995 over a one week period in June, and question whether senesce alone could account for that high rate of death over such a short period. It may be that older snails are more sensitive to dry-season stresses (e.g., high temperatures measured just prior to the greatest rate of survival decline), but the relative contribution to snail mortality during our field study could not be determined. On Lake Kissimmee, 48% of the stranded snails perished from predation, which precludes drawing conclusions about senescence and/or desiccation tolerance. Laboratory studies of survival as a function of dry down conditions and water temperature are presented in Chapter 5 of this report.

Caution should be exercised in interpreting movement patterns and survival as influenced by environmental and physiological conditions that vary seasonally (White and Garrot 1990), since many of these conditions change simultaneously; for example, photoperiod and temperature increase, water depth decreases, snail reproductive activity increases, and the population ages. Each of these parameters may affect movement patterns and survival. Our two main telemetry surveys (Lake Kissimmee and BCWMA East) included changes in all of these parameters, which makes conclusions about casual relationships difficult, if not impossible, to make. For example, we suspect that interpretation of the snail movement data was confounded by the fact that the majority of our study population eventually died, and likely were moribund for a week or more immediately prior to death. Evaluation of snail data (e.g., Figure 16), especially during the period of greatest mortality, should consider that moribund snails likely move less than robust individuals.

The results from our 1995-1996 telemetry and trapping surveys not only lead us to a greater understanding of apple snail ecology, but also raised questions which we explored in the second phase of research in 1996. In laboratory experiments we controlled for confounding factors such as predation and high temperatures in order to better understand snail survival during the dry season. The results of this effort are presented in the next chapter.

5.0 LABORATORY EXPERIMENTS OF SNAIL SURVIVAL

The results of field studies of two snail populations, BCWMA and Lake Kissimmee, demonstrated that apple snails do not seek out deep water refuge during the course of a dry down. Snails quit moving once water levels fall to approximately 10 cm and eventually become stranded when the water table drops to ground level. Confounding factors in the field studies, primarily predation and rising water levels, precluded quantifying snail tolerance to dry hydrologic conditions. The purpose of the experiments described in this chapter was to quantify apple snail survival in dry season conditions while controlling for confounding factors.

The dry down experiment was designed to emulate typical spring-summer dry season hydrology experienced by the snail population in BCWMA in 1995. We were interested in comparing snail survival in controlled laboratory conditions to survival in field conditions. We also investigated the potential impacts of substrate type and water withdrawal rate on snail survival in the lab. In addition, we looked at survival of snails following recovery from dry down conditions. We were interested in the recuperative capacity of snails and their potential to contribute to snail populations by continuing to grow or mature and therefore potentially reproduce.

High water temperature impacts on snails were also of interest based on observations of high temperatures and corresponding survival declines in BCWMA. We conducted two replicate experiments to determine the temperature that kills 50% of a snail study population.

5.1 Methods

Dry Down Experiment

The dry down experiment was conducted at the Florida Cooperative Fish and Wildlife Research Unit, Three Lakes Field Station (Kenansville, FL). Snails for this experiment were collected from BCWMA East approximately 500 to 1000 meters from our telemetry and crayfish trapping study site (Figure 1). All snails (n=520) were collected via crayfish traps and trap arrays from 29 April through 10 May 1996. Snails were immediately placed in tanks with aerated water, provided food, and held until initial loading for the experiment. Snails were loaded into the 24 experimental tanks on 12 May.

Tank construction

Each test unit consisted of a 120"L x 61"W x 46"H, 285 liter capacity polyethylene tank. During some preliminary work with dry downs in aquaria, we found a false bottom necessary to sufficiently dry the substrate to challenge snails. The false bottom was inserted 3" above the bottom of the tank and was constructed of 1/2" mesh polyethylene screen attached to 3" fiberglass beams. A two-inch layer of stone in a gradation from 1.9 cm (bottom) to 0.6 cm (top) was layered on top of the false bottom to hold the test substrates (sand or peat). The test substrate layer was 5" thick. Test units were outdoors and subject to ambient temperatures and limited sun exposure in a shaded hammock area. Plastic tarps on pvc frames were used to prevent rain from falling into the tanks. The water supply was from a well (pH 7.5, total hardness 3.7 grams per liter). Well water was passed through a 5 um particulate filter, through an activated carbon filter, and into a 950 liter holding tank. The

holding tank water was aerated and allowed to reach ambient temperature prior to distribution to the test tanks.

Animal Loading and Maintenance

Each tank held 255 L of aerated water, providing a 15 cm water depth above the substrate. A total of 250 to 300 grams of snails (20 to 25 snails) was placed in each tank resulting in an approximate loading of 1.1 grams / liter. Snails in the experimental population ranged in size from 25 to 43 mm (34.2 ± 3.0 mm). Fifty-two (10%) of the snails had shell lengths less than 30 mm.

We based our loading and maintenance regime on prior successful routines at our field station and on Hanning (1979) laboratory experiments with breeding snails. In general, however, we replaced water and checked for dead snails more often than in previously described efforts. Dead snails were identified as those floating, and which failed to withdraw into the shell when disturbed. Water was replaced in each tank every 3 to 7 days. Snails were fed unlimited bladderwort or hydrilla. Each tank received the same approximate wet weight of food. Uneaten food and waste were removed every 3 days with a net followed by a siphon pump.

Draw down regime

Twenty-four tanks were placed in two rows of six and one row of twelve and randomized for substrate type and draw down rate. A depth of 0 cm refers to a water depth at the substrate surface. To dry the substrate, the water level fell below the substrate surface.

Twelve tanks contained peat as a substrate, the other twelve contained sand. For each substrate (12 tanks per substrate):

3 Control Tanks: Water depths were maintained at 15 cm above substrate level for the duration of study.

3 Slow Drawdown Tanks: Water was dropped from 15 cm to 0 cm over 28 days (this is the approximate rate of water drop for the 28 day period prior to dry down conditions in BCWMA in 1995). Dry down conditions were achieved on 10 June 1996. One day later, all the water was drained and the substrate was allowed to dry.

3 Slow Drawdown Tanks with Vegetation: The same hydrologic regime as described under slow draw down applies. These three tanks contained vegetation to investigate their potential to enhance survival by retaining moisture. Sand tanks were planted with *Panicum hemitomon* and peat tanks were planted with *Sagittaria lancifolia.* Plants were purchased from Horticultural Systems Inc. (Parrish, FL) and were 30 to 60 cm high when planted. Stems of each plant type were planted with 5 to 8 cm between plants, which resulted in approximately 30 *Sagittaria* plants per peat tank and 100 *Panicum* plants per sand tank.

3 Fast Drawdown Tanks: Water was dropped from 15 cm to 0 cm over 1 day. One day later, all the water was drained and the substrate was allowed to dry. These tanks reached dry down conditions on the same day as the slow draw down tanks.

All tanks containing water were monitored daily to remove dead snails. All snails under dry down conditions were monitored every 6-8 days to check for mortality. [The procedure for identifying dead snails in dry down conditions was described in Chapter 4 under

Methods: Survival]. The status of snails in dry conditions can not be assessed without disturbing the snails; therefore, monitoring was less frequent than for snails in water (which generally does not require disturbing the snails). As opposed to snails in water, dead snails in dry down conditions cannot foul the tank environment (there is no water to foul). We can not be sure how long after death we are able to detect mortality, but we did observe active individuals (just prior to dry down) which died and putrified within the first week of a dry down. Substrate temperature, moisture, and humidity at substrate level (in dry down tanks) were measured three times each week. Humidity was measured using a hand held digital hygrometer (Oakton Instruments, CA). Substrate saturation was measured on a relative scale (0 to 100%) using a soil moisture meter (Lincoln Industries, NE) inserted to 2 inches below the substrate surface. The moisture meters were calibrated for each substrate type using saturated sand and saturated peat as a reference.

Snail Recovery from Dry Down

Snails exposed to dry down conditions for 3 and 7 weeks were replenished with water, first with 4 cm (for 24 hr) followed by 15 cm water depth. The initial 4 cm of water did not completely immerse the snail, permitting aerial respiration without the snail having to immediately crawl to the surface following weeks of "aestivation." We randomly selected no more than 20% of the draw down study population for the recovery study. We took snails from control tanks as well as dry down tanks to account for snail handling in the recovery procedure. To monitor individuals and from which experimental tank they came, we glued 2 mm x 3 mm plastic numbered tags to the recovery snail shells. Tagged snails were placed in

1 of 2 recovery tanks which had a sand substrate, and kept under conditions as described earlier. Survival of these snails was monitored for 8 weeks.

Lethal Temperature Experiment

The laboratory studies on temperature were conducted at the U.S.G.S, Biological Research Division facility in Gainesville, FL in the Fall of 1996. Snails were collected from BCWMA East via crayfish traps. For the initial lethal temperature trial, snails (n=93) were collected from 25 October - 28 October. For the second trial, snails (n= 104) were collected from 1 November - 4 November. Snails were immediately placed in tanks with aerated water, provided food, and held for 3 to 6 days until initial loading for the experiments.

Tank Design and Temperature Control

The temperature experiments were conducted in 30 cm x 61 cm x 30 cm, 57 liter tanks. Tanks were placed on 4 shelves, with 3 tanks per shelf. Temperature regimes were randomly assigned to the 12 tanks. All tanks were enveloped in 3/4" polystyrene insulation on all sides and the bottom. A lid constructed of the same insulating material covered the top of the tanks. We tested 6 temperatures, with two replicate tanks of each temperature. We used 500 watt heaters with adjustable temperature regulators (CLEPCO, Inc). All tanks were aerated so that low dissolved oxygen would not be a factor in survival. Test water was from a well (USGS-BRD facility, North Gainesville). Water was checked daily and adjusted to maintain a water level within 2.5 cm of the top of the tank. With the lid on, this water level prevented snails from crawling up the sides and escaping the high temperatures.

5 Day LT_{50}

Two replicates of 6 temperatures (26°C, 39°C, 32°C, 35°C, 38°C, 41°C) were used to determine the temperature that kills 50% of the study population in 5 days. Our upper temperature limit of 41°C was determined from a rangefinder test in which all snails at 41°C and higher died within 3 days. Snails were not fed during the study to prevent plant material from getting caught on the heaters. Seven to nine snails were placed in each of the 12 tanks. All tanks started at 26°C. We considered test initiation as the point at which we began to raise treatment tanks above 26°C. During the first 24 hours of the experiment, temperatures were raised at a rate of approximately 1.5°C per hour until the final target temperature was reached. Temperature and dissolved oxygen were monitored each day of the experiment. Temperature and DO data were taken from a point at mid- depth in the center of each tank. Temperatures were maintained within ± 1°C throughout each experiment. The first trial was conducted from 7 November - 11 November. The second trial was conducted from 15 November - 19 November.

Analysis

Dry Down Survival

Survival was calculated for each treatment type (n=60 to 65) using a Kaplan-Meier estimator (Kaplan and Meier 1958). Survival curves for each treatment were compared using a chi-square test as described by Pollock et al. (1989).

Lethal Temperature

In the second trial, only one temperature resulted in partial mortalities, so we were unable to perform probit analysis, which is the primary method recommended by the American Public Health Association (1985). The 5 day LT_{50} with 95% confidence limits was therefore determined by the moving average method (Finney 1964, American Public Health Association 1985, Baker and Heidinger 1996).

5.2 Results

Dry Down Survival

Apple snail survival dropped at approximately the same rate (approximately 10% per week in the first few and last few weeks, and up to 50% per week in between) for all treatment types, including controls (Figures 28 and 29). For both sand and peat tanks, control survival does remain 10 to 20 % higher than treatment tanks following the first day of dry down conditions. This difference between controls and treatments within each substrate type was not significant (Table 22). Vegetation did not affect survival in the peat tanks, but in the sand tanks the presence of *Panicum* appeared to result in lower survival (Table 22). Looking at the survival curves for the sand tanks (Figure 28), it appears that this difference may be due largely to survival differences prior to dry down conditions. Draw down rate did not result in different survival for peat or sand tanks when comparing slow draw down tank survival versus fast draw down tank survival. There was a rapid drop off in soil moisture in

Figure 28. Survival of snails in sand tanks. Vertical dotted line indicates the day on which water levels reached substrate level in treatment tanks (fast, slow and vegetation).

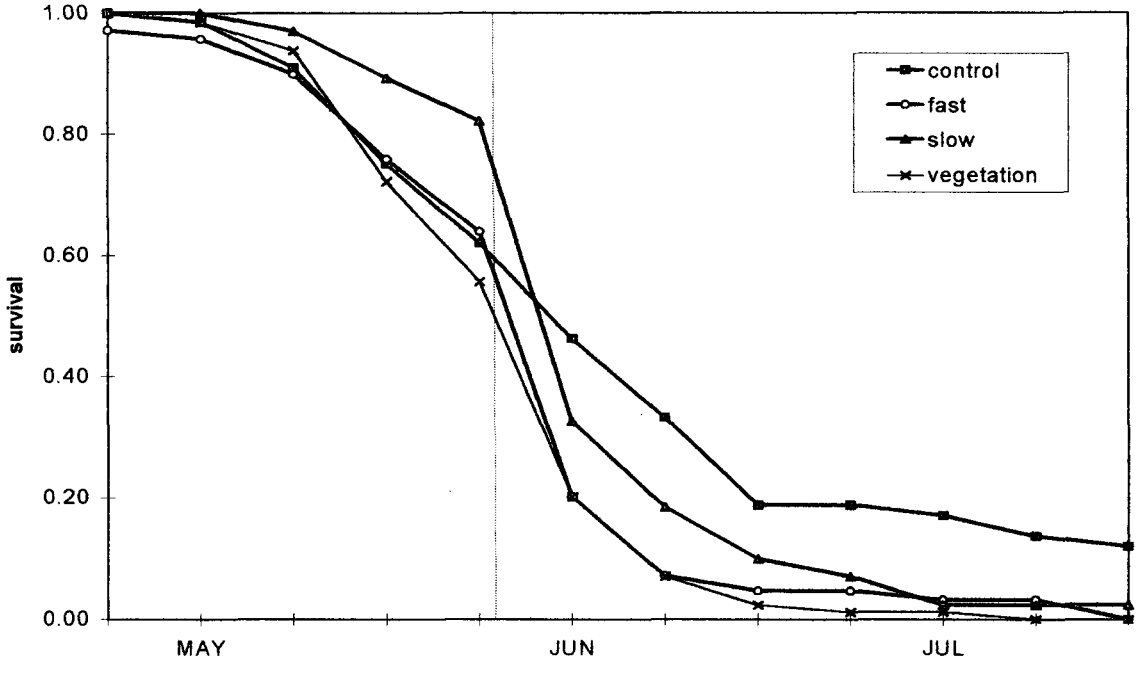


Figure 29. Survival of snails in peat tanks. Vertical dotted line indicates the day on which water levels reached substrate level in treatment tanks (fast, slow, and vegetation).

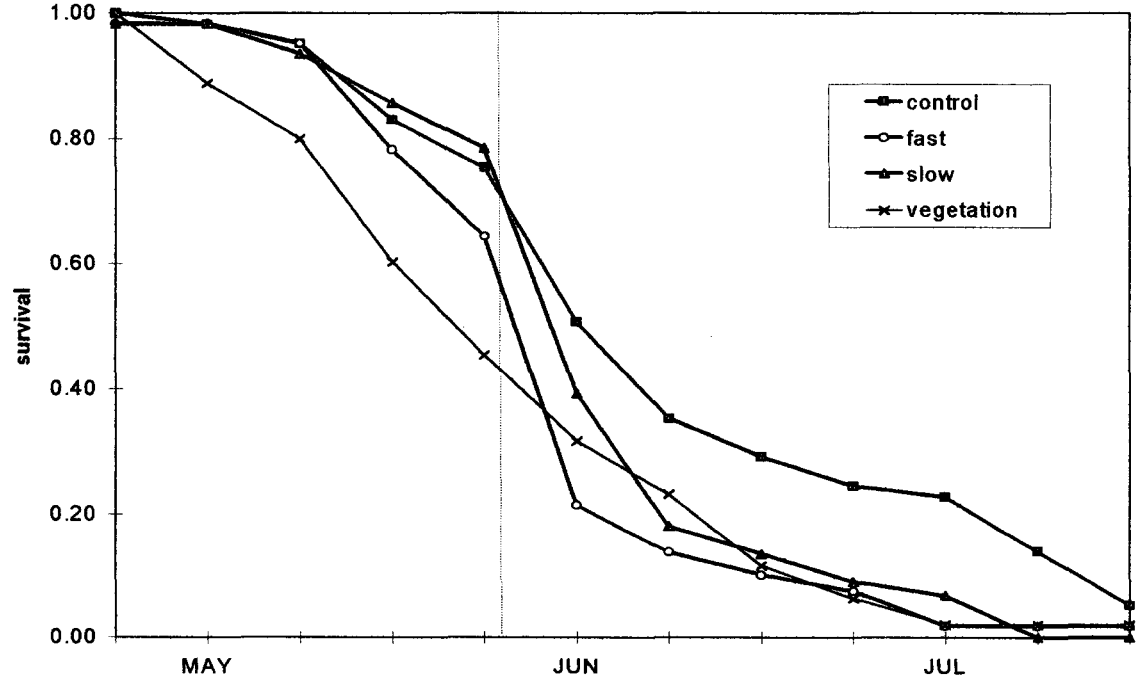


Table 22. Comparisons of survival between controls and dry down tanks (slow draw down tanks with no vegetation), slow versus fast draw down rate, dry down tanks with versus without vegetation, and sand versus peat substrates. For all comparisons (2 survival curves) df=1.

Comparison	$\chi 2$	PR> $\chi 2$
Control vs Slow Dry Down		
sand tanks	0.98	0.20
peat tanks	3.61	0.06
Slow vs. Fast Draw Down		
sand tanks	3.05	0.08
peat tanks	1.72	0.70
Vegetation vs. No Vegetation		
sand tanks	5.84	0.02
peat tanks	1.64	0.30
Sand Draw Down vs. Peat Draw Down	0.06	0.99

sand relative to peat tanks (Figure 30), but survival for snails in dry down sand versus dry down peat tanks did not differ (Table 22).

The survival of snails which were removed from control and treatment tanks, marked, and placed in recovery tanks initially showed the same survival rate as snails monitored in the first 12 weeks of the study (Figure 31). Interestingly, however, recovery snail survival stabilized during the last 4 weeks of the study, during which time no snails died. The ten snails still alive those last four weeks were smaller than the rest of the draw down study population ($T=4.29$, $df= 513$, $p<0.001$). The mean size (± S.D.) of these 10 snails was 30.1 ± 5.9, and we suspect most, if not all, of these snails were young of the year.

Figure 30. Relative substrate moisture in sand and peat dry down tanks following the dry down. Controls for both sand and peat tanks were saturated throughout the study (controls not shown). Moisture scale ranges from 0 (completely dry) to 10 (saturated). Relative humidity ranged from 50 to 90% throughout the study. Sample size ranges from 7 to 9 tanks per substrate. Standard errors shown.

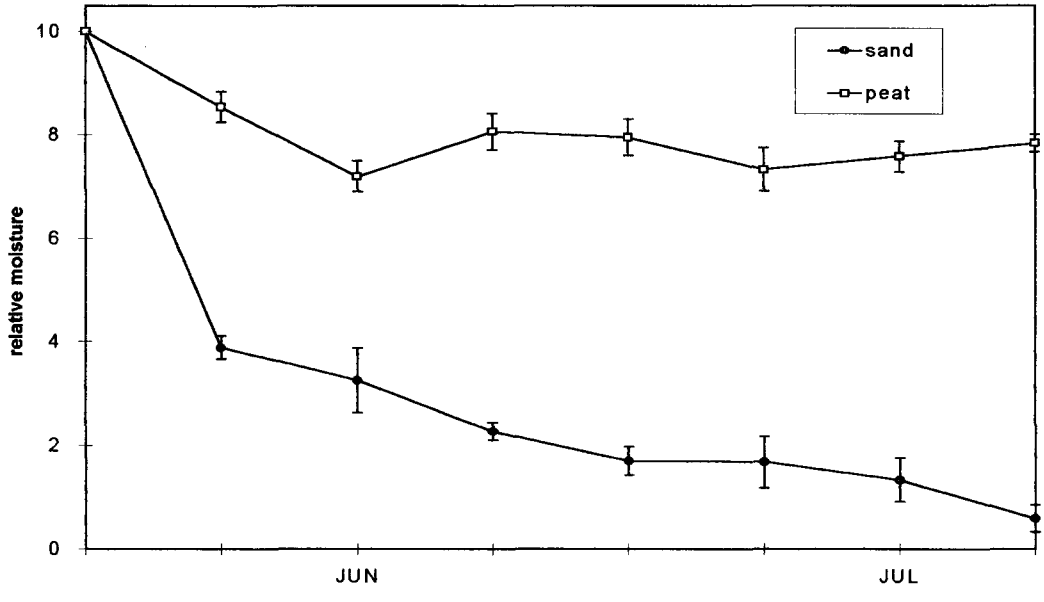
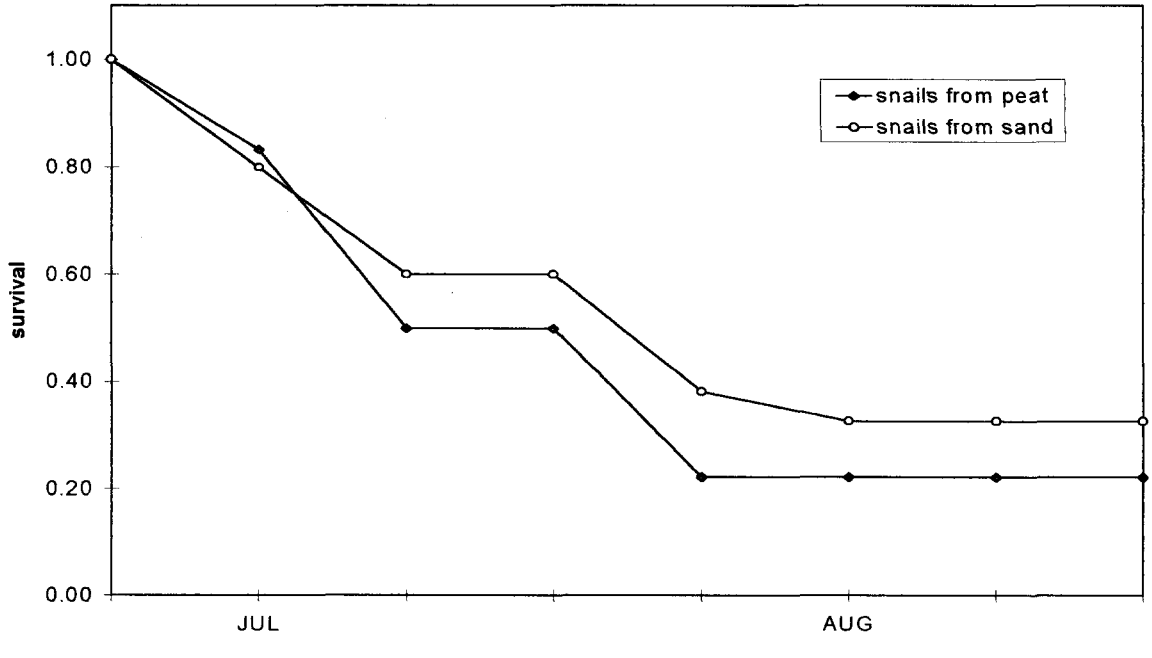


Figure 31. Survival of snails removed from control and draw down tanks and placed in recovery tanks.



Lethal Temperature

The results of the two trials differed substantially, but both studies indicated that water temperatures above 35°C cause extensive snail mortality (Figure 32). The dissolved oxygen in all tanks was maintained at near saturation level for each temperature (Figure 33). The LT_{50} for the first trial was 31.2°C with 95% confidence limits of 30.9 and 31.5°C. The LT_{50} for the second trial was 37.01°C with 95% confidence limits of 36.9 and 37.2 °C.

Figure 32. Median temperatures which killed 50% (LT_{50}) of snails in two study populations in two test trials. LT_{50} were calculated using the moving average method (Finney 1964). LT_{50} with 95% confidence limits for trial 1 indicated by symbol —■—. LT_{50} for trial 2 indicated by symbol —0—. For each temperature n = 15 to 17 snails.

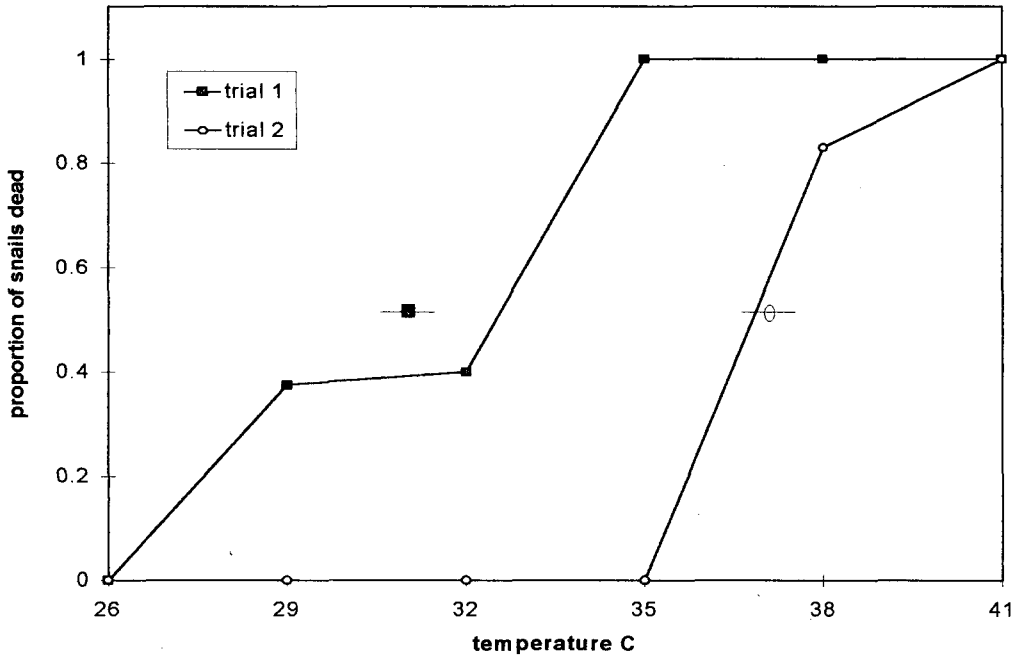
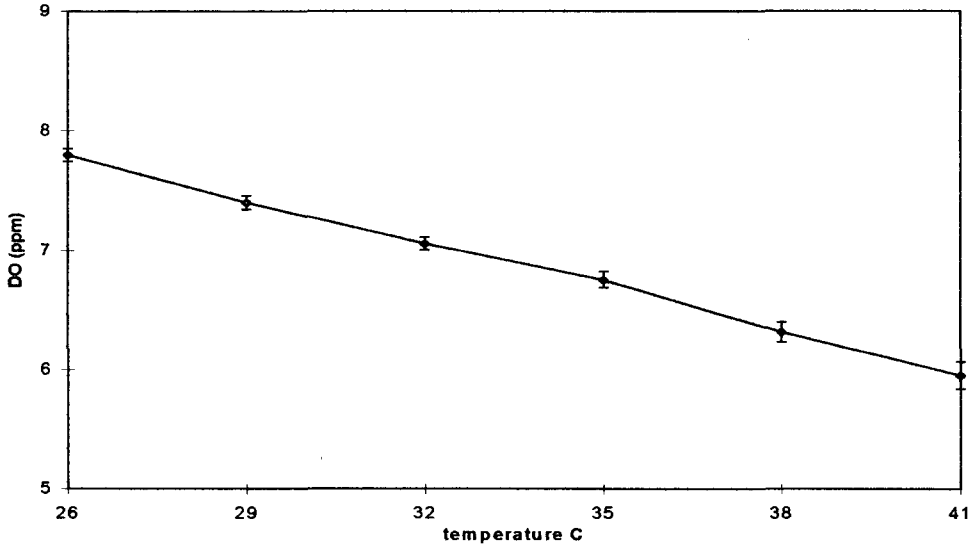


Figure 33. Mean dissolved oxygen levels (DO) for each tank temperature in the LT_{50} trials. For each temperature, n= 8 to 18 (DO was measured daily, but only for tanks with live snails remaining). Error bars are standard errors.



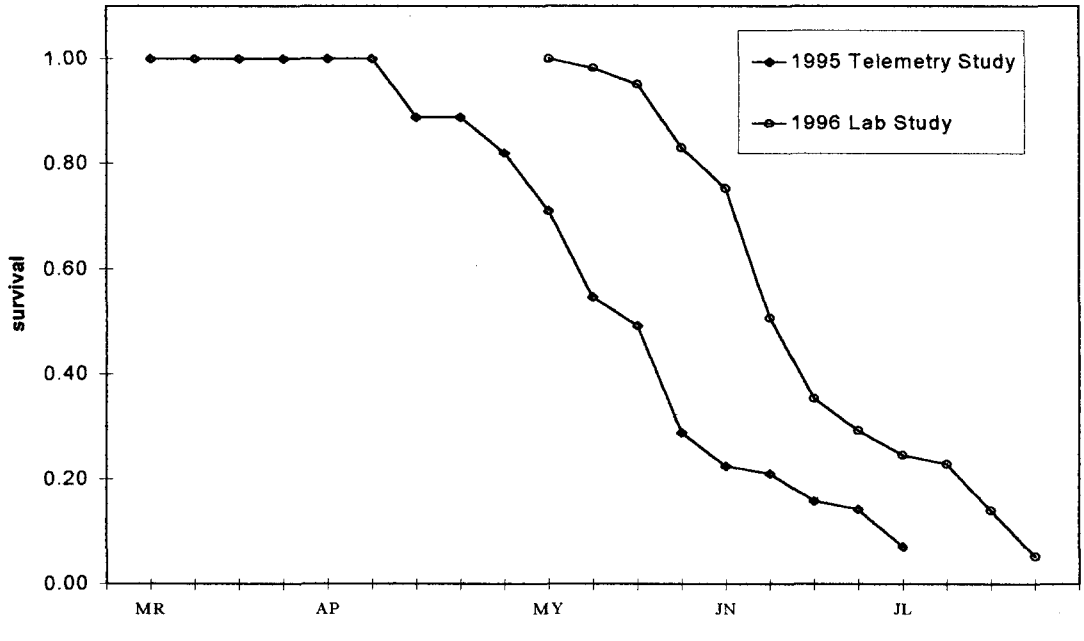
5.3 Discussion

The results of our dry down study concur with earlier reported apple snail tolerance to dry down conditions. Following exposure to dry down conditions, in our case after June 10, 1996, snail survival dropped by 50% or more after just two weeks. Turner (1994) found the same trend in his lab study. Little (1968) also reported a relatively low tolerance for *P. paludosa* to dry down conditions, but provided no survival data. Survival in dry down conditions on the order of a few weeks is considerably less than the months and even years reported for other Pilidae snails (Meenshaki 1964, Little 1968, Burky et al. 1972, Haniffa 1978a).

Our experiment demonstrates a very important caveat to purported tolerances of apple snails to dry down conditions; tolerance is contingent upon age/size or reproductive maturity.

Our study included control snails (always inundated with aerated water), which was not the case for the Turner (1994) and Little (1968) studies. Although control snail survival remained 10 to 20% higher than draw down tanks, in general it appears that regardless of environmental conditions, the adult population crashes over the course of 2 months in spring and summer. This is corroborated by our telemetry field study results. The control survival curves from the laboratory experiment look remarkably like those from adult snails monitored in BCWMA in 1995 (Figure 34). Our lab and field studies confirm earlier unsubstantiated reports that snails live 1 to 1.5 years (Hanning 1979, Ferrer et al. 1990). Given a 1 to 1.5 year life span, and knowing that the majority of egg cluster production for

Figure 34. Survival of snails monitored in the field (BCWMA East 1995) and for snails monitored in the lab study.



any given year occurs between March and July, this means that most overwintering adult snails will die from approximately April through August; this was demonstrated in our lab experiments and field studies (Chapter 4). It is significant that the Turner (1994) desiccation tolerance experiments were conducted in May and June.

Snails that survived in our lab through August (some in dry down conditions for 7 weeks) were significantly smaller than the snails that had perished earlier in the experiment. In another situation described earlier (section 4.2), we inadvertently left snails in traps under dry storage conditions, and these snails survived for 12 weeks. These snails were juvenile sized snails. We believe that snail size and/or snail reproductive condition dictates tolerance to desiccation. In the last four weeks of the lab study, these smaller snails exhibited 100% survival, indicating that they can fully recover from dry down conditions and potentially complete their life cycle through reproduction. Our observations of a rapid recovery of snails from dry down conditions were consistent with those of Meenshaki (1964) for Indian apple snails.

Our interest in the potential impact of high temperatures on adult mortality evolved from observations of 33°C to 38°C water temperatures in BCWMA in May 1995. Given our understanding of adult survival obtained from the telemetry and lab studies, we now realize that high temperatures likely were not the primary cause of snail deaths during the week immediately following our highest recorded water temperatures in BCWMA in 1995. Post-reproductive snails were likely dying from senesence during this period, but high temperatures may have exacerbated mortality in these snails.

The laboratory temperature study results support our hypothesis that high temperatures in the BCWMA may have made some contribution to snail mortality. A median lethal temperature for *Pomacea paludosa* of 31 to 38°C is consistent with that for other Pilidae snails (Meenakshi 1964, Burky et al. 1972, Frieburg and Hazelwood 1977, Haniffa 1978b). However, our lab experiments were conducted during the fall season, and therefore should be considered preliminary to understanding the impacts of temperature on snail populations in the spring-summer dry season. Snails and fish which are acclimated to higher temperatures have a higher temperature tolerance than conspecifics acclimated to cooler temperatures (Skoog 1976, Elliot et al. 1981, Baker and Heidinger 1996), so the LT_{50} could be higher for snails acclimated to typical spring water temperatures. We also did not apply a fluctuating temperature regime in the lab studies (due to the expense and difficulty of replicating temperature profiles), which would have more accurately reflected field conditions. Given that snail tolerance to dry down conditions appears to be based on age or reproductive conditions, we suspect that tolerance to high temperatures may also depend on the life stage of the snail.

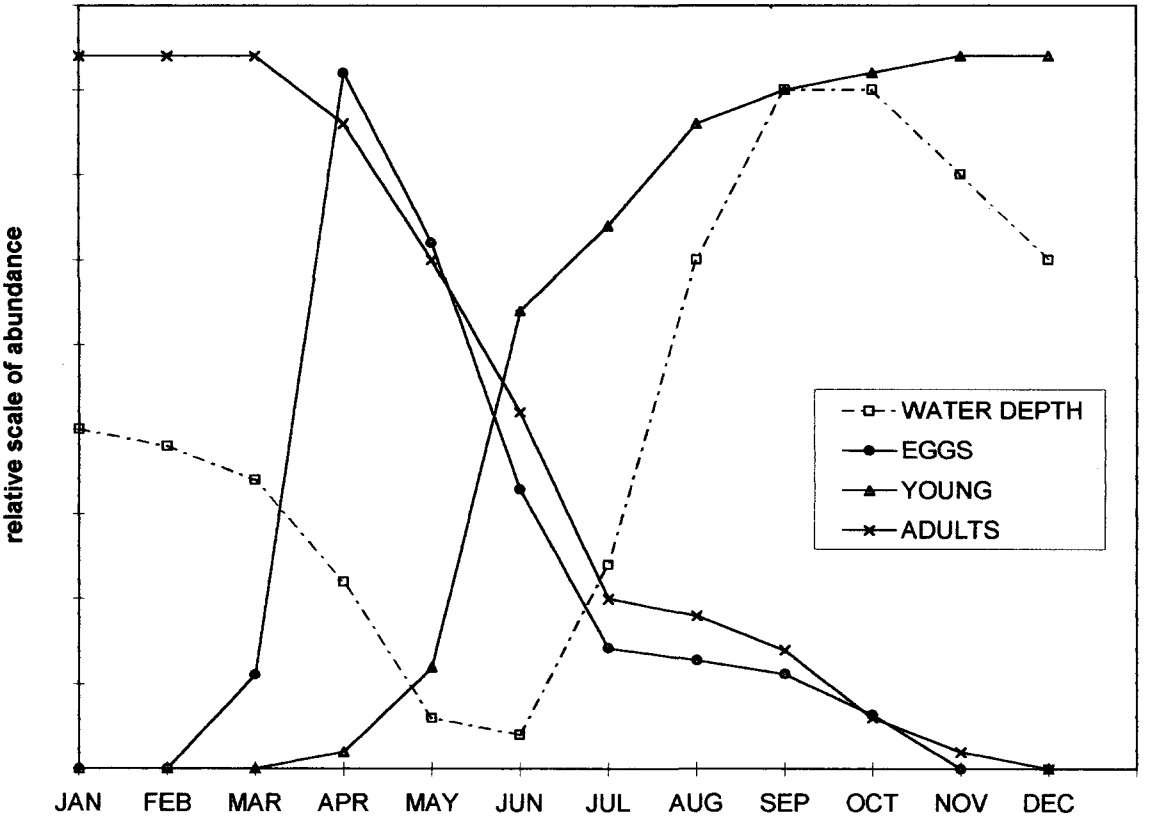
6.0 SYNTHESIS

Like the wetland environments in which they live, Florida apple snail populations complete a dynamic cycle on an annual basis. This synchronous flux in snail populations and hydrology poses a challenge for research and has strong implications for water management regimes designed to balance human needs and the integrity of wetland systems.

From season to season, and at times within a few weeks, snail populations undergo discrete transitions from eggs to juveniles, juveniles to reproductive adults, and finally reproductive adults to short lived post-reproductive adults (Figure 35). Our findings are consistent with earlier estimates of a life span of 1 to 1.5 years for apple snails (Hanning 1979, Ferrer et al. 1990). What we have elucidated is the relationship between reproductive activity, survival, and the timing of post-reproductive die-off, which occurs over an unexpectedly small window of time each year. For most of the year, it appears that just one or two of these life stages dominate. For example, reproductive adults dominate the population from January until June, at which time these adults undergo rapid senescence and disappear from the population. They are gradually replaced by young of the year, whose rapid early growth brings them to adult size within 2 to 4 months. This period of turnover (i.e., April through August), constitutes the greatest time of flux in the population, and coincidentally has been the period within which the bulk of our research and that of others (e.g., Turner 1994) has been conducted. It is imperative that the interpretation of data from dry down studies and different sampling protocols account for snail population dynamics.

A better understanding of snail life history has enabled us to explain some of the issues associated with determining apple snail density or relative abundance (Objective 1, as

Figure 35. Relative abundance of egg clusters (EGGS), young of the year snails (>25 mm)(YOUNG), and overwintering adult snails (ADULTS) as related to water depth for a one year period. Egg cluster curve based on BCWMA East 1996 data (this study), Odum (1957) and Hanning (1979). Young of the year snails (25 mm and larger) based on oviposition rates in BCWMA 1996 (this study), an incubation period of 22 days for eggs (Hanning 1979) and 2 month growth period to reach ≈ 25 mm (Hanning 1979). Survival of overwintering adults based on BCWMA 1995 telemetry data and draw down experiment (this study). Relative water depths are based on measurements taken in BCWMA East in 1995 and 1996 (this study). The relative abundance of snails assumes no deaths due to predation, disease or dry down conditions. *This graphic is based on the best available information, but the trends may appear different as more information becomes available.*



stated in Chapter 1). Techniques for sampling apple snails thus far have focused on snails of a certain size range (i.e., 25 mm and above). As stated earlier, it appears that particular size classes dominate the population from season to season. The labor and length of time required to complete a density assessment using throw trap techniques may, as it did in our studies, span the transitional periods where size dominance shifts in a snail population. If site to site comparisons are made in the spring and summer, it is imperative to control for time effects because of the rapid disappearance of post-reproductive snails. If different sites were sampled even two weeks apart in the spring and early summer, the results may reflect a difference in post-reproductive adult survival. For example, using Figure 35 as a reference, if a population of adult snails sampled in April were compared to a second population sampled in May, the second population may appear to have a lower density when in fact the survival of both populations dropped by 25% from April to May.

The demographic issues just described are one reason we recommend the use of mark-recapture techniques to determine snail density. Mark-recapture data provide information on survival, capture probability, and transients which are incorporated into models that provide a reliable density estimate. We recognize that mark-recapture studies may not always be feasible, and that throw traps may fulfill the needs of some studies. When throw trap sampling is used, we highly encourage the use of blind recovery studies to account for potential site differences in sampling efficiency that could be misinterpreted as a difference in snail density. This is more an issue of how habitat structure, rather than demographics, affects recoveries. However, as illustrated earlier by example, there could be a problem with demographic variability if throw trap sampling encompasses a shift in size

class dominance. We recommend that studies of snail populations, regardless of sampling technique, include shell length measurements of all snails sampled in order to help identify the contribution of juveniles relative to adults (there is a gray area between about 25 and 35 mm). With regard to throw traps, recovery tests for snails over the entire size range encountered should be conducted, because smaller snails would, we hypothesize, be harder to detect than larger snails. We did not test this hypothesis during the research reported herein, but this seems reasonable based on our experience with identifying snails among the vegetation and peat extracted from throw traps.

Density assessments using movement-based trap techniques are sensitive not only to the age/size structure of the population, but also to changes in snail behavior. We have evidence that snail movement patterns reflect the reproductive activity of adult snails (Chapter 4). These behavioral and surival effects likely contributed to the lack of a relationship between crayfish trap snail catch and snail density (Figure 6). We had success with trap arrays during fall sampling efforts, but their utility is limited to prairie and slough habitat. Controlling for time effects is crucial, since we do not yet understand the relationship between snail movements and the number of snails captured in trap arrays.

Temporal variation in egg cluster production proved problematic for using egg clusters as an index of snail abundance (Figure 11). Hanning (1979) found variation in egg cluster production between sites within the same system sampled at the same time, which further compromises their utility as an abundance index. Unfortunately, discrediting egg clusters as an index of abundance leaves only more labor intensive options for surveying snail populations. However, efforts to understand snail ecology are ill served by unreliable

estimates of snail abundance, and we encourage investigators to employ techniques which account for habitat and demographic variability, such as throw traps with blind recovery tests or preferably, mark-recapture grids.

While testing throw trap and mark-recapture trapping techniques we were able to draw some conclusions about the distribution of apple snails in graminoid marshes (Objective 2). Apple snails were found in all habitat types encountered during our research which included sawgrass, prairie, slough, and cattail. Densities among all sites sampled ranged from approximately 0.15 snails/m^2 to 1.0 snails/m^2. No consistent pattern of distribution among habitat types was observed in 5 sites sampled in WCA3A, although it appears that higher densities of snails are more likely to be found in prairie or cattail habitats in some sites (Figure 3 and Figure 10). Our egg cluster surveys and snail density assessments revealed that snails do inhabit densely vegetated areas (e.g., interior sawgrass and cattail habitats). Observations of snails with transmitters routinely moving in and out of sawgrass patches are consistent with the conclusions drawn from egg cluster counts and snail density assessments. These observations contradict earlier reported statements that snails have difficulty penetrating dense stands of vegetation and that habitat use is clearly skewed to more open habitats (Owre and Rich 1987, Turner 1996). Our results do not dispute the importance of the prairie/sawgrass or slough/sawgrass ecotones as being critical for oviposition (Figure 13) (Bennetts et al. 1988, Turner 1996). We would simply add that the interior of sawgrass and cattail plant communities should also be recognized as important habitat to apple snail populations. We agree with Turner (1996) that most favorable snail habitat would likely include a mosaic of densely vegetated and sparsely vegetated habitats

within a wetland system. Based on our experience with site to site variability in the habitat distribution of snails, we anticipate that a considerably greater effort will be required to make generalizations about snail distribution in different habitat types. Understanding snail use of habitat types remains an important issue related to natural resource management practices that affect the plant community (e.g., hydroperiod, fire, aquatic weed control, nutrient loading), and most certainly in turn, apple snail populations.

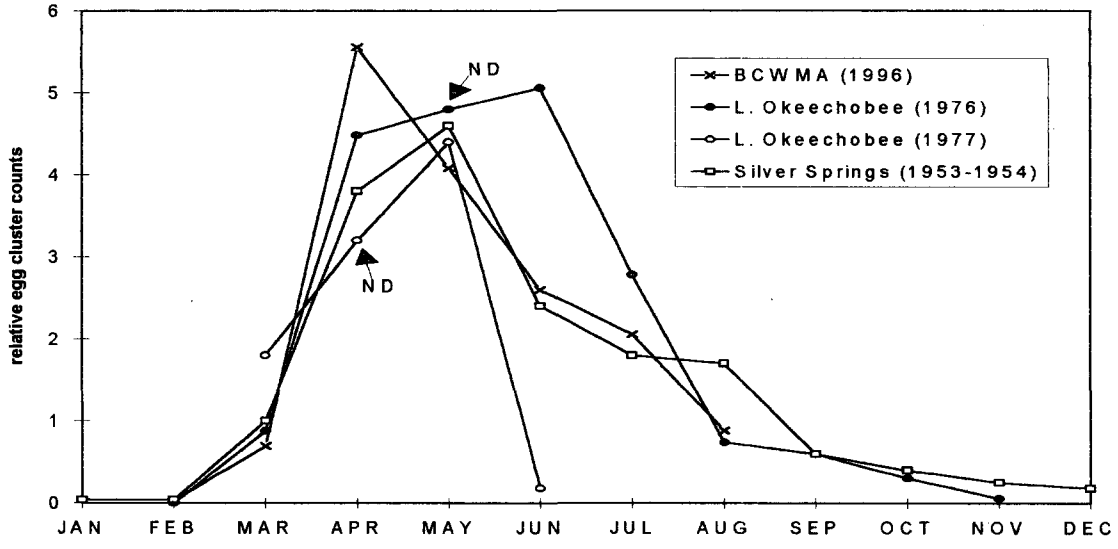
Realization of the seasonality associated with snail population structure (Figure 35) and behavior has an even larger impact on the way we interpret snail behaviors (Objective 3) and survival (Objective 4) during the dry season. We were able to attribute the movement patterns of male and female snails during this time of year to the breeding season, rather than to hydrologic conditions. We did, however, identify a hydrologic threshold for movements of approximately 10 cm water depth. Survival data interpretation was impacted considerably by the coincidental breeding and dry season. Because our collection procedures and the telemetry technique were limited to snails 25 mm or larger, our laboratory and telemetry studies of survival targeted adults, specifically reproductive and post-reproductive adults (we collected nearly all of our snails for the telemetry and lab survival studies in March, April and May). The same scenario impacted the results of a previously reported lab study (Turner 1994), wherein it was concluded that adult apple snails possess little to no tolerance for dry down conditions. In hindsight, it was fortunate that the BCWMA 1995 dry down was not extensive, because the decline in survival of snails in water revealed the rapid disappearance of post-reproductive adults regardless of hydrologic condition; we were able to corroborate this with our lab study. Based on our observation that snails surviving 7 to 12 weeks in dry

down conditions were subadult snails (i.e., less than 30 mm shell length), we believe that tolerance to dry down conditions varies with the size and/or reproductive status of the snail. We plan to test this hypothesis in 1998-1999 via another series of laboratory dry down experiments.

Understanding the relationship between dry down survival and snail physiological status will provide critically needed information about the impact of the timing and duration of dry downs under the control of water management districts. However, we already have information on the potential impacts of hydrologic regime on another critical factor regulating snail populations: recruitment. Our egg cluster surveys in the BCWMA in 1996 (this study), as well as earlier surveys done by Hanning (1979) on Lake Okeechobee and Odum (1957) in Silver Springs, reveal that peak egg cluster production consistently occurs between March and July, and the majority of eggs are laid over a 4 to 12 week period (Figure 36). Dry downs which encompass the period of time associated with peak reproduction and subsequent adult die off may reduce or eliminate recruitment in the effected area. We observed an 80% reduction of the Lake Kissimmee snail population following a habitat restoration effort which left the majority of the littoral zone dry from February through mid-June (Darby et al. 1997). During the Fall 1995 pre-draw down population assessment, 32% of the 1995 snail catch was juveniles (< 25 mm shell length) versus 3% in the Fall 1996 post-draw down assessment (Darby et al. 1997). These data suggest that recruitment was greatly reduced by the spring dry down conditions.

Our study results, coupled with earlier reports containing information on apple snail ecology (Odum 1957, Hanning 1979, Turner 1996), may offer an explanation of apple snail

Figure 36. Temporal variation in apple snail egg cluster production from three areas in Florida. Lake Okeechobee data is from Hanning (1979), Silver Springs data is from Odum (1957), and BCWMA data is from this study. Different survey techniques were used to sample egg clusters in these three studies, so the data from Hanning (1979) and Odum (1957) were transformed by multiplication with a constant in order to place the data on the same scale. ND refers to no data; these data points were extrapolated to complete the graph and therefore may not accurately reflect the actual peak production.



life history strategy that has important implications for water management during the dry season. It has been suggested that the reproductive strategy of apple snails evolved to deal with a combination of predation pressure, low dissolved oxygen, and the annual dry season (which may or may not result in dry down conditions) (McClary 1964, Andrews 1965, Aldridge 1983). Eggs laid on emergent vegetation avoid aquatic predation and low dissolved oxygen conditions (Andrews 1965, Turner 1996), which are typical in freshwater wetlands (Mitsch and Gosselink 1993). Submersion of eggs has been found to delay development and decrease embryo survival (Turner 1996). It seems reasonable, therefore, that apple snails would lay eggs during the year when water levels are most likely to be stable or falling, and

this would be the dry season. As invertebrates, the higher metabolic demand of reproduction requires increasing environmental temperatures, which may explain why egg clusters are not found from December through February, months included in the central and south Florida dry season (Chen and Gerber 1990, Duever et al. 1994). Instead, highest production occurs in April through June, when water levels are typically falling and ambient temperatures are rising. Elevated temperatures also decrease incubation time and enhance hatching success for apple snails (Hanning 1979). The combination of falling or stable water levels and rising temperatures is consistent with oviposition data that reveal the majority of egg production occurrs in March through June.

The large eggs deposited by apple snails produce shell bearing hatchlings of about 4 to 5 mm after 20 to 25 days of incubation. These hatchlings reach 25 mm in just 6 to 8 weeks (Hanning 1979). We believe rapid early growth enables snails hatched in March and April to reach sufficient size (we estimate "sufficient size" to be 15 to 25 mm) to survive dry down conditions, which occur most often around May. Rapid juvenile growth in order to reach sufficient size to handle a dry down has been hypothesized for other Pilid snails (Burky and Burky 1977, Haniffa 1978b). Our laboratory study results indicated that smaller snails (most likely juveniles) have the capacity to survive 2 to 3 month dry downs. Hydrologic models developed for south Florida indicate that most dry down occurrences under natural hydrologic conditions would be 3 months or less (Fennema et al. 1994). Although sufficient data has not yet been collected to substantiate our hypothesis, we believe that the reproductive strategy of apple snails is well adapted to periodic dry downs which occur in late Spring.

Although dry downs exceeding 3 months in duration (our best estimate thus far) would substantially depress apple snail populations, the timing of dry downs may be just as critical. Alterations to the timing of dry downs in the last 50 years have resulted in some areas going dry earlier than would occur naturally (Davis et al. 1994, Light and Dineen 1994). Suspected declines in snail abundance in some wetland systems may have as much to do with dry down impact on recruitment (an issue of dry down timing) as it does on survival (more an issue of dry down duration). Earlier than normal dry downs which encompass a substantial portion of the March - June peak in oviposition could nearly eliminate an entire year's recruitment. We do not suggest that dry downs be avoided, only that water management regimes consider their timing as well as duration.

Our research has revealed the importance of understanding how snail population dynamics can affect interpretation of study results, and how the timing of hydrologic changes affects survival and recruitment in snail populations. Balancing the distribution of water for human use and for maintaining sustainable snail populations may narrow down to a few weeks or months late in the dry season. The critical issue for sustaining apple snail populations, therefore, is not whether dry downs occur, but rather when and for how long they occur.

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