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1. **Project Title:** Life histories of the northern longear sunfish (*Lepomis megalotis peltastes*) and pugnose shiner (*Notropis anogenus*) in Minnesota, with examinations of other rare non-game fishes
2. **Project Period:** May 14, 2010– June 30, 2012

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SUMMARY

The northern longear sunfish (*Lepomis megalotis peltastes*) was recognized by the Minnesota DNR as a Species in Greatest Conservation Need due to its perceived spotty Minnesota distribution and its threatened status in Wisconsin. Except for the preliminary data on distribution and abundance gathered by Porterfield and Ceas (2008), nearly all other life history data comes from studies that were conducted outside of Minnesota on stream-dwelling populations (Cooke and Phillip, 2009), versus the lake-dwelling populations that we find in Minnesota. The pugnose shiner (*Notropis anogenus*) is listed as a Species of Special Concern in Minnesota, as a Threatened Species in Wisconsin, and as an Endangered Species in Ontario. Very little is known about this species. This study, funded by the Minnesota State Wildlife Grant program examined the life history of lake-dwelling populations of both species.

Northern longear sunfish (LES) were quite restricted in the lake habitat they used. Characteristics of the lakes where we found LES are : clear water (an average secchi disk reading of 9' or greater), shorelines that have relatively undisturbed stretches of emergent aquatic plants, extensive shallows (< 3' depths), and a firm substrate that was usually rich with organic detritus and submerged plants. However, adult LES were not present in the shallow shoreline waters after ice-out until aquatic plant regrowth had begun. LES remained in the shallows during the warmer months of the year (mid-May – August), but once the aquatic vegetation began to die back in late Summer/Fall the fishes disappeared from the shallows. Juvenile sunfishes (generally < 30 mm) were found scattered along the vegetated shorelines, and were generally associated with areas of thick bulrush beds or dense muskgrass. Shoreland development, including the presence of boat docks/piers, did not appear to be a limiting factor compared to the large-scale removal of aquatic vegetation (or alteration of the aquatic vegetation by extensive nutrient input or soil erosion). Otolith analysis revealed that, on average, about six growing seasons are required for northern longear sunfish to reach 100 mm total length (TL). During the summer spawning season the distinctive saucer-shaped nests could be found in clearings within the plant beds, often right next to the shoreline, at depths of 6-36 inches (15-92cm). Larger/older individuals (Age 4+) tended to move into the shallows first and spawn in June. Once these older individuals left the breeding ground then the younger/smaller individuals moved in to spawn. We did not observe colonial nesting or cuckoldry, as has been reported for some riverine populations (Dupuis and Keenleyside, 1988), but nesting densities were quite low when compared to that riverine study. Stomachs of LES contained mostly Amphipods (Scuds – *Gammerus spp.*), and caddisfly (Trichoptera) larvae in 2010, and Amphipods and midge (Chironomidae) larvae in 2011, and on a given sampling date these prey were often consumed by most LES examined. When looking at trends, females and males had similar diets. As expected, LES would actively take prey from the water column and from the sides of plants and rocks. A different feeding behavior had the LES using its lower jaw to frequently scoop up substrate and we assume that this technique is used to capture scuds. Through stable isotope analysis we noticed a shift in feeding location from the pelagic to littoral zone, which correlates with the appearance of LES in the shallows in mid-May. The trophic feeding position did not change significantly throughout the summer, indicating that as LES moved into the shallows they did not change where they were feeding on the food chain.

Pugnose shiners (PNS) were also were quite restricted in their habitat use, requiring clear water lakes with shorelines that have relatively undisturbed stretches of emergent aquatic plants. Fishes were found in May in deeper (4-6') water, which corresponded to 15-30m from shore. As the summer progressed, and as the aquatic vegetation grew thicker in the shallower waters, pugnose shiners were then found closer to shore in waters 3-4' deep. Unlike LES, however, pugnose shiners were rarely found in the very shallow waters (1-2' deep) immediately adjacent to shore, which can make sampling via backpack electrofishers problematic. PNS were always associated with dense aquatic vegetation, and were not found in open waters. As with the LES, pugnose shiners were found in the shoreline shallows from approximately mid-May to mid-October. The results of the stable isotope data suggest that as April turned into May the fishes were migrating from the deeper portions of the littoral zone into the shallow portions. In the field and in aquaria pugnose shiners are an extremely wary/skittish fish, which made

behavioral observations difficult, and spawning was not observed. Females of Age 1 (35mm TL Ave. length) contained abundant Mature/Mature Ripe ova by mid-May in 2010, 2011, and 2012 and were believed capable of spawning. Age 2 males were sexually mature by mid-May. The testes of Age 1 males became mature by early July, and thus Age 1 males were potential spawners during late summer. Snelson (1971) hypothesized that pugnose shiners were herbivorous given the elongated intestine, but Suttkus and Bailey (1990) stated that the serrations on its pharyngeal teeth may be an adaptation to feeding on microcrustaceans. Our observations on a much larger data set demonstrate that pugnose shiners feed on both microcrustaceans and filamentous green algae, and that there are times when the species feeds almost exclusively on specific prey items. Stomachs of PNS contained mostly Ostracods, filamentous algae, water mites (Hydracarina), and Cladocerans, with Ostracods making up the majority of the diet overall, but there were a few sampling periods when the stomachs of pugnose shiners would contain large quantities of filamentous algae or water mites. Juvenile pugnose shiners were not found in the study areas. During spring and summer 2013 when we return to Fish Lake to capture and observe spawning individuals, we will also make a conscious effort to find juveniles and fill in the gaps in the early life history of the species.

INTRODUCTION

NEED AND JUSTIFICATION FOR THIS STUDY

Life History Studies – Minnesota's State Wildlife Action Plan referred to as the Comprehensive Wildlife Conservation Strategy (CWCS, 2006) lists three Priority Conservation Goals. Priority Conservation Goal II Strategy IIB recognizes in part the importance of "researching the life histories of some SGCN, or groups of SGCN, that are closely tied to key habitats" and that such life history data "may provide essential information for management" of the species and habitats.

Northern Longear Sunfish (LES). Prior to 2006 the northern longear sunfish (*Lepomis megalotis peltastes*) was recognized by the Minnesota DNR as a SGCN due to its perceived spotty Minnesota distribution and its threatened status in Wisconsin. More recent publications (Cooke & Phillip, 2009; Page and Burr 2011) have elevated this taxon to full species status as *Lepomis peltastes*, but we will continue to refer to it as *Lepomis megalotis peltastes* during this project to avoid confusion within the DNR.

Our recent studies focused on CWCS Goal I, Strategy IA (identify key SGCN habitats), Goal II Strategy IIA (survey SGCN populations and habitats), and Goal II Strategy IIB (research important aspects of SGCN habitats). We determined the species' range and confirmed its extremely spotty distribution, relative abundance, population genetics, and basic habitat requirements (Porterfield and Ceas, 2008). Most importantly we determined that the species is quite habitat-specific and dependent on a high-quality near-shore littoral zone (e.g., it can be abundant around boat docks of "clean" lakes, but only if the submerged aquatic vegetation has been left relatively intact and has not been "clear-cut" by weed rollers, etc.). We recommended that it be listed as Special Concern in Minnesota and noted that continued habitat degradation would necessitate its listing as Threatened.

As partial fulfillment of Goal II Strategy IIA we have mapped the locations of known LES spawning beds in the survey lakes and have made estimates of LES relative abundance. However, we have only the beginnings of a true understanding of its life history requirements. During our previous sampling efforts we preserved specimens from representative lakes throughout the LES's range in Minnesota, to begin to conduct a detailed life history study. From this sampling we have determined some basic information regarding growth rates and food habits of LES (M. McInerny, MNDNR, pers. comm., 2010) however we are lacking other basic life history information such as reliable estimates on the length and timing of its spawning season. Nearly all other life history data known for LES comes from studies that were conducted outside of Minnesota on stream-dwelling populations (Cooke and Phillip, 2009), versus the lake-dwelling populations that we find in Minnesota. Our research will be the first attempt to examine the life history of lake-dwelling populations of the species, and will be the first life history study of the species in MN.

Pugnose Shiner (PNS). The pugnose shiner (*Notropis anogenus*) is listed as a Species of Special Concern in Minnesota, as a Threatened Species in Wisconsin, and as an Endangered Species in Ontario. One of the issues that confound our ability to improve PNS populations is that very little is known about this species besides that it spawns during the spring (Eddy and Underhill, 1974; Craft et al., 2006; New York State Department of Environmental Conservation Fact Sheet 2012) and there has never been an adequate life history study conducted on this species. We hope that our research will expand upon the life history of this distinctive little fish and improve our ability to manage their populations into the future. Within the past two months we became aware, courtesy of K. Schmidt, of an unpublished report (Doeringsfeld, 1993) that details some of the life history traits of the pugnose shiner in Fish Lake, LeSeuer County, MN (one of our study sites). To further our knowledge of the biology of the pugnose shiner we have incorporated information from that unpublished report into this report.

Goals and Objectives of this Study

Our objective is to develop a clear understanding of the life histories of the northern longear sunfish and pugnose shiner, which will then directly help us to manage both the species and their aquatic habitats. The essential metrics for a life history study of nongame fishes are well known and are the basis for the objectives below.

1. Determine habitat requirements and seasonal movements of northern longear sunfish and pugnose shiner.
2. Document spawning period, habitat, behavior; reproductive cycle; age/size at sexual maturity, and estimated fecundity for northern longear sunfish and pugnose shiner.
3. Document the population ecology of northern longear sunfish and pugnose shiner including a description of larvae; development & growth; age composition & density; and longevity.
4. Document the food habits of the northern longear sunfish and pugnose shiner.

Study Areas

Prior to the commencement of the project we had identified a number of potential lakes that could serve as study areas. Once the field season began we initially targeted Cameron Lake, ca. 4.5 mi south of Scenic State Park, Itasca County (DOW # 31-0544). In visits prior to 2010 we found both target species as well as blackchin shiner, blacknose shiner, banded killifish, and least darters. The shoreline is undeveloped with undisturbed habitat, however, the access road to the lake was in very poor condition and limited our opportunity to sample the lake and therefore we abandoned this lake as a viable study lake for this project.

In life history studies of nongame fishes, the project is traditionally focused on examining fishes from one defined site (e.g., Johnston and Haag, 1996). We decided to sample two sites/lakes per species in hopes of seeing if there were any differences in life history traits among sites. We further supplemented this information by traveling to additional lakes throughout the state to make brief observations on the target species at those lakes, in an attempt to determine whether our findings could be used to generalize among populations across the state.

Northern Longear Sunfish Study Sites - Turtle and Movil Lakes

Turtle Lake (DOW# 04-0159) and Movil Lake (DOW# 040-152) are located within seven miles of the City of Bemidji (Figure 1). They are part of a chain of eleven lakes known as the Turtle River Chain and are in the Mississippi River Headwaters Watershed. Turtle Lake flows directly into Movil Lake at the Hwy 22 bridge crossing and along with many of the lakes in the Turtle River Chain, are managed with a special regulation for northern pike (24 to 36-inch protected slot limit).

Turtle Lake is a 1,436-acre lake (718 acre littoral area) with a maximum depth of 45 feet. About half of the shoreline is undeveloped, with lake homes and small seasonal resorts present on the remaining shoreline. The sport fish community of consists of walleye, northern pike, panfish (including black crappie), largemouth bass, yellow perch, sucker/redhorse and bullhead species (MNDNR 2011).

Movil Lake is a 923-acre lake (513 acre littoral area) with a maximum depth of 50 feet. There are a fair number of lake homes surrounding this lake, perhaps lining 2/3 of the lake perimeter. There is no public water access on Movil Lake, so boaters must access from Turtle Lake, which is directly upstream and connected by a navigable river channel. The sport fish community of Movil Lake consists of northern pike, walleye, panfish (including black crappie), largemouth bass, yellow perch, tullibee, redhorse/sucker and bullhead species (MNDNR 2011).

The two study areas are located across Hwy 22 from each other, to the east of the Hwy 22 bridge, which made field observations and sampling much more efficient. The Turtle Lake study site (Figure 2a) is located on the southern shore of Turtle Lake, quite well protected from W/SW/S/SE winds. There is a small family-run cabin resort that includes a series of boat docks and a small swimming beach. With the exception of the swimming beach, much of the aquatic vegetation is left fairly well intact so that there is a nice complement of wild rice, bulrush, muskgrass, narrow-leaf pondweeds, coontail, and white water lily. The owners of the resort (White Pines Resort, Jerry and Rebecca Hummel) are very proactive in the protection of the aquatic vegetation within the shallows because they understand the importance of such vegetation not only to shoreline erosion but also supporting robust fish populations. Their assistance helped greatly with sampling this site.

The Movil Lake study site (Figure 2b) is located on the northern shore of Movil Lake, and is quite well protected from NW/N/NE/E winds. There was once a small family-run resort located here as well, but the resort has been closed since at least 2006, and the remaining main lodge and cabins were removed in 2009. There are no boat docks or other artificial structures present. Wild rice, bulrush, muskgrass, narrow-leaf pondweeds, coontail, white water lily, and other aquatic plants are also present along this shoreline.

The substrate in both lakes consisted of the remains of previous years' decaying vegetation combined into a firm mixed sand/gravel bottom. Water clarity for both lakes is above 9 feet, but the shallows can, and do, become quite turbid when the winds are blowing steady from the appropriate direction, or with near shore boat traffic.

PUGNOSE SHINER STUDY SITES - FISH AND LITTLE SWAN LAKES

Fish Lake (DOW# 40-00510 in Le Sueur County is a small, 78 acre lake (33 acre littoral area) north of the city of Elysian, and within the Cannon River Watershed (Figure 1). A DNR-owned public access is located on the southeast end of the lake off County Road 14, and the main study site (Figure 3a) was in the shallow waters that found immediately to the east and west of the boat ramp (MNDNR 2009). The aquatic vegetation was summarized by Perleberg and Brown (2005):

> "Fish Lake supports an abundant and diverse native aquatic plant community, despite the fact that the exotic curly-leaf pondweed (*Potamogeton crispus*) has invaded the lake. The lake is buffered by tracts of deciduous forest and wetlands with relatively little development and water clarity is relatively high for this region of the state. A mixed band of wild rice (*Zizania aquatica*), bulrush (*Scirpus* sp.), yellow waterlily (*Nuphar variagata*) and white waterlily (*Nymphaea odorata*) rings the entire shoreline and submerged vegetation extends to a depth of 19 feet. While curly-leaf pondweed is present in Fish Lake, it co-dominates the submerged plant community and does not form dense and monotypic beds. Native species that are equally as abundant in the lake include narrow-leaf pondweed (*Potamogeton* sp.), flat-stem pondweed (*Potamogeton zosteriformis*) and coontail (*Ceratophyllum demersum*). A total of 18 native submerged plants were found in the lake, including several species that are more typical of clear water, northern lakes."

Little Swan Lake (77-0034) is a small, 149 acre (55 acre littoral area) riverine lake on the SW edge of the town of Pillsbury located within the Swan River watershed (Figure 1). The lake has a maximum depth of 67 feet with submersed vegetation growing out to a depth of 14.5 feet. There is a public swimming beach on the southeast side of the lake that attracts many local residents, and the lake's outflow is approximately 200m north of this beach (MNDNR 2006). The study site (Figure 3b) was focused on that 200m stretch of shallow shoreline located between the public beach and the lake outflow. The most common species of submergent vegetation were claspingleaf pondweed, coontail, flatstem pondweed, and northern milfoil. Sand was the most common shoal water substrate around the lake (MNDNR 2006). Precipitation and runoff upstream in the Swan River watershed can have considerable influence on water quality in the lake; during dry periods the lake clarity is over 9 feet, but during windy/stormy conditions the eastern shallows (where the study area is located) can become quite turbid.

METHODS

During May 2010 – July 2010, May 2011 – July 2011, and May 2012 – July 2012 field observations and sampling at each of the four study sites were conducted approximately every other week at each of the four study areas. Although data was collected during July 2012 and included in this report, expenses associated with data collected during July 2012 were not charged to this project as that occurred after the project end date of June 30, 2012. Additional site visits were made during August-October of 2010 and 2011. One ice dive by P. Ceas in February 2011 was made in Fish Lake to attempt to locate pugnose shiners during winter conditions. To maximize travel efficiency, Movil, Turtle, and Little Swan lakes were sampled during one particular week and then Fish Lake was sampled during the alternate week. Weather conditions would occasionally alter this basic sampling calendar, but we strived to stay as close as possible to this alternating series of weeks. When we were confronted with inclement weather (high winds, rain, cold) we still attempted to (safely) observe the fishes in the field since we were curious to learn more about how these fishes behaved under such conditions. A variety of capture techniques were tried, including a small-mesh 40' bag seine, backpack electrofisher, boat electrofisher, minnow traps, and a 12" x 18" x 16" (deep) dipnet. Field sampling and observations were focused on the four study areas, but during each visit we also motored around the entire shoreline/perimeter of the lakes to ensure that we were not missing other significant concentrations of these fish. We also visited other lakes in Minnesota to determine if our field observation on these lakes could be generalized across the state. Fin clips were taken for future genetic studies and vouchered specimens will be given to the Bell Museum for permanent storage at a future date. Fishes were preserved in the field using a 10% formalin solution, and in the lab rinsed in water before being transferred into 70% ethanol for permanent storage

Behavioral observations in the field were made from above the water while wearing polarized sunglasses and from within the water while snorkeling, and were attempted every time we visited a study site, including during times of inclement weather. These behavioral observations included feeding, nest site preparation and guarding, courtship, spawning, and predator avoidance. Given the habitat heterogeneity (i.e., aquatic vegetation), the species' impressive maneuvering capabilities, combined with boating activity creating clouds of suspended matter in the water column, it proved to be difficult and time-consuming to observe these small fishes in the field. Northern longear sunfishes were photographed and videotaped while underwater, and these images were reviewed in the lab. Even though repeated attempts were made, no useful underwater images of pugnose shiners were obtained. Behavioral observations in the lab were made by placing fishes into aquaria that ranged from 30-70 gallons (114 – 265 L). The aquaria were situated to either natural day length or timed lighting conditions, and contained assorted small patches of the more common native aquatic plants found at the study sites, including wild rice, muskgrass, narrow-leaf pondweeds, coontail, and white water lily.

Age and growth relationships were developed using otoliths and scales (M. McInerny, MNDNR, pers. comm.). Estimates of population composition, density, and survival rates were completed in each lake by dividing the study areas into shoreline segments. Within each shoreline segment repeated bag-seining was conducted until individuals were not captured in at least three consecutive seine hauls. For LES, sampling block nets were placed in the water and extended from the shore out into the lakes perpendicular to the shore, thereby separating each shoreline segment. The lake-side border of these 300 m^2 sampling areas were not blocked off (due to a lack of netting), so this effort does not qualify as depletion sampling, but given that the block nets extended into the lake beyond where we saw LES we felt confident that immigration and emigration by fish into the sampling areas were minimal during the relatively short sampling period (half-day per lake). Fishes captured in each particular shoreline segment were kept alive in aerated containers until all sampling was completed, and only then were fishes returned to the lake. Block nets were not used when sampling for PNS because the thick vegetation made it impossible to ensure that the nets were actually touching the bottom. To ensure that the block nets would have touched the bottom would have meant the removal of much aquatic vegetation and an alteration of the habitat, and we determined that such degradation of the habitat was not a worthy "expense" to the environment. Sampling for LES for the population composition estimates occurred during our June 30, 2011, and July 18, 2011 visits, which based on our observations from previous years is a time frame when LES are generally most common in the near-shore habitat.

To determine potential seasonal changes in diet, stomachs were examined from fishes collected May-August in 2010 and 2011; all specimens used in the diet study were collected in the 10am-3pm time frame of a given day. Stomachs of 235 *L. m. peltastes* from Turtle and Movil lakes and 373 *N. anogenus* from Fish and Little Swan lakes were dissected out, and food items were identified to the lowest taxonomic order possible. A cursory examination of food items based on sizes of different adult age classes did not reveal any noticeable difference in diet, so adult age classes were combined. For the filamentous green algae that were commonly found in pugnose shiners, we counted each individual piece as one food item. On bar charts presented in Figures 19 and 29, the error bars listed represent +/- one Standard Error. Stomachs from 5-10 individuals of select other lakes throughout the state, collected during the same season and a similar time of day as the study lakes, were also examined to provide comparative diet data. These fishes were adults, falling in the Age 1-2 (PNS) or Age 3-4 (LES) range. For LES the lakes sampled were Little Bemidji Lake (Ottertail River), Potato Lake (Crow Wing River), Cameron Lake (Big Fork – Rainy River), Balsam Lake (Prairie River), Woman Lake (Boy River), and Rush Lake (Whitefish Chain - Pine River); for PNS the lakes sampled were Cameron Lake (Big Fork – Rainy River), Movil Lake (Mississippi River – Headwaters), Baby Lake (Boy River), and Pillager Lake (Crow Wing River).

Gonads of both sexes were also dissected out and examined; however, during the final data analysis for this report we discovered that data set had been lost. The data is being gathered again (i.e., new ova counts, measurements, etc.) for use in a future publication. For the Reproductive Ecology section of this Report we have included discussions of our field observations on the reproductive conditions of the species and referrals to observations from the literature, as well as some quick spot-checking of dissected gonads. To describe ova development we used the terminology of Heins (1995). Instead of merely counting the number of "immature ova" and "mature ova" as an estimate of reproductive condition, ova are divided into more distinct categories (Latent, Early Maturing, Late Maturing, Mature, Ripening, and Ripe), which allowed for a better understanding of which females were actively spawning.

With the recent purchase of an Isotope Ratio Mass Spectrometer by St. Olaf College, we had the opportunity to develop an insight into these fishes' diet during the early (Spring) season when they are not easily caught, which then might allow us to make assumptions on the species movements and feeding ecology. Light element stable isotopes such as ^{13}C and ^{14}N are commonly used to determine trophic position in aquatic communities (Wada et al., 1991). There is a consistent enrichment of stable nitrogen isotope, ^{15}N, between prey and predator, and this allows for determination of trophic position (Vander Zanden and Rasmussen, 1999). Stable carbon isotope, ^{13}C, shows a difference between littoral and pelagic communities (Vander Zanden et al., 1999). This is useful for determining the source of production for the consumers or where the organisms are actually feeding in the body of water. Furthermore, there is a delay in time from the actual consumption of the ^{13}C and ^{14}N until assimilation into the tissue; therefore, a strength of conducting stable isotope analysis is that it allows a researcher to essentially look back in time and determine the trophic position of a fish prior to the actual collection date. It is more complicated, but for North American freshwater fishes one can assume a lag time of approximately 1-2 months (S. Schmidt, AK Fish & Game, pers. comm., 2011), so individuals collected in June are actually being tested for their trophic position from April-May. The primary goal of the stable isotope analysis component of this study was to determine where the northern longear sunfish and the pugnose shiner were feeding in the food web, and to examine any differences between lakes. Tissue samples were obtained from specimens of LES collected in 2010 from Movil and Turtle lakes on June 10, July 13, and July 26, and from PNS from Little Swan Creek on June 24 and July 26. Dorsal muscle tissue was dissected out of ten individuals from each collecting date for each fish species. Samples were also prepared for pugnose shiners from Fish Lake, but a power failure while the samples were in the spectrometer resulted in the loss of useful data. Tissue samples were dried for 24-48 hours at 55°C. Samples were weighed and $\delta^{13}C$ and δ^{15} N stable isotope analysis were performed using the Isotope Ratio Mass Spectrometer.

Potential predators (perch, northern pike, and smallmouth bass) were captured in the field in June 2012 in a cursory attempt to determine if northern longear sunfish or pugnose shiners fishes are preyed upon in a regular manner. Gastric lavage was performed in the field, and any fish-like items were returned to the lab for examination.

RESULTS & DISCUSSION – NORTHERN LONGEAR SUNFISH

HABITAT PREFERENCES OF NORTHERN LONGEAR SUNFISH

With the exception of the one known Turtle River locality, northern longear sunfish in Minnesota are associated with deep lakes (i.e., depths > 15') possessing the following characteristics:

- clear water lakes with an average secchi disk reading of 9' or greater.
- shorelines with relatively undisturbed stretches of emergent aquatic plants (Figure 4a) such as wild rice and bulrush (*Schoenoplectus sp.*) combined with extensive shallows (e.g., the water that is 3' deep or less at distances of 150' from shore). Extensive snorkeling efforts confirmed that northern longear sunfishes are not generally found in vegetated shoreline waters in which the depth increases relatively quickly away from shore (e.g., in waters that are 6' deep within 4' feet of shore). In these deeper sites (Figure 4b) we observed an abundance of pumpkinseed sunfishes, which are also frequently present in typical longear habitat, yet did not find northern longear sunfishes present.
- firm substrates that are generally a mixture of sand/marl/silt and often have submerged plants present such as bushy pondweed (*Najas flexilis*), coontail (*Ceratophyllum demersum*), flat-stem pondweed (*Potamogeton zosteriformis*), and Canada waterweed (*Elodea canadensis*). From our observations in lakes throughout the LES range in Minnesota, muskgrass (*Chara sp.*) is almost always the most abundant submerged aquatic plant present in LES Habitat. Mixed beds of wild rice and bulrush are excellent indicators of potential habitat, however shoreline with dense beds that are predominantly bulrush are not necessarily good indicators of habitat.

LES are not present in shallower water after ice-out and do not return to this habitat until aquatic plant regrowth has begun. LES remain in the shallows during the warmer months of the year (mid-May – August) and once the aquatic vegetation begins to die back in late Summer/Fall the fishes once again disappear from the shallows. During the summer spawning season the distinctive saucer-shaped nests can be found in clearings within the plant beds, and often right next to the shoreline.

Shoreland use, including the presence of boat docks/piers, does not appear to be a limiting factor compared to the large-scale removal of aquatic vegetation (or alteration of the aquatic vegetation by extensive nutrient input or soil erosion). We frequently found northern longear sunfish in Turtle Lake and elsewhere spawning near and even under boat docks as long as the aquatic vegetation has been left relatively intact. In lakes where LES are present, if landowners have removed aquatic vegetation from the shoreline area, we do not find northern longear sunfish present (Figure 4c).

Some lakes may appear superficially to provide suitable habitat since these lakes have a combination of extensive shallows and mixed bulrush beds; however, instead of having a substrate of mixed sand/silt/marl these lakes have a substrate primarily made up of sand. These types of lakes often have large numbers of fishes in the shallows (mainly perch, bluegill, pumpkinseed, and banded topminnow) and are clearly high-quality waters, but do not appear to support populations of LES.

Northern longear sunfish are known to occur at one locality in the Turtle River just within the western boundary of the Chippewa National Forest (at CR 207, known locally as Three Culverts Rd); however, the microhabitat in which the individuals are found are pooled waters along the stream margin, which is what other researchers (Johnston and Smithson 2000; Taylor 2000) have noted for *Lepomis. m. megalotis*. The bridge site is comprised of three medium-sized culverts; downstream of the bridge the river is a moderately-flowing shallow stream of exceptional water clarity and quality. Upstream of the bridge the culverts have restricted the water flow and have converted the river into a slower-moving pooled system (Figure 5). The margins of the upstream shorelines have been clearly stabilized by wetland and aquatic vegetation for many decades and were lined with wild rice and bulrush beds, with well-defined shallow-pool habitats dispersed along these margins. Both northern longear sunfish and pugnose shiners are found within these pools, but the two species' distribution within the river appears to be severely restricted to these pools and neither species are found in area with current (up or downstream of the bridge). This bridge is designated for eventual replacement by a bridge that will span the entire stream; this will change the upstream river segment to a more lotic/flowing environment, at which time both species will likely no longer be found there.

SEASONAL MOVEMENTS

Even though the ice out dates varied during the three years of this study, northern longear sunfishes were not found in the shallows of Movil and Turtle lakes until mid-May of all three years, and then only in very small numbers (five individuals in 2010, two individuals in 2011, and 10 individuals in 2012). We did not find any correlation between ice out dates and the time when sunfishes were first found in the shallow shoreline waters. The median date (1965-2008) for ice-out on Turtle and Movil lakes is April 23; the earliest known date was April 4 (1981) and the latest date was May 10 (1996). Ice out in 2010 occurred in early April (April 1-7), in 2011 ice out occurred in late April (April 22-28), and in 2012 ice out occurred on March 23, which represents a new earliest date for ice out. Northern longear sunfishes were common in the shallows in early June in both 2010 and 2012, but were not common in the shallows in 2011 until mid-June. Northern longear sunfishes begin to become much less common in these shallows by mid-August in all three years.

Water temperature and aquatic vegetation cover appear to influence when the fishes begin to move into the shallows. Water temperature was in the 58°F (14.4°C) range in late May in all three years. In 2010 and 2012 it increased quickly to the 65°F range (18.3°F) by early June, but did not reach that temperature in 2011 until mid-June. Although other aquatic vegetation was producing new growth, the emergence of wild rice floating leaves was identified as a good indicator of LES seasonal appearance in the near-shore littoral zones in the study lakes (Figures 6 and 7 demonstrate growth of aquatic vegetation over time at the study areas). This movement event was monitored by volunteer shoreline observers and MNDNR staff who made numerous trips to the spawning sites in the spring to help us determine when the sunfishes were arriving in the shallows.

GROWTH, AGE, AND SURVIVAL

Otolith analysis was conducted to complete growth and age and survival estimates (M. McInerny, Minnesota DNR, pers. comm.). Unlike the related Central longear sunfish (*Lepomis m. megalotis*), Northern longear sunfish (*Lepomis m. peltastes*) do not reach as large a size, and growth is slower. On average, about six growing seasons are required for northern longear sunfish to reach 100 mm total length (TL). The table below shows growth data for the sexes combined.

	Age (years) and Total Length (mm)					
	1	2	3	4	5	6
Mean TL	37	59	71	80	89	98
Minimum TL	32	49	63	72	78	85
Maximum TL	42	66	81	89	99	118

When examining the sexes separately, female average size at a given age are as follows: Age 1 (33.79 mm); Age 2 (54.21 mm); Age 3 (66.80 mm); Age 4 (74.16 mm); Age 5 (79.68 mm); Age 6 (82.12 mm). Male average size is somewhat larger: Age 1 (35.65 mm); Age 2 (57.96 mm); Age 3 (69.36 mm); Age 4 (81.50 mm); Age 5 (91.20 mm). Although there are reports of LES reaching upwards of 150 mm, the largest individuals that we have seen are in the 105-110 mm range. We have been able to examine a number of specimens in the 120-150 mm range that were identified as LES, but all have turned out to be either hybrid pumpkinseed x northern longear sunfish hybrids, or hybrid pumpkinseed x green sunfish.

Factors affecting growth of LES in Minnesota are not understood, but are certainly influenced by differences in forage and growing season. A growth index (opercular flap growth divided by pelvic fin spine growth) of males of the closely-related *Lepomis m. megalotis* that were fed a larger ration were greater than those of males fed a smaller ration, although the difference was marginally non-significant (Goddard and Mathis 2000), and males with longer opercular flaps were reported to out-compete/dominate males with shorter flaps for food.

Larval Growth and Identification. We did not collect eggs from the field, nor did we observe spawning in the lab. Eggs in the related Central longear sunfish (*L. m.* megalotis) are demersal and adhesive (Yeager 1981). In aquaria, embryos hatch 2 days after fertilization in 25^{o}C water, and swim-up and feeding begin after 7 days (Smith 1975). The following two paragraphs were kindly provided by M. McInerny, who is writing the species account for the northern longear sunfish for the upcoming "Fishes of Minnesota" publication. These descriptions are based on northern longear sunfishes from the Lake Michigan basin:

> Protolarvae (2–5 mm) newly hatched; caudal fin rays developing. Myomere count 32 (14 +18). Preanal length 46% TL. Pigment lacking. Ovoid yolk sac yellowish with single oil globule posteriorly. Meso- and metalarvae (6–11 mm) have myomere count of 27–32 (10–14 + 17–18). Pectoral buds well developed at 6 mm; dorsal fin spines developing at 8 mm, pelvic fins begin developing at 9 mm. Pigmentation limited to a few melanophores on dorsal surface of swim bladder, belly (6 mm), head, and abdomen, and faint spot on upper part of operculum (6–7 mm). At around 11 mm, pigmentation on head and body has increased and melanophores begin appearing on caudal, dorsal, and anal fins and upper jaw. Yolk sac absorbed at 5–6 mm. Juvenile (13–19 mm) pigmentation composed of melanophores over posterior portion of body, patch behind eye, melanophores on spinous dorsal fin (13 mm), melanophores on all fins except pelvics, and about 10 vertical bands (pigment bands wider than unpigmented interspaces) laterally between head and caudal fin (19 mm). Description based on Yeager (1981) and Tin (1982e).
>
> Proto-, meso-, and metalarvae and juvenile longear sunfish are not reliably distinguishable from other sunfish at similar life stages. At about 25 mm TL and longer, small mouth size (jaw

does not reach eye) is useful for separating longear sunfish from green sunfish and warmouth in Minnesota waters (Tin 1982e). Pectoral fins in sunfish appear fully developed at around 35 mm TL; thus, the short rounded pectoral fin separates longear sunfish from pumpkinseed and bluegill. The 10 rather wide vertical bars found on longear sunfish 13 to 19 mm TL distinguishes this species from orangespotted sunfish (Tin 1982e); however, it is unclear if this holds true for juveniles 20 to 40 mm TL. The earflap starts elongating to adult form after longear sunfish reach 32 mm TL (Yeager 1981), which separates this species from all other Minnesota sunfish.

DEMOGRAPHY

Composition. The Turtle Lake study area was divided into four sampling areas (each area with approximately 15m of shoreline and extending 20m away from shore, a 300 m^2 area); these four sampling areas (Figure 8) were separated from each other by the series of boat docks that run out perpendicular from the shore, and block nets were placed hanging from the boat docks to restrict movement between the four sampling areas. For each sampling area repeated bag-seining was conducted until individuals were not captured in at least three consecutive seine hauls. Sites A and B were dominated by beds of muskgrass (*Chara sp.*) and wild rice (*Zizania aquatica*) which from our previous observations was identified as preferred habitat for northern longear sunfish (Porterfield and Ceas, 2008); Site C was dominated by muskgrass with scattered lily pads (*Nuphar variegata*); Site D had a carpet of muskgrass but this vegetation was fairly impacted by boat propeller activity as this area is bordered by the two docks that get the most use at this small resort.

The Movil Lake study area was divided into three sections, using the three dominant beds of aquatic vegetation (Figure 9): Site A started near the Hwy 22 bridge and included a stand of bulrush; Site B encompassed an area that was dominated by muskgrass; and Site C was dominated by a mixed bulrush/lily pad bed. Each site contained approximately 10m of shoreline and extended 20m away from shore.

Larger/older (age 4+) individuals of both sexes were present in the study area in June, but were absent in July except for one 4 year-old female in Movil Lake (Table 1). The number of 3 year-old females increased in Movil Lake in July, but the number of 3 year-old males in Movil Lake and the number of 3 year-old males & females in Turtle Lake decreased. Conversely, the number of 1 and 2 year-old fishes increased from June to July. Overall larger/older individuals move into the shallows first and tend to spawn in June. Once these older individuals leave the breeding ground then the younger/smaller individuals move in and spawn. These trends correspond to our observations in previous years, both in these two lakes as well as in lakes throughout the species' range in Minnesota.

Juvenile sunfishes (generally < 30 mm) were not common in the study areas. They could be found scattered along the vegetated shorelines, and were generally associated with areas of thick bulrush beds or dense muskgrass (P. Ceas, pers. observ). Given the habitat heterogeneity and the species impressive maneuvering capabilities, it can be difficult to capture these small individuals. The best success was using minnow traps, or simply snorkeling for long periods of time after which the fishes would sometimes allow the snorkeler to observe and identify juveniles.

Density and Abundance. Although LES can appear to be quite abundant when pulling a bag seine through the water, the total number of fishes captured and their overall density is actually quite low. Even on dates when the species was observed to be abundant in the shallows the density was in the 0.25-0.33/m^2 range.

Species Conservation Status. During site visits to the study areas and to all lakes visited in 2010-2012, we would actively search the shoreline/perimeter of the lakes to find all active nesting areas. Regardless of the lake, we usually found one or perhaps two main nesting areas in a given lake, and the locations of these nesting areas remained fairly constant from year to year. There were occasionally some additional small clusters of nests in a lake where we would find perhaps 15-20 individuals. For example, in addition to the primary spawning site on Turtle Lake, we know of two other shoreline locations that have each had a consistent but small "population" of breeding individuals since at least 2006. The important message is that when northern longear sunfish are present in a lake, large colonies of breeding individuals are not found throughout the shoreline, and the species is never as abundant as other sunfish species such as bluegill (*Lepomis macrochirus*) and pumpkinseed sunfish (*Lepomis gibosus*). This reaffirms the perception that the northern longear sunfish is a rare species within Minnesota, and lends support to its continued listing as a species deserving of some level of conservation protection.

Survival Rates. Survival rates of longear sunfish in Minnesota and elsewhere are not well known. Individuals as old as 7 years have been collected in Minnesota (M. McInerny, pers. observ.), but it is possible that some older individuals exist. Given the overall small numbers of fishes that we found on the primary breeding sites within these two lakes we did not sacrifice larger longear sunfish to collect otoliths for aging and therefore it is difficult to construct a survivorship curve. We did measure and sex all individuals captured during the depletion sampling and these results show a considerably larger number of younger (Age 1-3 year) vs. older (4+ years) fishes (Table 1). Given that these fishes were collected during the spawning season over spawning beds, if a large percentage of fishes survive until the Age 4+ (and were reproductively active) then one would expect to find quite a few older fishes in these samples, but that was not the case.

REPRODUCTIVE ECOLOGY

Reproductive Cycle of the Female.

Appearance of Females. Female coloration did not change much during the spawning season. There may be an intensification of body patterns, and the red earflap may brighten a bit, but the primary change in appearance is due to the belly as it becomes increasingly distended as ova mature (Figure 10a). Females that had a clutch of ripe ova and were ready to spawn were easy to identify by very gently squeezing the distended belly, which would result in the release of the golden-yellow ova. Once a clutch of ripened ova were spawned the female's belly would become soft and deflated for a period of time before the next clutch of ova ripened, making it quite easy during the spawning season to determine if a female had recently spawned.

Ova development. Ovaries of females collected in mid-May were classified as Early Maturing to Late Maturing. Oocyte development proceeded quickly at this point. By early June the ovaries of individuals of Age 3+ contained Mature oocytes, and in 2010 and 2012 both Mature Ripening and Ripe ova were present (fishes were spawning at this time). In 2011 Ripe ova were not present until mid-June. Figure 10b shows an Age 3 female (64mm TL) captured on June 13, 2011 that does not have ripe ova. Figure 10c shows a photo of an Age 4 female (73 mm TL) that has been partially dissected to show the ovaries, which contain an abundance of Mature and Mature Ripe ova. Ripe ova diameter ranged from 1.0 to 1.5 mm. These diameters are similar to those found by Yeager (1981) for Central longear sunfish (*L. m. megalotis*).

The larger/older females produced considerably more Mature/Mature Ripe ova. For specimens captured on June 22 2010 and June 30 2011, counts for Age 4+ females ranged from 1266 – 3560 and averaged 2298; counts for Age 2-3 females ranged from 480 – 911 and averaged 723.

Size at Maturity. Females of Age 2 (43 – 58 mm TL) contained abundant Mature/Mature Ripe ova in July, and were believed capable of spawning.

Reproductive Cycle of the Male.

Appearance of Males. Males of Age 2+ exhibited high breeding color patterns for which members of the *Lepomis megalotis* species complex are so well known. The colors can be subdued somewhat during periods of inclement weather or when the fishes are not spawning, but there is a certain "wow" factor when one captures a male that is in full breeding color (Figure 10e-h). Males that are not spawning during one time of the day (for example, the morning) can in a short time, perhaps even minutes, "turn on" the iridescent and brilliant colors associated with these fishes when they are spawning. In addition to the high breeding colors, males that were ready to spawn, or were already spawning, were easy to identify because the slightest pressure on the belly would release a stream of milt.

Testes Development; Size/Age at Sexual Maturity. Testes of sexually mature males (Figure 10d) are greatly enlarged, opaque white, and spongy. We did not collect any males from the months immediately preceding the spawning season, but testes collected in Aug - Oct are small and translucent. Jennings and Philipp (1992) stated that testes develop in precocious sneaker male northern longear sunfish when they reach 45–55 mm TL, but testes develop in parental males only after they reach 60 mm TL or longer. Males 48 – 72mm TL that were collected in June and July in this study were all sexually mature and thus were potential spawners. If there where small "parental" males that were not sexually mature in the study lakes then they likely were not present in our study area.

Spawning Period. Although pinpointing an exact water temperature when nest construction began was not possible, nest construction and spawning activities were in full-swing by the time water temperatures reached 68°F (20°F). This corresponded to early June in 2010 and 2012, and to mid-June in 2011. Although a few males were found guarding nests in mid-August, it appears that most spawning activities ended in late July each year. Water temperatures in these shallows at this time of year fluctuated considerably depending on the weather. A daily fluctuation in water temperature between 77-82°F (25-27.8°C) was not uncommon, but sunfishes were found to be guarding nests and spawning throughout this temperature range. What seemed to have a much greater influence on spawning activity were the daily weather patterns, as fish can be highly sensitive to changes in the barometric pressure (Markum et al., 1991). During periods of stormy/rainy weather the fishes would suspend spawning, and often the males would leave the nests. When the wind was blowing strong enough to form larger waves, the fishes would generally disappear from the shallows, only to reappear a day later if the weather had improved.

Nest Construction, Size, and Location; Colonial Nesting. Males Age 3+ constructed nests in exactly the same manner as described by others (Boyer and Vogele, 1971; Keenleyside 1978; Bietz 1981), with the male using a sweeping motion of his tale (Figure 11a) to create a shallow circular/saucer-shaped depression. Nests were always associated with cover; if found on open sandy areas then cover was available within a few feet (Figure 11b). The majority of nests were typically created by the male creating a clearing in a patch of aquatic vegetation, usually muskgrass, and often near taller cover such as wild rice and/or bulrush (Figure 11c, d). Nests were not located deep within a stand of bulrush, but were frequently found within beds of wild rice. In lakes throughout the state nests were often found next to, or even under, fallen tree trunks (Figure 11e), but even when such cover was present and located at the preferred depth it was not always used. The substrate within the saucer pit was variable depending on the bottom substrate, but typically all vegetation & detritus was cleared out, creating a firm bottom of some combination of sand/silt/gravel/snail shells (Figure 11f).

Average nest diameter (N = 50) was 34.9cm with a range of 24.13cm – 41.91cm; this is comparable to the 33cm average that other researchers have found (Dupuis and Keenleyside, 1988). Nest diameters are approximately 3-4 times the TL of the occupying male. Nest depth (N = 50) was fairly consistent between spawning seasons and ranged from 6-36 inches (15-92cm) with an average depth of 12 inches (30.50cm). Nests were located typically within 0.5 – 5 m of the shoreline, which correlates with nesting depth since these shallow waters tend to increase in depth gradually as one moves further away from shore. This is important because people swimming and wading in these lake shallows can inadvertently walk right through a nest, completely destroying the nest and potentially scattering the eggs or burying the eggs in a layer of substrate.

In two studies done on the Thames River in Ontario, Canada (Bietz 1981; Dupuis and Keenleyside, 1988), LES were found to be selective colonial nesters, building nests in dense aggregations of rim-to-rim hexagonally shaped nests (20-100 nests) similar to what is typically observed in nesting bluegill colonies in Minnesota. During our observations of spawning sites throughout Minnesota we never observed such tightly packed nests, and never observed what could be considered rim-to-rim nests. On any given day we observed at most 10-20 nests scattered throughout each spawning area. Perhaps if nesting densities were to increase considerably then there might be such colonial aggregates in Minnesota lakes. Instead, there were often what might be considered "clumps" of nests where perhaps 3-5 nests were found within a 3m diameter (Figure 11c), but we did not observe tight colonies of breeding males as found in the Thames River (Dupuis and Keenleyside, 1988).

Nest Guarding. Once a male had finished constructing a nest he remained very alert, constantly staring at anything that approached the nest. These males seemed able to recognize particular organisms as harmless; for example, we observed a few times when a solitary large leech (*Macrobdella decora*) would swim within inches of a nest, but the male sunfish would simply ignore it. When another male sunfish (regardless of species), or even the hand of the snorkeler, approached to within about three feet of the nest the territorial male would stare directly at the potential intruder. As the intruder moved closer the male would move towards the forward rim of his nest and look directly at the intruder. If the intruder came "too" close (usually within a foot) then the nest-guarding male would typically begin exhibiting any number of agonistic behaviors, including the flaring of his opercles while either remaining stationary or rushing over to the intruder, turning to his side and performing tail-wags once within body contact of the intruder, or actually biting the intruder. If the intruder retreated, the territorial male would either return/remain at his nest or chase the intruder for about 5 more feet. Disturbance of the nest by smoothing out the saucer pit resulted in the guarding male becoming very "agitated" and commencing the rebuilding of the nest using vigorous tail-wags. It is unknown if this resulted in any previously-laid eggs being dislodged from the nest or being shifted down into the deeper substrate. If this does happen, then the disturbance of these sunfish nests could result in reduced hatching of a particular clutch of eggs. Males are reported to guard nests for up to 9 days depending on development rates of their offspring (Jennings and Philipp, 1994).

Spawning Behavior. Unlike females and juveniles, it was rather easy to observe nest-guarding males. After just a few minutes of kneeling/floating fairly motionless in the water the male would ignore the observer unless the observer moved in such a way as to provoke the territorial/guarding response of the male. As is typical for members of the genus *Lepomis*, spawning behavior by northern longear sunfish involved nest defense by the territorial male and elaborate courtship displays. The larger, older (Age 4+) males were the first to build nests and spawn. These fishes seemed to complete their spawning by the end of June/early July, and then the smaller 3+ males would construct nests and spawn. We observed very few instances of smaller males (Age 2 or 3) attempting to sneak into the nests of these solitary males and spawn with females. This lack of cuckoldry was also observed in solitary nests by Dupuis and Keenleyside (1988) and Jennings and Philipp (1991).

Nesting males have been reported to make gruntlike or popping sounds when courting females (Gerald 1971), but we were unable to hear such sounds, presumably because of the background noise of wind, water, etc. The actual courtship and spawning act of members of the *Lepomis* sunfishes has been well documented so will not be repeated here.

DIET (EXAMINATION OF STOMACH CONTENTS)

Stomachs of 235 LES contained mostly Amphipods (Scuds – *Gammerus spp.*), and caddisfly (Trichoptera) and midge (Chironomidae) larvae, but there was some variation between lakes and even among sexes and dates. The mean number of food items per stomach, and the percent of stomachs in which a given food item occurred, are summarized by date and lake in Tables 2 and 3.

Relative Abundance: By Year and Lake. Based on the relative abundance (percent occurrence) of total food items in the stomach (Figure 12), scuds were the most prevalent food item across both years and both lakes.

- 2010: Movil Lake: Scuds comprised 45%, and caddisfly larvae 28%, of the total items found in the stomachs.

Turtle Lake: Scuds comprised 35%, and caddisfly larvae 54%, of the total items found in the stomachs.

- 2011: Movil Lake: Scuds comprised 37%, midge larvae 25%, and mayfly larvae 19% of the total items found in the stomachs.
  Turtle Lake: Scuds comprised 37%, midge larvae 37%, and caddisfly larvae 8%, of the total items found in the stomachs.

Midge larvae were present in 2010 stomachs but comprised a very small percentage of the total food items found. Conversely, caddisfly larvae were found in stomachs in 2011 but did not approach the levels of abundance seen in 2010. The reason(s) for this change in diet is uncertain; one possible explanation might be correlated with the difference in lake levels and thus available habitat between 2010 and 2011 since the lake levels were approximately 18" lower in 2010 compared to 2011.

Relative Abundance: By Collecting Dates & Lake. When examining the relative abundance of total food items by collecting date and lakes (Figures 13 and 14), there are some interesting results for each lake:

- 2010: Movil Lake: Scuds comprised the majority of food items in June with both scuds and caddisfly larvae sharing a similar abundance in July.
  Turtle Lake: Scuds comprised about third of the diet all summer, and caddisfly larvae comprised at least half the diet all summer.
- 2011: Movil Lake: Midge larvae comprised about one-half of the diet in May; Midge larvae, mayfly larvae, and scuds each comprised about one-third the diet in early July; Scuds comprised 30-60% of the diet during June and mid-July.
  Turtle Lake: Midge larvae comprised about one-half of the diet in May, with a continually decreasing abundance as the summer progressed; Scuds were a consistent part of the diet (32-41% of prey abundance) throughout the season.

When considering the percent of individuals in which the food items occurred (Tables 2 and 3), scuds, caddisfly larvae, and midge larvae were the most relatively abundant total food items in 2010 and 2011, but they were often consumed by most individuals on a given sampling date. For example, in Turtle Lake in 2010, of the 37 individual stomach examined, scuds and caddisfly larvae were present in 68% and 78%, respectively, of the 37 stomachs.

Mean Number of Prey Items per Fish: By Year and Lake. When examining the mean number of prey items per fish, by lake and year (Figure 19a), Movil Lake fishes are fairly consistent from 2010 (6.57 items/fish) to 2011 (7.07 items/fish). Turtle Lake saw a considerable increase in mean prey numbers from 2010 (4.97 items/fish) to 2011 (11.77 items/fish), and fishes in Turtle Lake in 2011 appeared to eat more prey items than compared to Turtle Lake in 2010 or Movil Lake for either year.

Mean Number of Prey Items per Fish: By Collecting Date and Lake. When examining the mean number of prey items per fish, by collecting date and lake (Figures 19b, c), there are a few trends that emerge.

- 2010: Movil Lake: The fewest number of food items per stomach were found in late July.
  Turtle Lake: The mean number of prey items appeared to be highest in mid-July.
- 2011: Movil Lake: The mean number of prey items in late June appeared higher than the other sampling periods.
  Turtle Lake: The Turtle Lake May mid-June samples appeared to have higher numbers of prey items compared to the same Movil Lake samples.

Mean Number of Prey Items per Fish: By Year, Lake, and Sex. When examining the mean number of prey items per fish, by year, lake, and sex (Figure 19d), the only difference was that in Turtle Lake, in 2011, where females consumed on average more prey items (12.40) than males (8.13). Otherwise males and females consumed the same average number of prey items, regardless of lake or year. When looking at trends, it could be said that in Movil Lake in 2011 (as with Turtle Lake 2011), females consumed more prey items (11.20) than males (4.77). Another potential trend is that Movil females, Turtle females, and Turtle males all consumed more prey items in 2011 vs. 2010.

Relative Abundance: By Collecting Date, Lake, and Sex.

- 2010: Movil and Turtle lakes: For a given date the diets of each sex were somewhat similar to the diet of the sexes combined. For example, comparing Figure 13a (sexes combined, June 10 2010) to Figure 15a (Movil females, June10) and Figure 15e (Movil males, June10) shows that scuds comprised the largest proportion of the diet for each sex on that date, and for the sexes combined on that date. This same trend holds for each of the remaining three Movil Lake dates in 2010: scuds dominate in the diet on June 22 (Figure 13b vs. Figures 15b and 15f); scuds and caddisfly larvae are the most abundant food items on July13 (Figure 13d vs. Figures 15g and 15h). The results – sexes share similar diets on similar dates – is the same for Turtle Lake: (Figure 13e vs. Figures 16a and 16e); (Figure 13f vs. Figures 16b and 16f); (Figure 13c vs. Figures 16c and 16g); (Figure 13d vs. Figures 16d and 16h).

- 2011: Movil Lake: Males were not found during the May or June collecting dates. The diet of females sampled during July (Figures 17c, d) was similar to the results of the combined-sexes data (Figures 14c, d), with scuds being the most abundant prey item. The diet of males in July (Figures 17g, h) was fairly evenly split among scuds, midge larvae, mayfly larvae, and Haliplidae (Crawling Water Beetle larvae).
  Turtle Lake: The diet of both females and males (Figures 18b-h) were also similar to the diet of the combined-sexes data (Figures 14f, g, h).

Mean Number of Prey Items per Fish: By Collecting Dates, Lake, Sex. When examining the mean number of prey items per fish, by collecting date, lake, and sex (Figures 19e, f), there did not appear to be any overall differences. This lends further support that, overall, males and female LES consumed a similar diet.

- 2010: In 2010 (Figure 19e) there are certain groups on a given date that appear to show differences in mean prey number (e.g., July 26 for Turtle females, Movil Males, and Movil females, collectively compared against July 26 Turtle males), but these three July 26 groups do not appear to be different than the June 10 groups, and the June 10 groups also do not appear to be different than the June 22 and July 13 groups.

Juveniles. The diet of juvenile sunfishes was quite similar to the adults in that scuds, caddisfly larvae, and midge larvae comprised the majority of the prey items found. One exception is that Cladocera were also fairly common prey items, and could sometimes make up 25% of the prey abundance on a given collecting date.

Diet in Other Lakes. A limited examination of sunfish stomachs (3-10 fishes per lake) from specimens collected throughout the state indicates that the diet of the northern longear sunfish is relatively uniform across Minnesota.

Feeding Behavior. Given that Amphipods such as scuds are often found mixed within the substrate, we used snorkeling to visually observe LES feeding. Under the few instances with optimal conditions of clear skies and no wind we were able to observe LES feeding. As expected, sunfishes would actively take prey from the water column and from the sides of plants and rocks. These prey included caddisfly and midge larvae as we saw in our diet study, but we did not see any scuds being consumed. A different feeding behavior had the sunfish using its lower jaw to frequently scoop up substrate and we assume that this technique is used to attempt to capture scuds. Whether the sunfish was able to detect the scuds within the substrate or if it had simply evolved the habit of scooping is unknown, but from our observations it appears to be the technique that LES use to capture scuds.

DIET (ANALYSIS USING STABLE ISOTOPES)

Through stable isotope analysis the trophic position was determined for LES (Figure 20). There was a significant difference in source carbon in Movil and Turtle lakes between June 10 and July 13, and between July 13 and July 26. This shows a shift in where the fishes were feeding. The more negative numbers suggest an autochthonous carbon type found in the pelagic zone whereas the less negative numbers suggest more allochthonous carbon type from the littoral zone (Vander Zanden and Rasmussen, 1999). The change-over to the littoral zone carbon source is consistent with seasonal movements/breeding patterns of the northern longear sunfish (Bietz, 1981; this paper). The trophic position (TP=3) did not change significantly, indicating that as the northern longear sunfish changed habitats they did not change where they were feeding on the food chain. This information is supported by the results of the stomach analysis. Stable isotope analysis is an extremely promising discipline since conducting such analyses on both freshly-captured fishes, or even fishes that have been preserved for years in museum holdings, could provide a wealth of information regarding trophic feeding levels and location of food sources that otherwise might be problematic to obtain.

INTERACTIONS WITH OTHER ORGANISMS

The northern longear sunfish was associated with 31 other fish species in 16 Minnesota lakes sampled with trap nets and gill nets since 1975 (MNDNR, unpublished data). The most common sportfish associates were northern pike and yellow perch (sampled in 100% of these lakes); rock bass, pumpkinseed, and black crappie (92%); bowfin, brown bullhead, bluegill, and largemouth bass (85%); and yellow bullhead and walleye (77%). In 15 samples taken from three Minnesota counties in 1991–2002, the northern longear sunfish was associated with 26 other fish species (K. P. Schmidt, pers. comm.).

Rare/indicator species or species of special concern that are commonly found with the northern longer sunfish include the blackchin shiner, blacknose shiner, Iowa darter, banded killifish, least darter, and pugnose shiner. After sampling lakes across the state for numerous years we have discovered what appears to be a reliable indicator of northern longear sunfish absence: lakes that contain “large” numbers of bluntnose minnows *Pimephales notatus* (i.e., they were as prevalent as blackchin/blacknose shiners), rarely contain northern longear sunfishes.

Potential predators (northern pike, and smallmouth bass) were captured in the field in June 2012 in a cursory attempt to determine if LES are preyed upon in a regular manner. Gastric lavage was performed in the field on two northern pike (16” TL each) and three largemouth bass (8 – 12” TL). No sunfish remains were found in the stomachs; however, given that these piscivorous species are commonly found in the vegetated shallows of the study area, it is certainly to be expected that northern longear sunfish occasionally end up as a meal.

Results & Discussion – Pugnose Shiner

Habitat Preferences of Pugnose Shiner

Pugnose shiners were found in May-July concentrated in shallow near-shore waters, generally in the thickest aquatic vegetation present. Our observations on the conditions of lakes in which we found pugnose shiners in Minnesota support statements made by previous researchers (e.g., Bailey, 1959) that pugnose shiners are intolerant of turbidity and "poor" water quality. In both Fish and Little Swan lakes, fishes were found in May in deeper (4-6') water, which corresponded to 15-30m from shore. As the summer progressed, and as the aquatic vegetation grew thicker in the shallower waters, pugnose shiners were then found closer to shore in waters 3-4' deep. Unlike northern longear sunfishes, however, pugnose shiners were rarely found in the very shallow waters (1-2' deep) immediately adjacent to shore.

In this 3-6' deep water the thickly-vegetated near-shore waters tend to have a soft substrate into which a person wearing waders can expect to sink at least 6-12 inches. During the 2010 field season we quickly discovered that sampling while walking in waders at these depths is problematic at best, and it was much easier and more effective to wear a wetsuit instead of waders while attempting to catch this species.

Aquatic plants and muskgrass seem to be a more important limiting factor than substrate type. We have found pugnose shiners in lakes in Minnesota with a variety of substrate types, including sand, gravel, mud, and detritus; however, within such lakes the shiners are always associated with dense aquatic vegetation, and are not found in open waters.

Predator Avoidance; Sampling techniques

In the field and in aquaria pugnose shiners are an extremely wary/skittish fish, and this made behavioral observations difficult. When the fishes are approached we observed that both aquarium-held and field fishes have a habitat of sinking down towards the bottom and hiding among the thickest root masses (also noted by Becker, 1983), in a likely predator-avoidance strategy. Fishes were held in aquaria during all three field seasons and during the 2010-2011 and 2011-2012 winters, but they never became habituated and remained wary at the slightest movement or noise within the lab spaces.

Seasonal Movements

As with the LES, pugnose shiners were found in the shoreline shallows from approximately mid-May to mid-October. We did not find individuals in the shoreline shallows in the weeks immediately following ice out, and we did not find any correlation between ice out dates and the time when sunfishes were first found in the shallow shoreline waters. There was a noticeable drop in the number of fishes beginning in late July, and the species was uncommon at best in the shallows by late September when we found very few fishes. The results of the stable isotope data (see below) suggest that as April turned into May the fishes were migrating from the pelagic zone to the littoral zone (or at least from the deeper portions of the littoral zone into the shallow portions). During the February 2011 ice dive, P. Ceas observed black-striped minnows hovering near the drop-off of the shoreline "shelf", among the deep-water edge/limits of last year's growth of aquatic vegetation (10-15' deep). Given the low light conditions that day due to the thick ice layer, positive identification could not be made, but based on swimming behaviors and body size these fishes were likely a combination of pugnose shiners, blacknose shiners, and blackchin shiners.

Growth, Age, and Longevity

Pugnose shiners live at least three years, with perhaps a relatively few individuals surviving into a fourth year. Doeringsfeld (1993) used scale annuli to age fishes, and back-calculated lengths were estimated from 65 adults collected from May - July. Fishes Age 0 reached an average size of 26.6 mm TL by late July/early August; Age 1, Age 2, and Age 3 fishes (sexes combined) averaged 35.2, 42.5, and 48.0 mm TL, respectively. The largest specimen we have captured was a 51.2 mm TL male from Little Swan Lake on July 1, 2011. We did not find larvae, so descriptions of larvae remain unknown. During Spring-Summer 2013 we plan to continue to study the PNS and hope to collect and describe larval development.

DEMOGRAPHY

Composition. Setting block nets in Fish and Little Swan lakes proved too problematic given the extensive aquatic vegetation, so the respective study areas were seined repeatedly until pugnose shiners were not found in three consecutive seine hauls (approximately 10 minutes elapsed between seine hauls to allow fishes that might have sought cover in the deepest vegetation to reenter the water column). Given the sensitive nature of this species we did not want to keep the fishes confined to aerated containers for extended periods of time, so we divided the specimens by age class, preserved approximately 1/2 of the specimens from each age class, and returned the remaining fishes to the water. Length, sex, and age classes were noted in the lab.

Fish Lake individuals were almost exclusively Age 1 females and males (Table 4). Females were much more abundant in the June collections in both 2010 (Date #'s 1 and 2) and 2011 (Date # 4); males were common to abundant during both June and July samples.

Little Swan Lake specimens were represented by both Age 1 and Age 2 individuals of both sexes (Table 4). For females, the greatest numbers of individuals captured was in late July 2010 (Date #3); conversely, there were almost no females captured in mid-July 2011. An initial thought might be that spawning had tapered off earlier in the season in 2010 compared to 2011, but other factors might have been in play: heavy rains had fallen the day before in 2011, and there was an impressive abundance of juvenile and Age 1 bluegills and perch in the shallows on this day. We also noticed that the other minnows that are commonly captured in the shallows (blacknose and blackchin shiners) were also quite rare. Males outnumbered females in both lakes.

Juveniles. Juvenile pugnose shiners were not found in the study areas.

Density and Abundance. Pugnose shiners are relatively rare compared to other co-occurring minnow species. Doeringsfeld (1993) stated that pugnose shiners were collected together with blackchin shiners in a ratio of 0.86:1; however, during those dates reported in Table 4 we would capture 100's of blackchin shiners in the same seine hauls as the relatively few pugnose shiners, and blacknose shiners were also more abundant than pugnose shiners.

REPRODUCTIVE ECOLOGY

Reproductive Cycle of the Female.

Appearance of Females. Female coloration did not change during the spawning season; the primary change in appearance is due to the belly as it becomes increasingly distended as ova mature (Figure 21a). Females that had a clutch of ripe ova and were ready to spawn were easy to identify by very gently squeezing the belly, which would result in the release of the golden-yellow eggs. Once a clutch of ripened ova were spawned the female's belly would become soft and deflated for a period of time before the next clutch of ova ripened, making it quite easy during the spawning season to determine if a female had recently spawned.

Ova Development. Ovaries of females collected from Fish Lake in mid-May in 2010, 2011, and 2012 contained Mature Ripening and Ripe ova, and females were in reproductive condition.

Doeringsfeld (1993) stated that Age 2 females contained more mature ova than Age 1 females. In Fish Lake, our observations on a limited number of Age 2 individuals supports this statement. Age 2 females in Little Swan Lake also contained more mature ova than Age 1 females. Counts range from 130 – 766 ova.

Size and Age at Maturity. Females of Age 1 (35mm TL ave. length) contained abundant Mature/Mature Ripe ova, and were believed capable of spawning.

Reproductive Cycle of the Male.

Appearance of Males. Males exhibit a bright golden yellow color across their entire body and fins when in peak reproductive condition (Figure 21b), so when captured it was quite easy to determine the reproductive readiness of males. In addition to the high breeding colors, males that were ready to spawn, or were already spawning, were easy to identify because the slightest pressure on the belly would release a stream of milt.

Testes Development; Size/Age at Sexual Maturity. Testes of Age 2 males collected in mid-May and June were greatly enlarged, opaque white, and spongy. It appeared that mainly Age 2 males were sexually mature during May and most of June, but the testes of some Age 1 males from both lakes were also enlarged, opaque white, and spongy by early June. The remaining Age 1 males became mature by early July, and thus Age 1 males were potential spawners. We did not collect any males from the months immediately preceding the spawning season, but the testes of males collected in Aug - Oct are small and translucent.

Spawning Behavior. Spawning behavior, including courtship, was never observed in the field or in the lab. To our knowledge spawning behavior has never been observed. As stated above, these small fishes are extremely wary/skittish when approached, which very likely is part of the reason why no one has had success observing courtship and spawning. Fishes were held in aquaria during all three field seasons and overwintered during 2010-2011 and 2011-2012, but they never became habituated and never seemed to “settle in.” Even placing a video recorder into a room that had no human traffic did not allow us to capture courtship and spawning activities.

All species of *Notropis* for which spawning behavior has been observed are broadcast spawners either on unprepared substrates or in the nests of other fishes (nest associates); therefore, it is likely that pugnose shiners also broadcast their eggs (Johnston and Page 1992; Lane et al. 1996). Given that May and June adults are found only in the nearshore vegetation mixture of coontail, pondweeds, wild rice, etc., we concur with others (Doeringsfeld 1993; Leslie and Timmons 1993, 2002) in stating that submerged vegetation likely plays a role in spawning habitat and/or embryo development. During Spring-Summer 2013 we plan to continue to study the PNS and hope to eventually discover its spawning secrets.

Spawning Period. Although spawning/courtship behavior was never observed, an estimate of the spawning period can be inferred from the conditions of the gonads and the body coloration. Pugnose shiners likely begin spawning in May and complete spawning generally by the end of July in Minnesota. Females gravid with ripe ova, and males in breeding coloration and with enlarged testes were present from mid-May to mid-July. By the middle of July the Age 2 males were loosing their breeding color.

DIET (EXAMINATION OF STOMACH CONTENTS)

Stomachs of 373 *N. anogenus* contained mostly Ostracods, filamentous algae, water mites (Hydracarina), and Cladocerans, with Ostracods making up the majority of the diet overall, but there were a few months when the stomachs of pugnose shiners contained large quantities of filamentous algae. Snelson (1971) hypothesized that pugnose shiners were herbivorous given the elongated intestine, but Suttkus and Bailey (1990) stated that the serrations on its pharyngeal teeth may be an adaptation to feeding on microcrustaceans. Doeringsfeld (1993) examined the stomachs of 15 individuals and found primarily Cladocrans and filamentous green algae. Our observations on a much larger data set lend further support that pugnose shiners feed on both microcrustaceans and filamentous green algae, and that there are times when the species feeds almost exclusively on specific prey items. The mean number of food items per stomach, and the percent of stomachs in which a given food item occurred, are summarized by date and lake in Tables 5 and 6.

Relative Abundance: By Year and Lake. Based on the relative abundance (percent occurrence) of total food items in the stomach (Figure 22), Ostracods were the most prevalent food item across both years and both lakes, with the exception of Fish Lake in 2010.

- 2010: Fish Lake: Filamentous algae comprised 40%, water mites 24%, Ostracods 14%, and Cladocerans 13% of the food items found in the stomachs. The prevalence of filamentous algae is discussed below (“by Collecting Dates

and Lakes")
Little Swan Lake: Ostracods comprised 61% of the diet, and Cladocerans Comprised 16%.

- 2011: Fish Lake: Ostracods comprised 61% of the diet, and water mites, Cladocerans, and unidentified organic matter (U.O.M.) each made up 11%.
  Little Swan Lake: Ostracods comprised 60% of the diet, followed by water mites (16%), Copepods (10%), and Cladocerans (9%).

Relative Abundance: By Collecting Dates & Lake. When examining the relative abundance of total food items by collecting date and lakes (Figures 23 and 24), Ostracods were still quite prevalent overall, but a better picture emerged concerning the changing diet over time:

- 2010: Fish Lake: From June to late July/August there is a noticeable shift from small invertebrates to a diet comprised mainly of filamentous green algae, and the stomachs of 11 of the 40 individuals examined in late July and early August contained only filamentous algae.
  Little Swan Lake: In early June 75% of the diet was split between Ostracods and Cladocerans; filamentous algae comprised 50% of the total food items during late June; and Ostracods made up nearly all the diet during July (85%).
  For the Sept 22 sampling date, 11 of the 20 stomachs were empty and the remaining stomachs had very little food. A total of only 18 prey items were found: 10 Ostracods, 3 Cladocerans, 2 Copepods, 2 "items" of unidentified organic matter, and 1 water mite.
- 2011: Fish Lake: In June, Ostracods comprise at least 61% of the diet; for the one July sample water mites were most abundant. Only a few individuals were collected in late July – September, and all were released into the lake.
  Little Swan Lake: Ostracods were the dominant food item during all three sampling dates, comprising at least one-half of the food items for each date, with water mites, Cladocera, and Copepods making up the remaining food items.

When considering the percent of individuals in which the food items occurred (Tables 5 and 6), not only were Ostracods the most abundant total food items in 2010 and 2011, but they were often consumed by most individuals on a given sampling date. For example, Tables 5 and 6 contain data from 15 samples from Fish and Little Swan lakes; in seven samples Ostracods were present in at least 70% of the stomachs examined, and they were present in over 50% of the stomachs examined for two additional samples.

Mean Number of Prey Items per Fish: By Year and Lake. When examining the mean number of prey items per fish, by lake and year (Figure 29a), Little Swan Lake fishes are fairly consistent from 2010 (4.37 items/fish) to 2011 (4.14 items/fish). Fish Lake had a noticeable increase in mean prey numbers from 2010 (6.95 items/fish) to 2011 (9.53 items/fish), which was due to that rise in the number of Ostracods consumed in 2011 vs. 2010 (Table 5).

Mean Number of Prey Items per Fish: By Collecting Date and Lake. When examining the mean number of prey items per fish, by collecting date and lake (Figure 29b, 29c), the numbers are relatively similar across dates, lakes and even years (most sampling dates hover around an average of 4 food items/per stomach), but there are a few notable exceptions.

- 2010: Fish Lake: The mean number of prey was fairly steady throughout the summer, with the one exception being that the mean number of prey items was considerably higher in late June. This is explained by the abundance of algae present in stomachs.
  Little Swan Lake: The mean number of prey items is highest in early June.

- 2011: Fish Lake: The mean number of prey is highest for the June and June/July sampling periods, and corresponds to large numbers of ostracods in the diet (Table 5).
  Little Swan Lake: The mean number of prey items/fish did not fluctuate, with the average number of prey items/stomach being around four.

Mean Number of Prey Items per Fish: By Year, Lake, and Sex. In general, females and males consumed similar numbers of prey items (Figure 29d). There were two cases when the comparisons were significantly different, and in both cases the females consumed on average more prey items than males: Fish Lake in 2011 (females [12.99 items] vs. males [7.34 items]); and Little Swan Lake in 2010 (females [8.99 items] vs. males [3.89 items]). Although not significantly different due to a large error bars, in 2011 females in Little Swan Lake also consumed on average more prey items than the males. This was visually noticeable when dissecting the stomachs.

Relative Abundance: By Collecting Date, Lake, and Sex. The relative abundance of prey items in the stomachs of females was quite similar to that of males.

- 2010: Fish Lake: The diets of each sex were nearly identical, even as the season progressed. For example, in early June (Figure 25a, 25e) the relative abundance of water mites (over 59%) and Cladocera (24-25%) are quite similar, and this trend holds when comparing sexes for early July (Figure 25b, f), mid-July (Figure 25c, g), and early August (Figure 25d, h).
  Little Swan Lake: The diets of each sex converge as the season progresses (Figure 26), primarily because both sexes shift to having the vast majority of the diet being Ostracods.
- 2011: Fish and Little Swan lakes: Both sexes consumed primarily Ostracods at Fish Lake in July when both males and females shifted to a variety of other food items (Figures 27 and 28).

Mean Number of Prey Items per Fish: By Sex, Collecting Dates & Lake. As for LES, Figures 29 e and f show in greater detail that the mean number of prey items/per fish by date and by sex, does not vary much through the breeding season/summer.

Juveniles. Juvenile pugnose shiners were not found in the study areas. During spring and summer 2013 when we return to Fish Lake to capture and observe spawning individuals, we will also make a conscious effort to find juveniles and fill in the gaps in the early life history of the species.

Diet in Other Lakes. A limited examination of pugnose shiner stomachs (3-5 fishes per lake) from specimens collected in a few lakes in other parts of Minnesota indicate that the diet of the pugnose is relatively uniform across Minnesota. Stomachs from specimens from Cameron Lake (Big Fork – Rainy River), Movil Lake (Mississippi River – Headwaters), Baby Lake (Boy River), and Pillager Lake (Crow Wing River) all contained the same main prey items discussed above, with Ostracods accounting a majority of the prey items.

Feeding Behavior. Given the skittish/wary behavior of pugnose shiners we were not successful in observing pugnose shiners feeding in the field, but we were able to observe fishes feeding in aquaria. When frozen bloodworms (dipteran larvae) were placed into the aquaria, the fishes readily consumed the food items. With its upturned mouth, the pugnose shiner would drop down below the bloodworms as the bloodworms slowly sank through the water column, then once below the food item the fish would rise up and take the food in its mouth. Given the relatively long length of the bloodworm (longer than the fish's head), the fish would need several to take several "gulps" before the food gradually disappeared into the fish's mouth. When live microcrustaceans (*Daphnia*) were placed into the aquaria the fish would slowly swim up to the swimming food and pluck it from the water column, sucking it into its mouth. We also observed fishes using their mouth to pick at the underside of the aquatic vegetation; given the upturned mouth of the pugnose shiner, we assumed that this is a normal feeding behavior for the species.

DIET (ANALYSIS USING STABLE ISOTOPES)

Through stable isotope analysis the trophic position was determined for *N. anogenus* (Figure 30). There was a significant difference in source carbon in Little Swan Lake between June 24 and July 26. This shows a shift in where the fishes were feeding. The more negative numbers suggest an autochthonous carbon type found in the pelagic zone whereas the less negative numbers suggest more allochthonous carbon type from the littoral zone (Vander Zanden and Rasmussen, 1999). This suggests that the fishes were migrating from the pelagic zone to the littoral zone as May turned into June, which is consistent with our observations on increasing numbers of pugnose shiners as the warm season progresses. The trophic position also changed significantly, indicating that as the pugnose shiner changed habitats they also changed where they were feeding on the food chain. These results lead us to believe that the pugnose shiner changes ecological niches as the summer months commence, which seems to be somewhat supported by the increased uptake in filamentous algae as the season progresses.

INTERACTIONS WITH OTHER ORGANISMS

Bluegill, pumpkinseed, and yellow perch were abundant at the study sites. Northern pike and largemouth bass were also present. Rare/indicator species or species of special concern that were commonly found with the PNS included the blackchin shiner, blacknose shiner, Iowa darter, banded killifish, and least darter. Pugnose shiners are found in many of the known LES lakes.

Potential predators (northern pike, yellow perch, and smallmouth bass) were captured in the field in June 2012 in a cursory attempt to determine if pugnose shiners fishes are preyed upon in a regular manner. Gastric lavage was performed in the field on 4 northern pike (7-13" TL) and three largemouth bass (6 – 8" TL), and five yellow perch (4 – 6" TL). The partially digested remains of blacknose shiners were found in the stomachs of all three species, but no evidence of PNS were found in the stomachs. Regardless, it is certainly to be expected that PNS occasionally get captured by predators.

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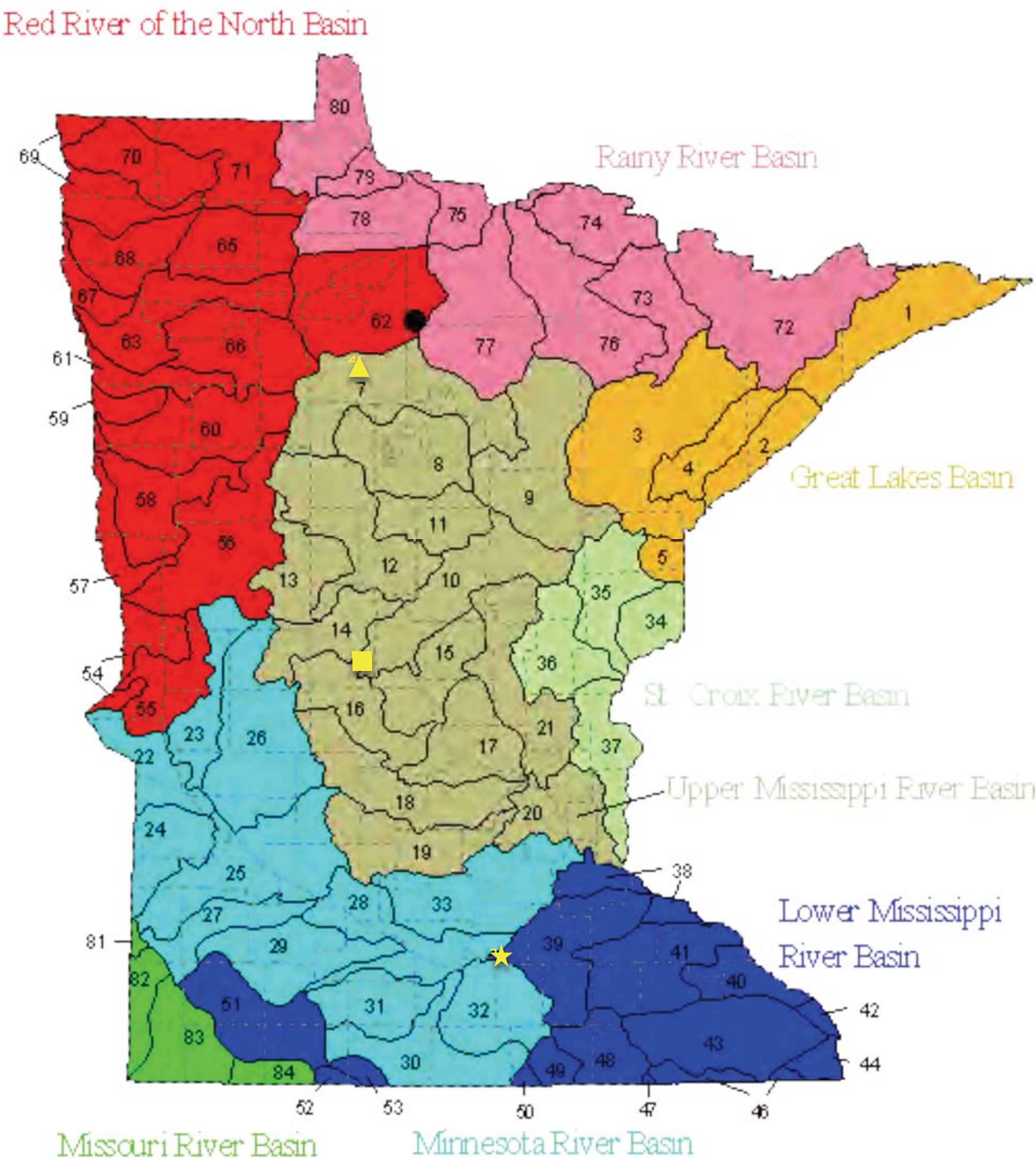


Figure 1. Approximate locations of the four study lakes. Yellow triangle = Movil and Turtle lakes; yellow square = Little Swan Lake; yellow star = Fish Lake (black circle near the #62 is an unknown dot on the original map).
Map taken from : http://www.dnr.state.mn.us/watersheds/map.html.

a) Turtle Lake study area, June 22, 2010.

b) Movil Lake study area, June 22, 2010.

Figure 2. Turtle Lake and Movil Lake study areas.

a) Fish Lake study area, June 16, 2010.

b) Little Swan Lake study area, June 11, 2010.

Figure 3. Fish Lake and Little Swan Lake study areas.

a) Baby Lake, July 13, 2010. LES was abundant in these shallow shorelines. Note the extensive muskgrass.

b) Movil Lake, July 13, 2010, directly to the right (west) of the images in Figure 5. LES is absent from these deeper (6-10' depths) shoreline; Pumpkinseed sunfishes abundant here.

c) Rush Lake, June 21, 2010. LES is absent from these shorelines where nearly all traces of aquatic vegetation have been removed from the shallow shoreline waters.

Figure 4. Shoreline photos showing locations where LES were and were not present.

Figure 5. Turtle River at CR 307.

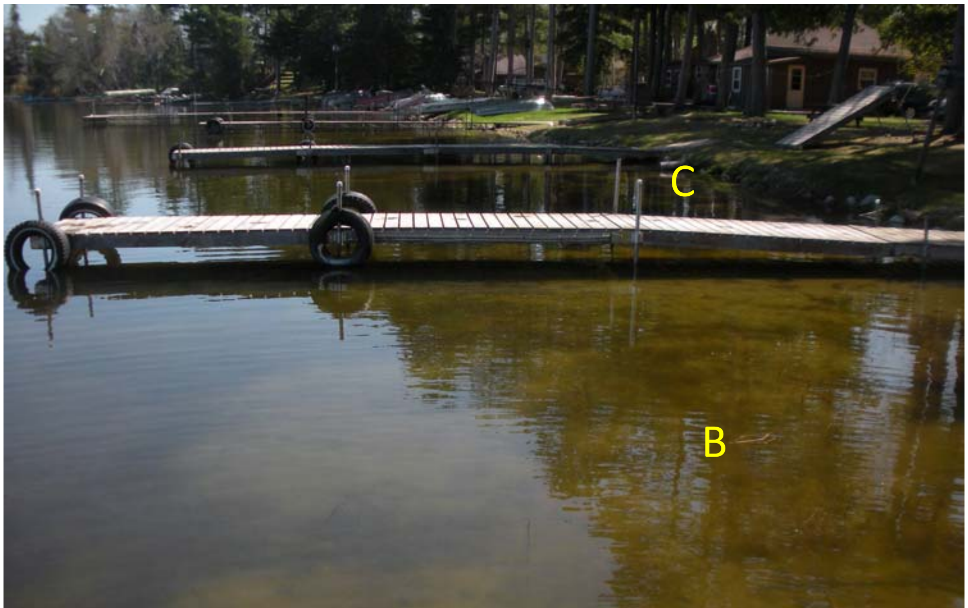
a) Movil Lake, May 17, 2011.

b) Movil Lake, June 14, 2011.

c) Movil Lake, July 1, 2011.

d) Movil Lake, July 18, 2011.

Figure 6. Movil Lake study area during May, June, and July 2011, showing the progression of aquatic vegetation over time.

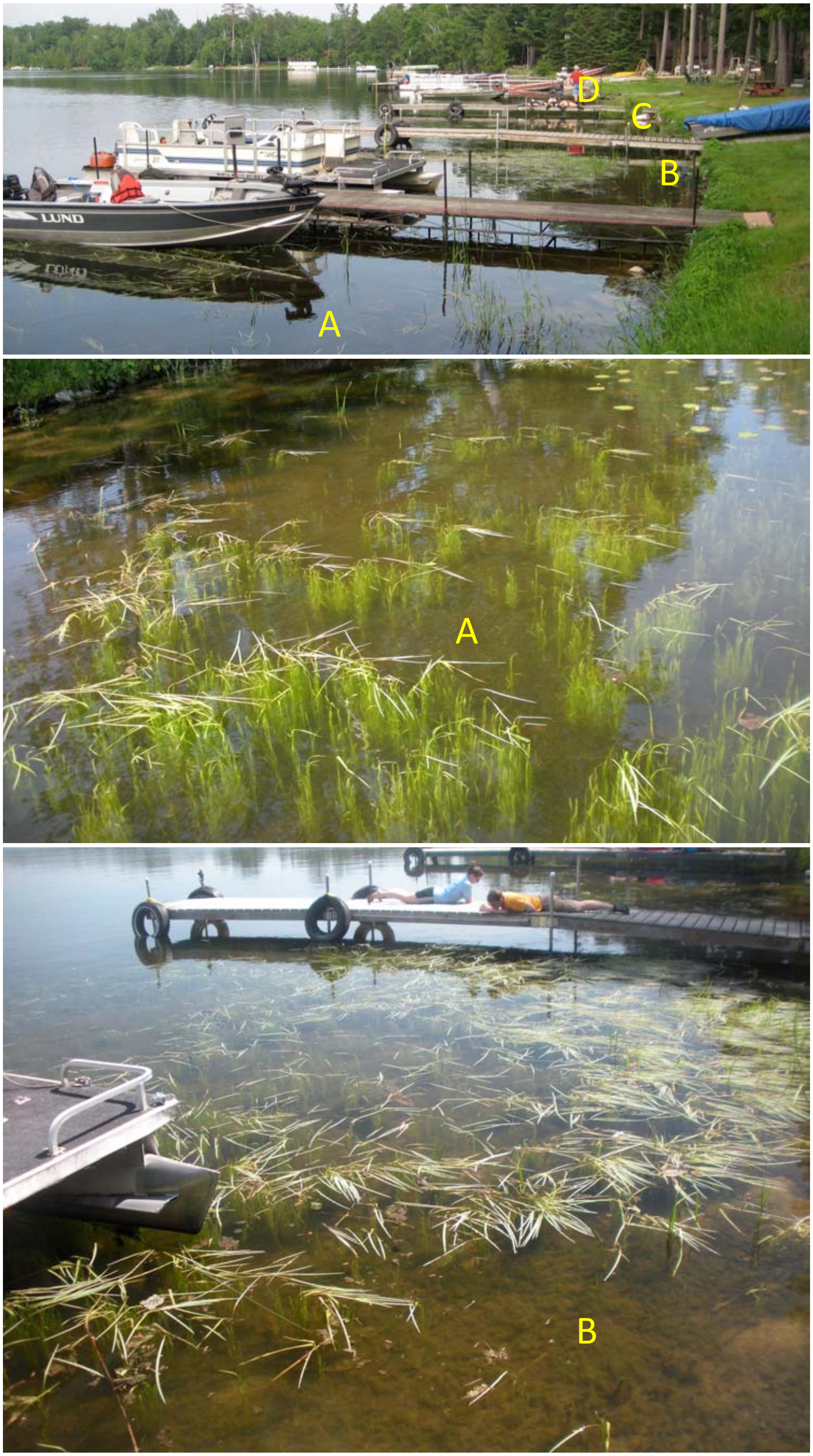


a) Turtle Lake, May 17, 2011.

b) Turtle Lake, June 14, 2011.

c) Turtle Lake, June 29, 2011.

Figure 7. Turtle Lake study area during May, June, and July 2011, showing the progression of aquatic vegetation over time. Notice how the floating leaves of wild rice did not reach the surface until the latter half of June, 2011. Yellow letters are for reference and discussed in “Demography” section.



a) Turtle Lake, June 30, 2011.

b) Turtle Lake, June 29, 2011.

c) Turtle Lake, June 29, 2011.

Figure 8. Turtle Lake study area during late June 2011. Yellow letters are referenced in the "Demography" section.

Figure 9. Movil Lake study area, June 22, 2010. Yellow letters are referenced in the "Demography" section.

a)

b)

c)

d)

e)

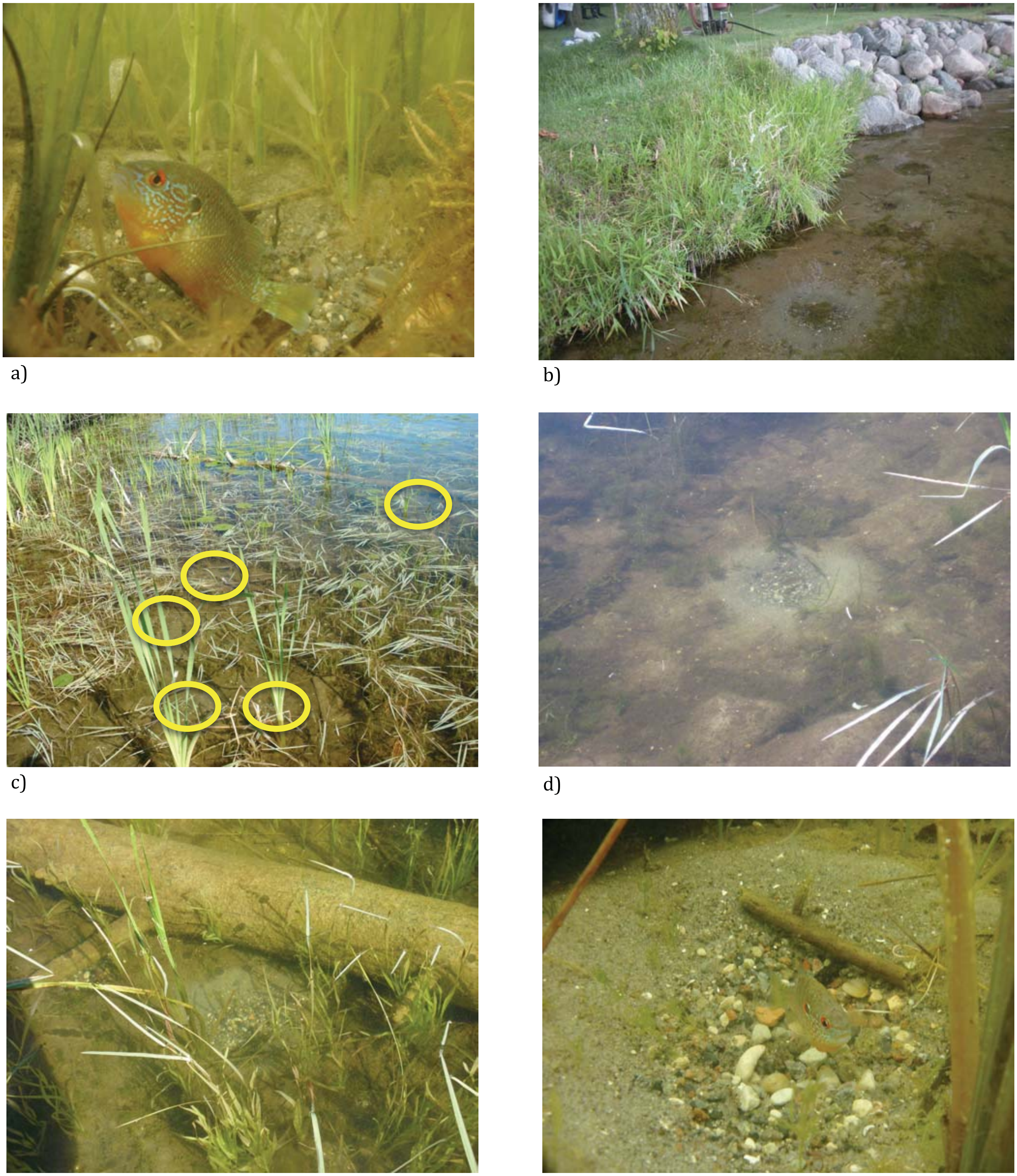
f)

g)

h)

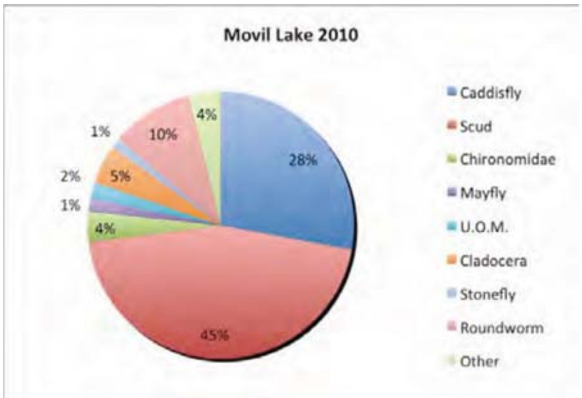
Figure 10. Photos of LES:

a) Gravid female, 67 mm TL (Age 3), Turtle Lake, June 22, 2010.
b) Nongravid female, 64 mm TL (Age 3), Turtle Lake, June 13, 2011.
c) Partially dissected female showing gravid ova, 73.4mm TL (Age 4), Turtle Lake, June 22, 2010.
d) Partially dissected male showing mature testes, 74.5mm TL (Age 3), Turtle Lake, June 22, 2010.
e) Mature male, 100.5mm TL (Age 6), Turtle Lake, June 28, 2011.
f) Mature male, 109 mm TL (Age 7?), Turtle River, June 22, 2010.
g) Mature male, 59.5mm TL (Age 2), Turtle Lake, June 22, 2010.
h) Mature male, 83mm TL (Age 4), Turtle Lake, Turtle Lake, June 22, 2012.

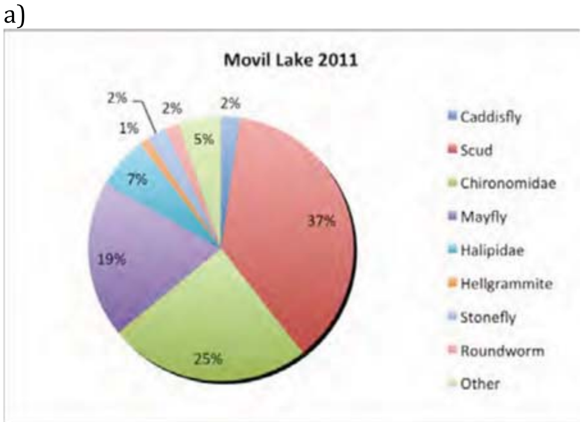


a) b) c) d) e) f)

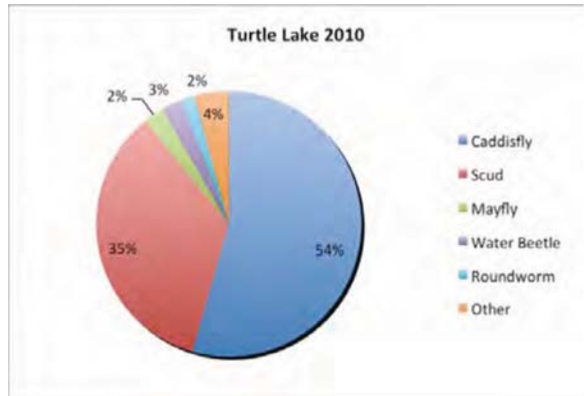
Figure 11. Photographs of nests of LES. Figures a, c-f from Turtle Lake, June 22, 2012. Figure b from Island Lake, June 15, 2010. For Figure c, the yellow circles indicate nest locations.



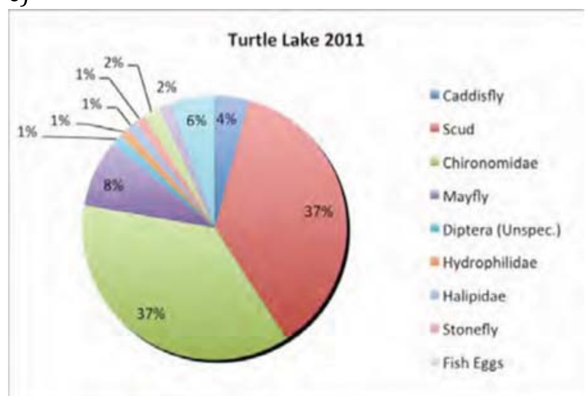
a)



b)



c)



d)

Figure 12. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *L. m. peltastes*, separated by lake and year.

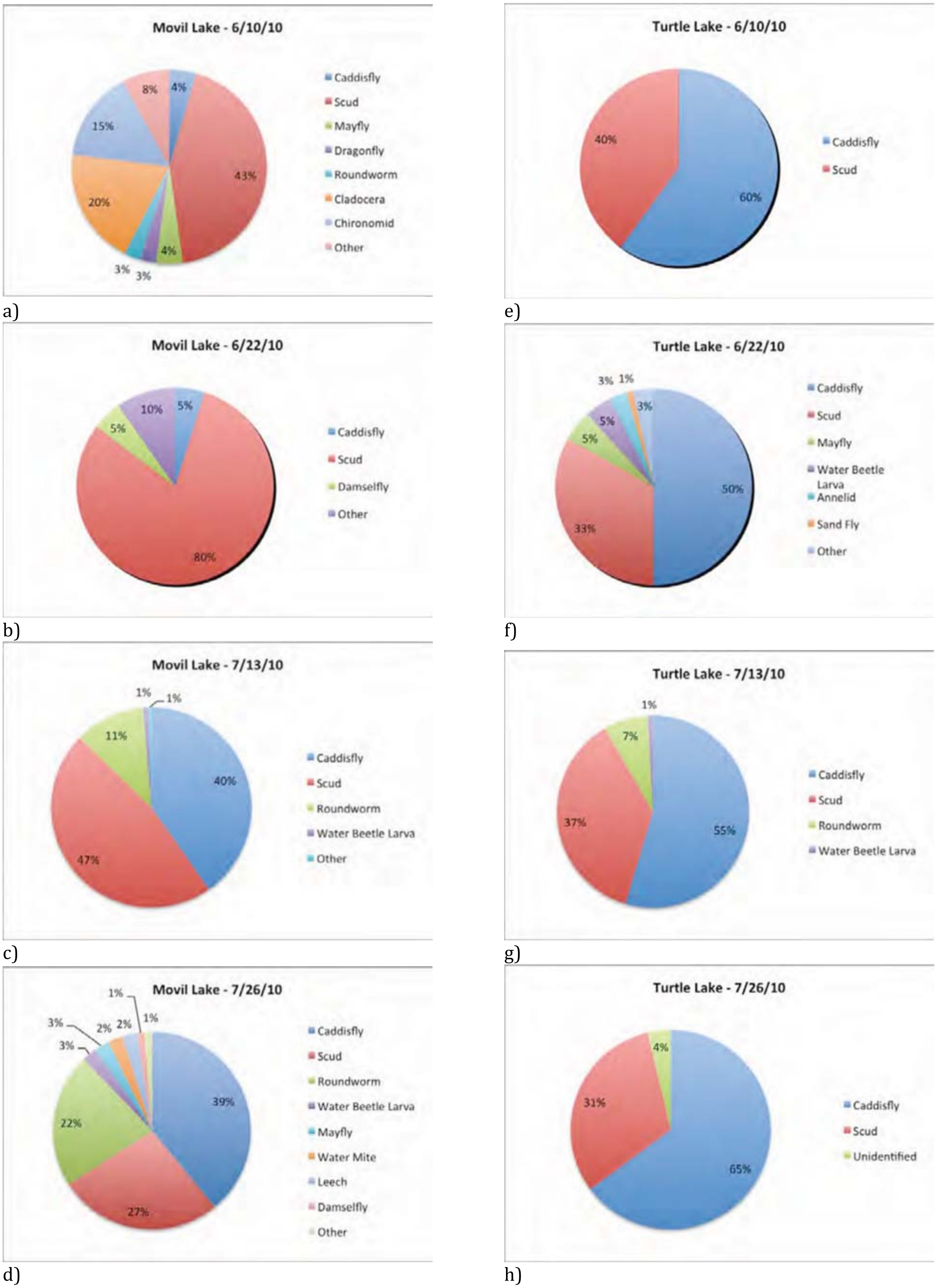


Figure 13. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *L. m. peltastes*, 2010, separated by lake and collecting date.

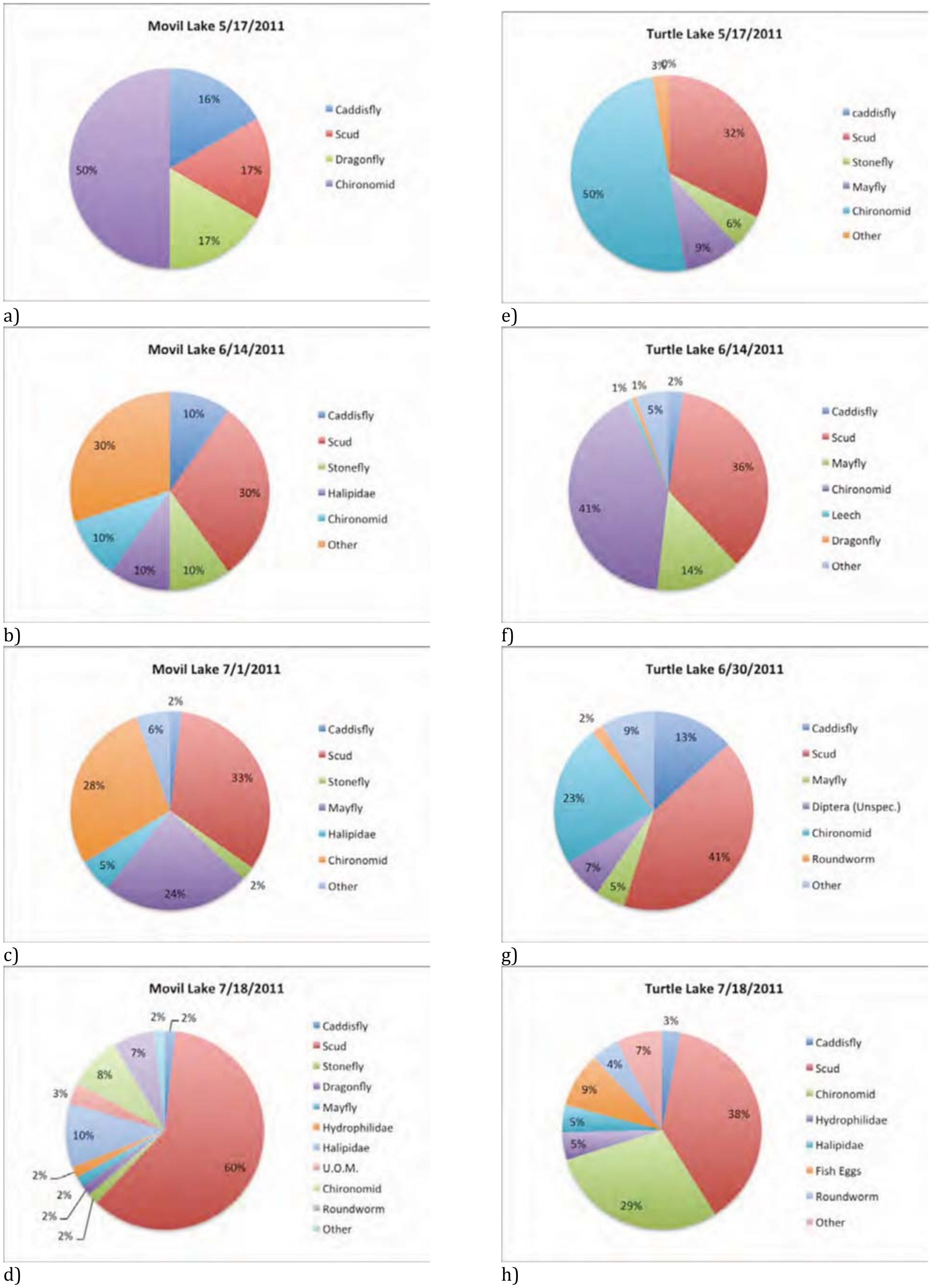


Figure 14. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *L. m. peltastes*, 2011, separated by lake and collecting date.

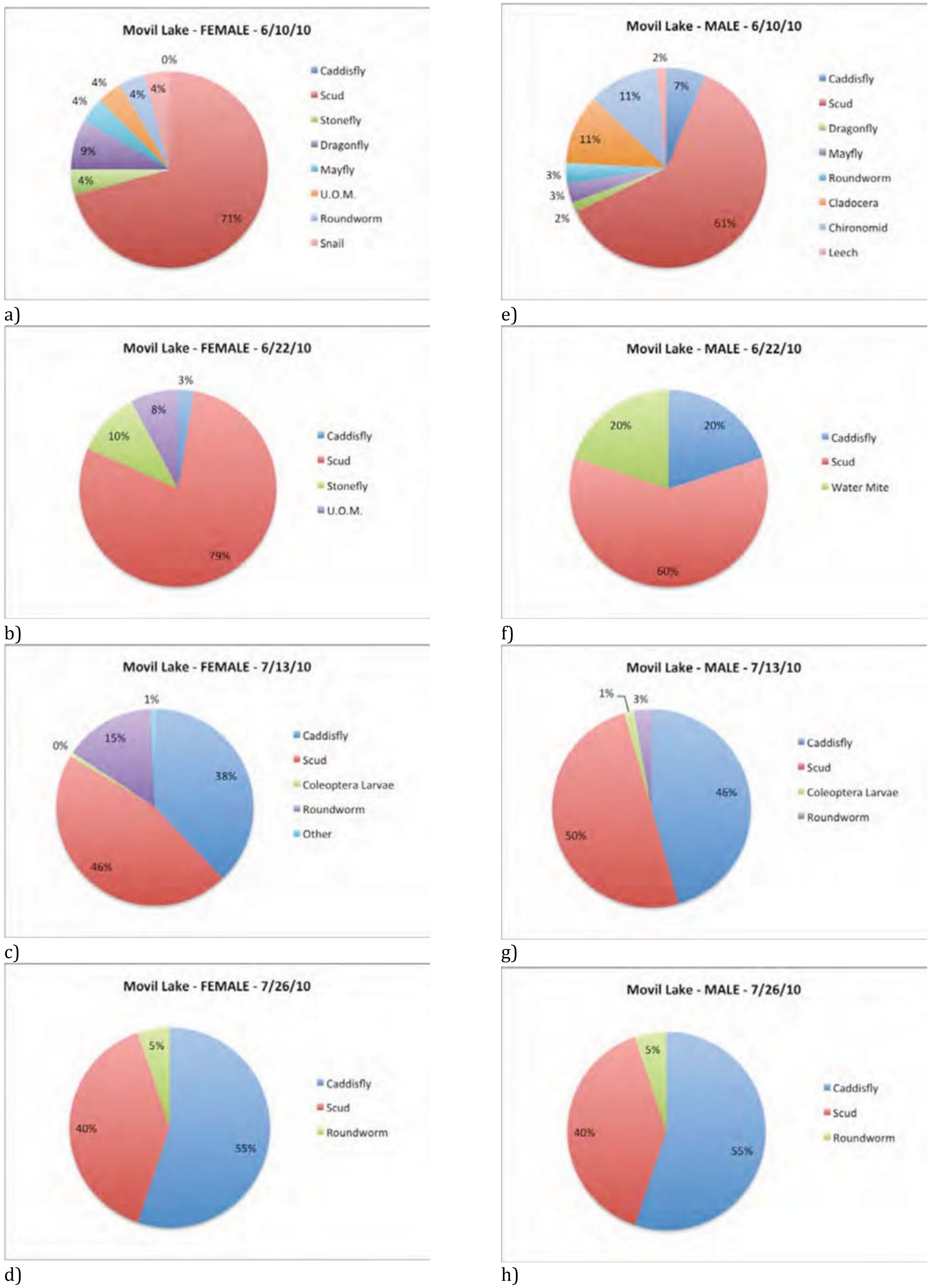


Figure 15. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *L. m. peltastes*, Movil Lake 2010, separated by sex and collecting date.

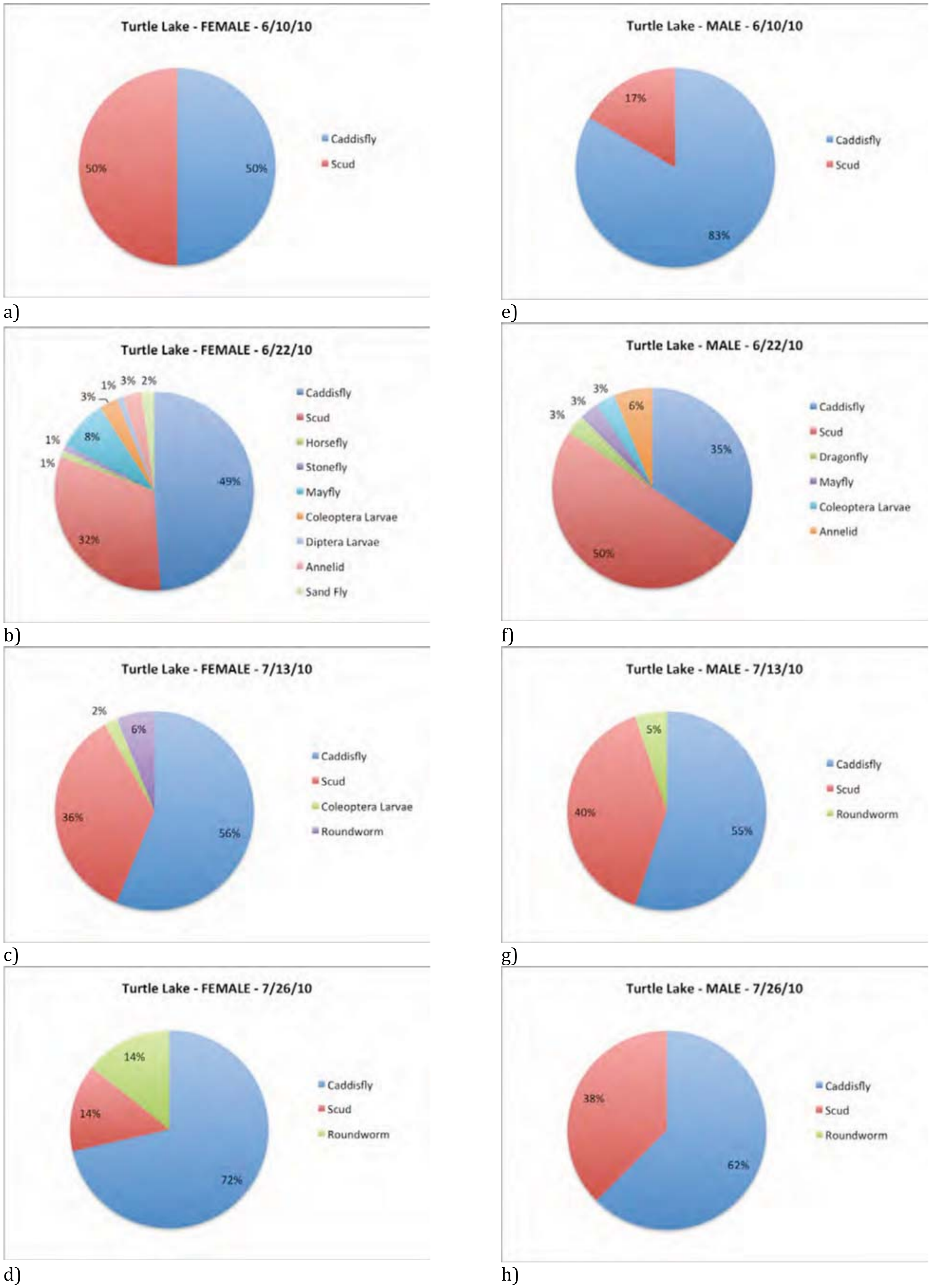
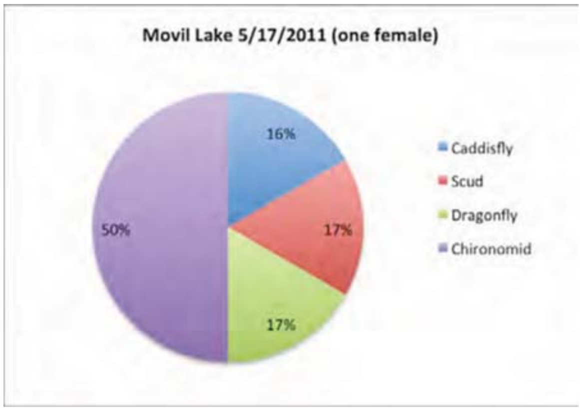
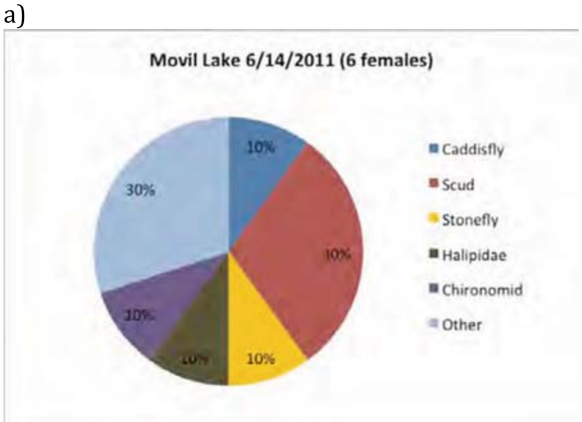


Figure 16. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *L. m. peltastes*, Turtle Lake 2010, separated by sex and collecting date.



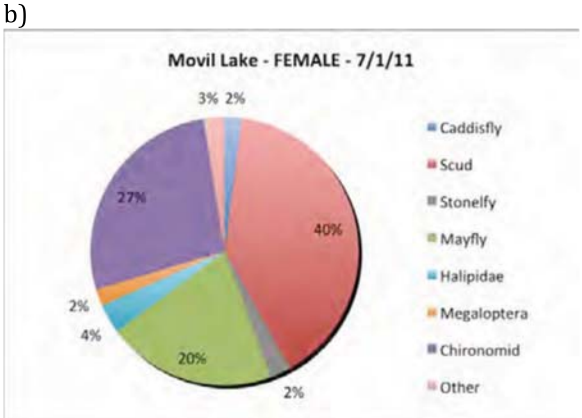
a)

e) Movil Lake 5/17/11 – no males

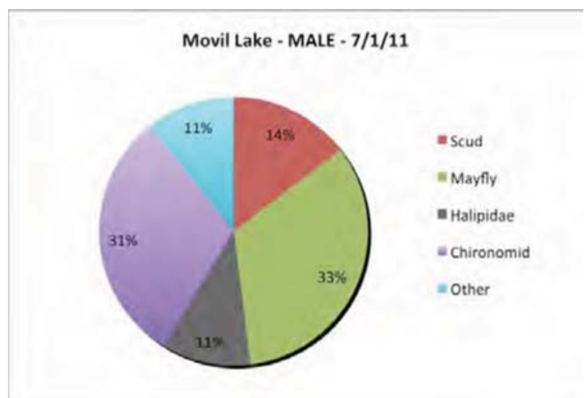


b)

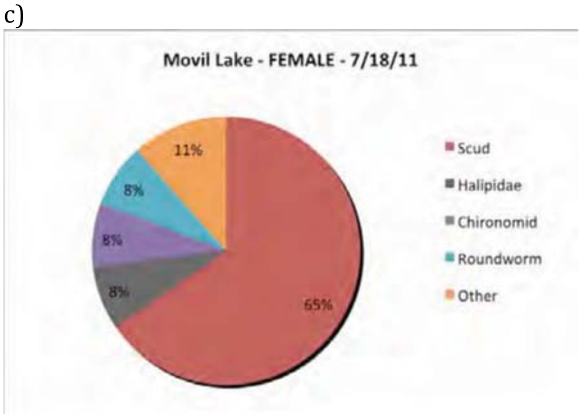
f) Movil Lake 6/14/11 – no males



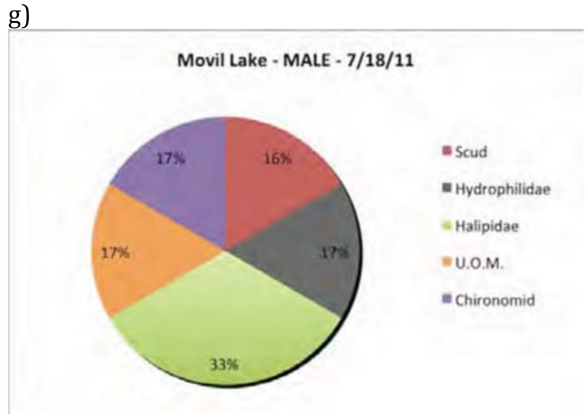
c)



g)

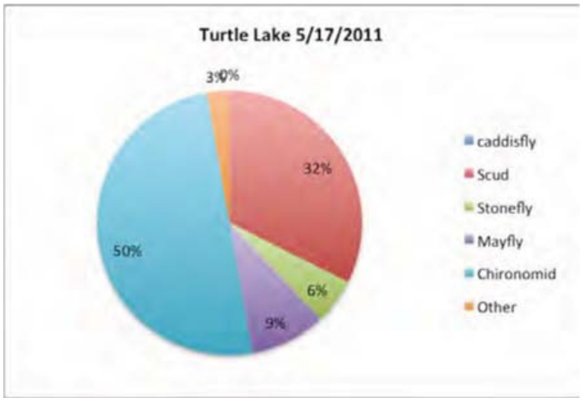


d)



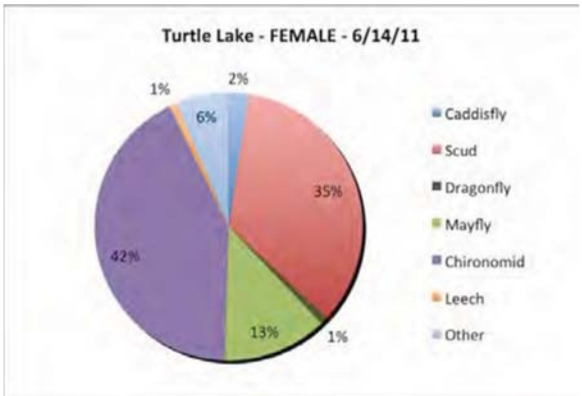
h)

Figure 17. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *L. m. megalotis*, Movil Lake 2011, separated by sex and collecting date.

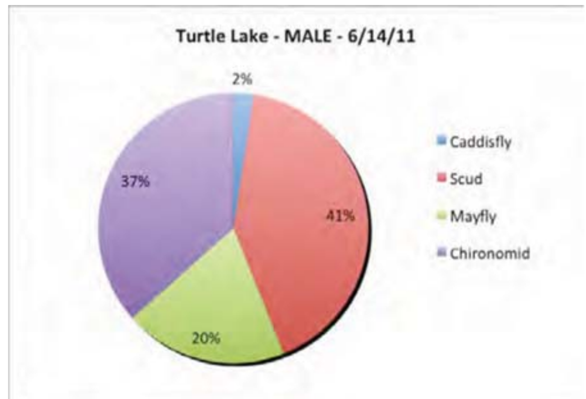


a)

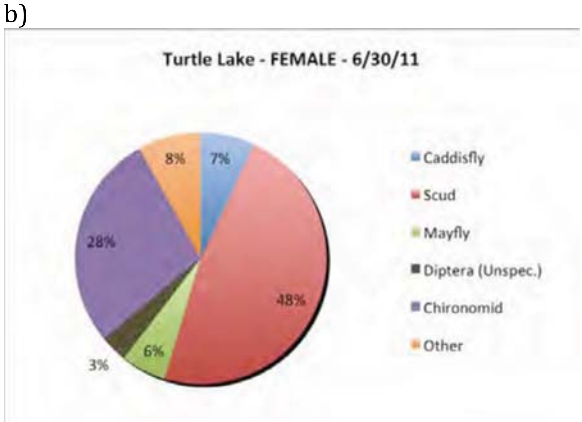
e) Turtle Lake 5/17/11 – no males



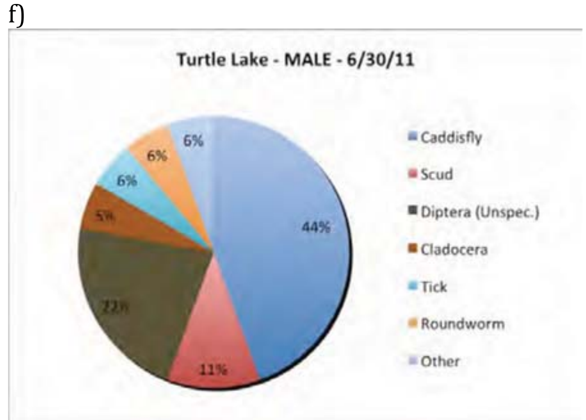
b)



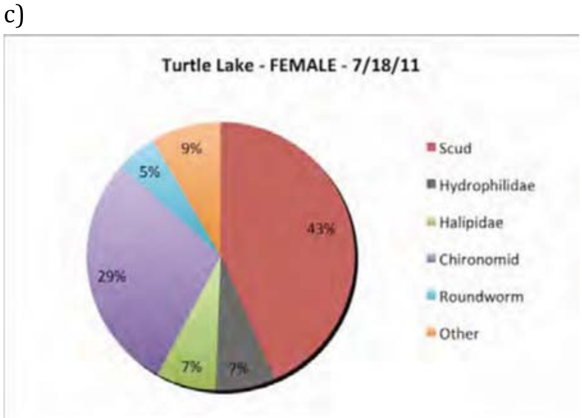
f)



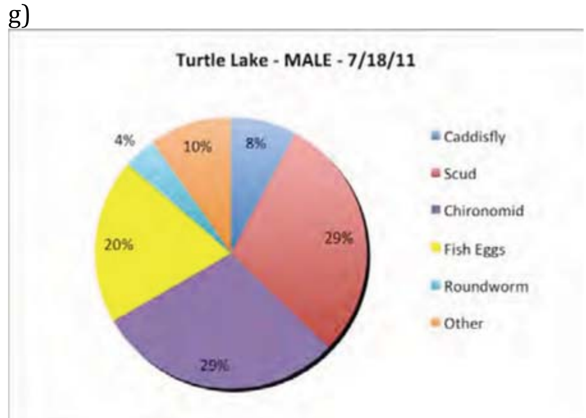
c)



g)

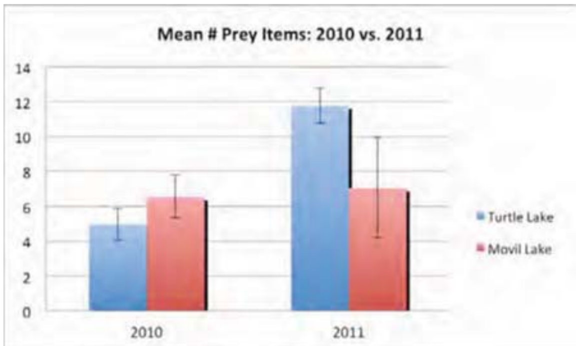


d)

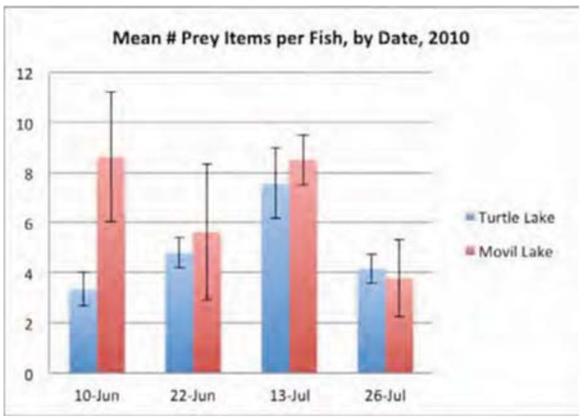


h)

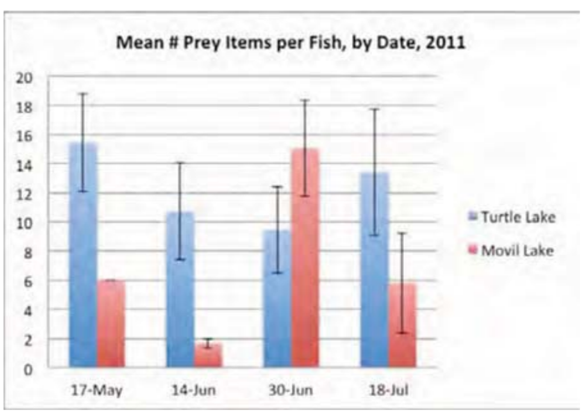
Figure 18. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *L. m. peltastes*, Turtle Lake 2011, separated by sex and collecting date.



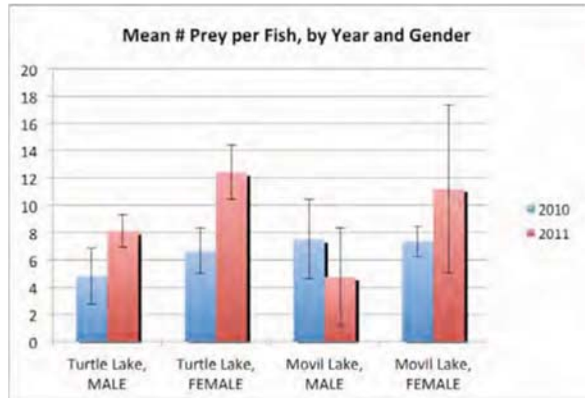
a)



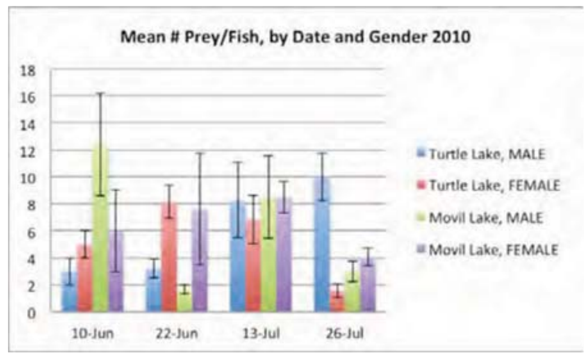
b)



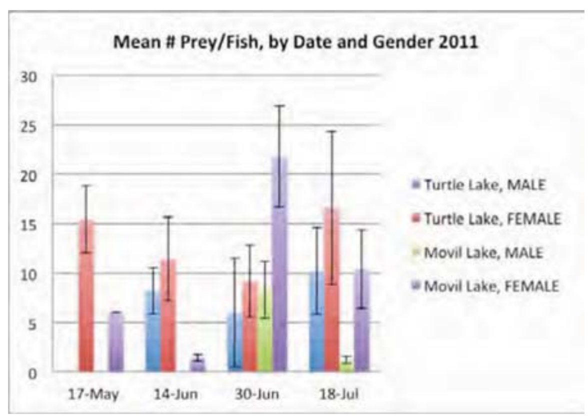
c)



d)



e)



f)

Figure 19. Mean # of prey items in the stomachs of individuals of *L. m. peltastes*.
Error bars represent +/- one Standard Error.

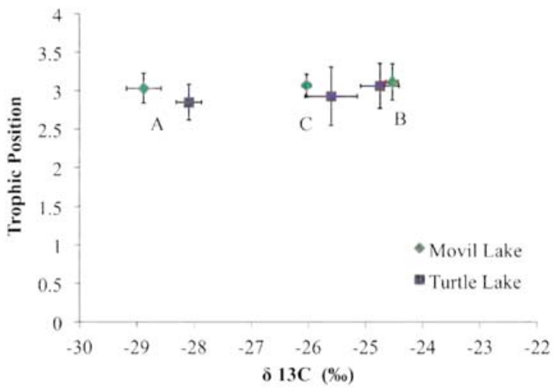


Figure 20a.
δ 13 C (‰) vs. Trophic Position of LES in 2010.
Error bars show + / - one standard error.

A= June 10, 2010
B = July 13, 2010
C = July 26, 2010

Trophic Position

- All fish fed at the same trophic level (TP=3).
- Significant difference in carbon sources in Movil Lake between A (6/10), B (7/13) and C (7/26).
- Difference in carbon sources in Turtle Lake between A (6/10) and C (7/26); B (7/13) and C (7/26).

Comparison	Significance p-value
Movil: Weeks A, B, C:	p>0.01
Turtle: Weeks A, B, C:	p=0.01483
Movil Turtle Week A:	p=0.5311
Movil Turtle Week B:	p=0.675
Movil Turtle Week C:	p=0.1041
Movil: Weeks A and B:	p>0.01
Movil: Weeks B and C:	p>0.01
Movil Weeks A and C:	p>0.01
Turtle: Weeks A and B:	p=0.03718
Turtle: Weeks B and C:	p>0.01
Turtle: Weeks A and C:	p>0.01

Figure 20b. Comparisons & p-values for Figure 9a.

- Overall, Movil Lake diet was significantly different than Turtle Lake diet ($p<0.001$ at $\alpha=0.05$).
- Movil Lake collection dates were significantly different from each other ($p<0.001$ at $\alpha=0.05$).
- Turtle Lake collection dates were significantly different from each other ($p=0.014$ at $\alpha=0.05$).
- For each collection date between Movil and Turtle Lakes the diet was not significantly different ($p>0.1$ at $\alpha=0.05$).

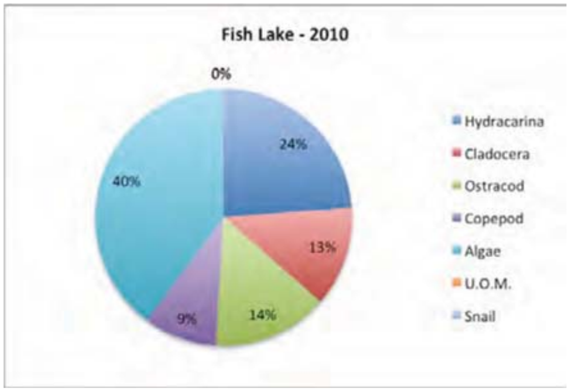
Figure 20. Stable Isotope results for *L. m. peltastes*.


a)

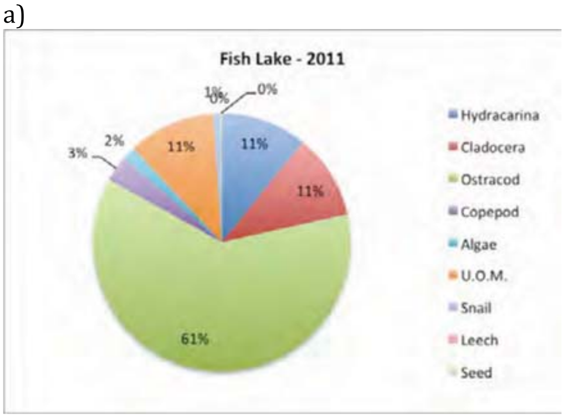

b)

Figure 21. Photos of PNS:

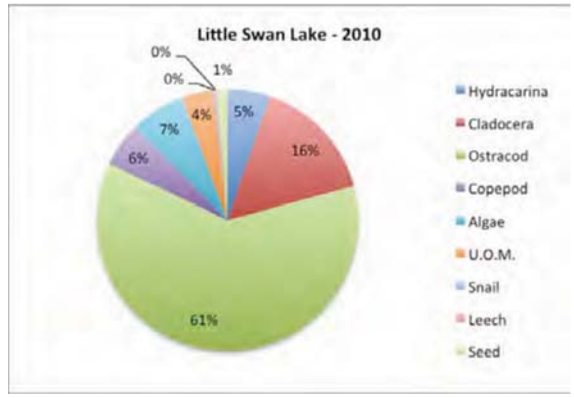
a) Gravid female, 44 mm TL (Age 2), Little Swan Lake, June 11, 2010.
b) Mature male, 42 mm TL (Age 2), Little Swan Lake, June 11, 2010.



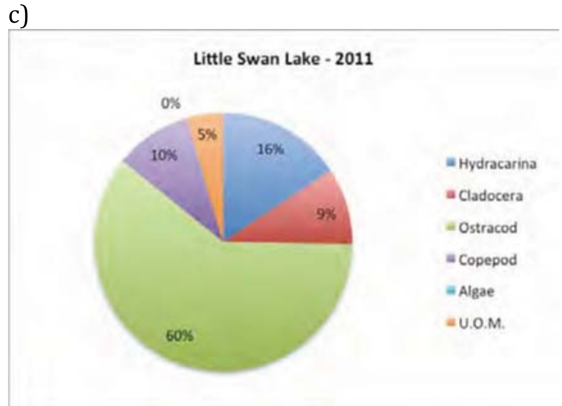
a)



b)



c)



d)

Figure 22. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *N. anogenus*, separated by lake and year.

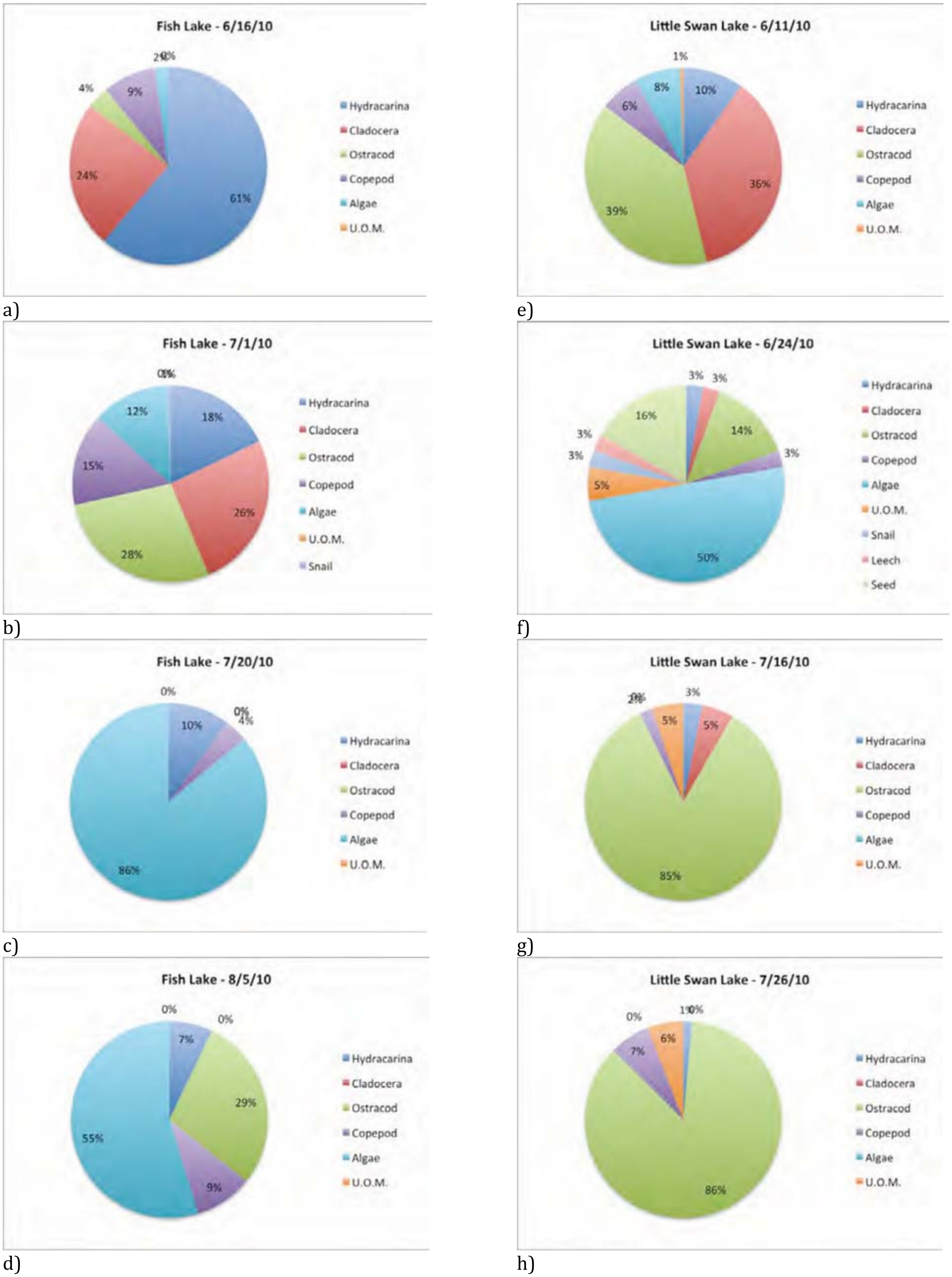
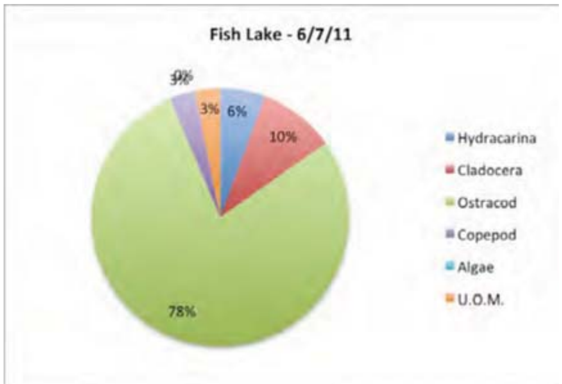
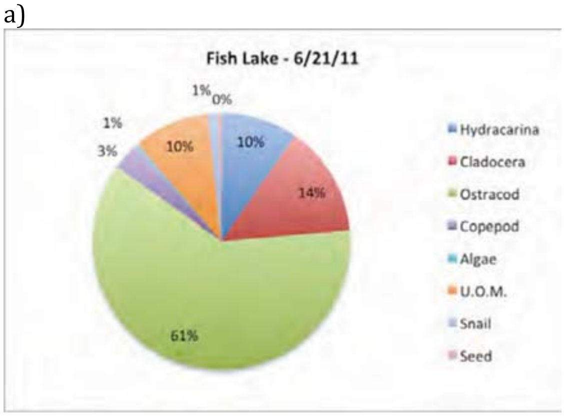


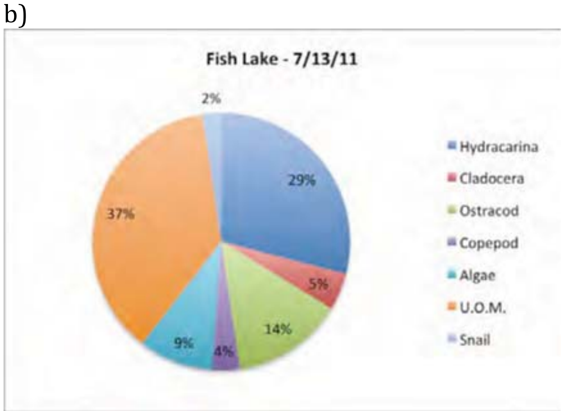
Figure 23. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *N. anogenus*, 2010, separated by lake and collecting date.



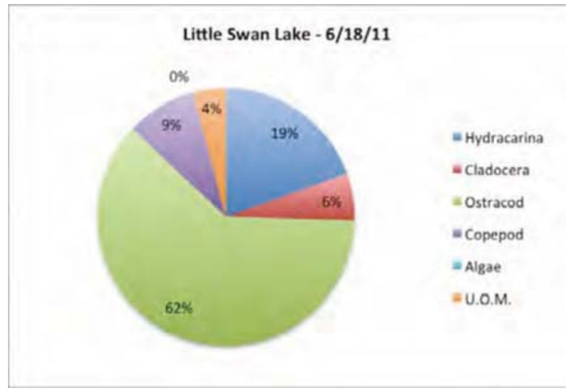
a)



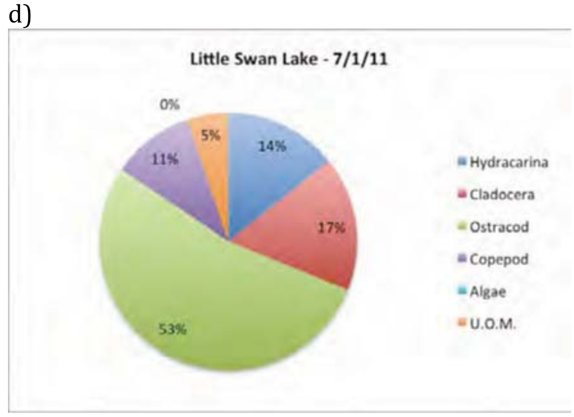
b)



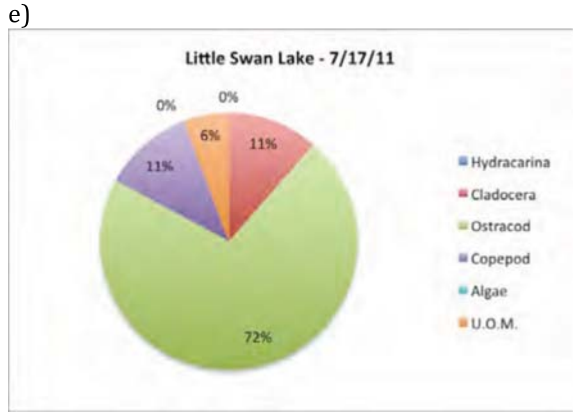
c)



d)

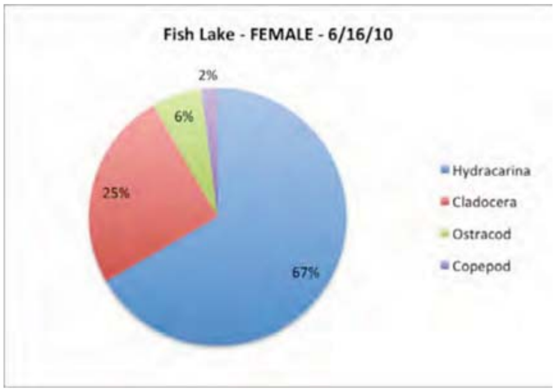


e)

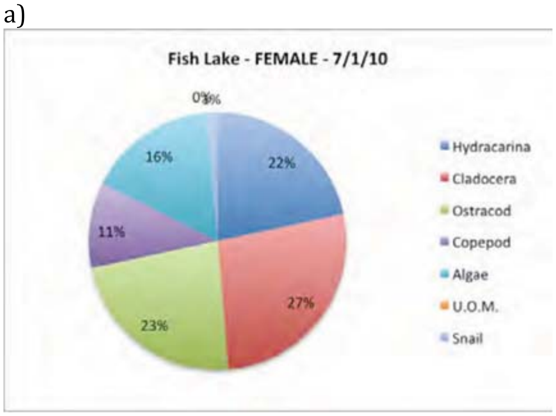


f)

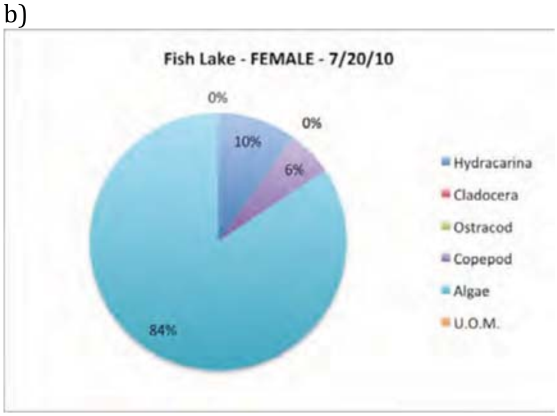
Figure 24. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *N. anogenus*, 2011, separated by lake and collecting date.



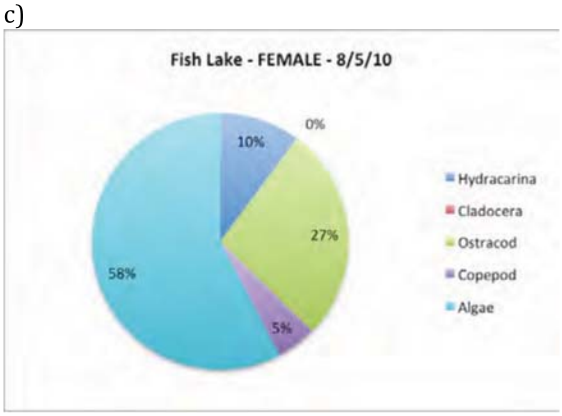
a)



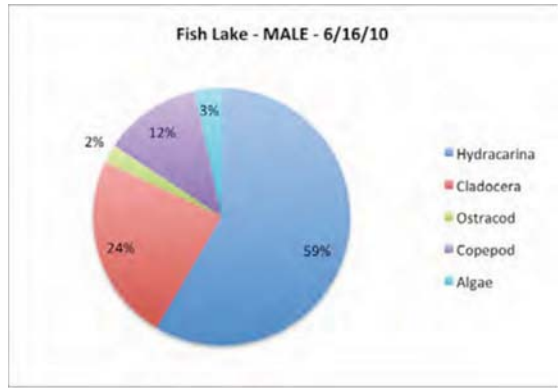
b)



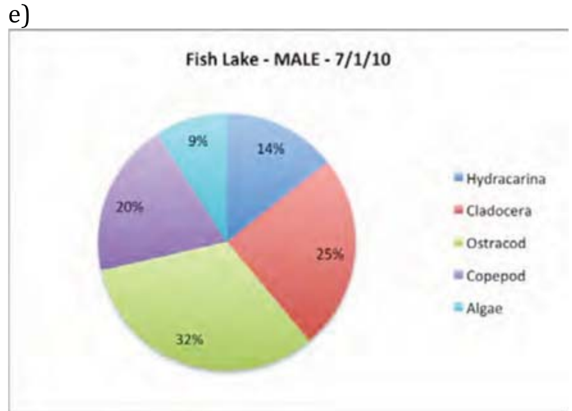
c)



d)



e)



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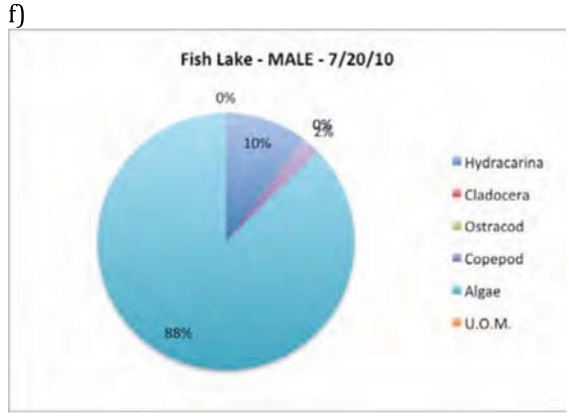
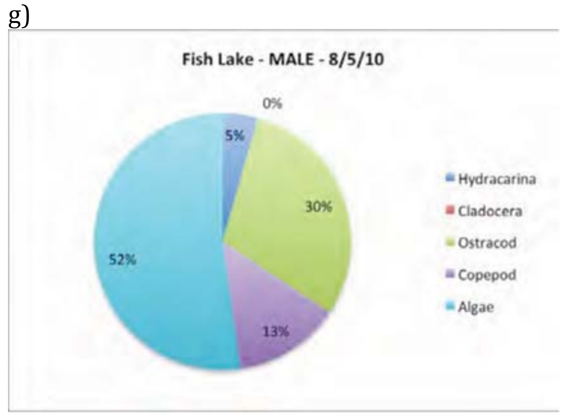


g)



h)

Figure 25. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *N. anogenus*, Fish Lake 2010, separated by sex and collecting date.

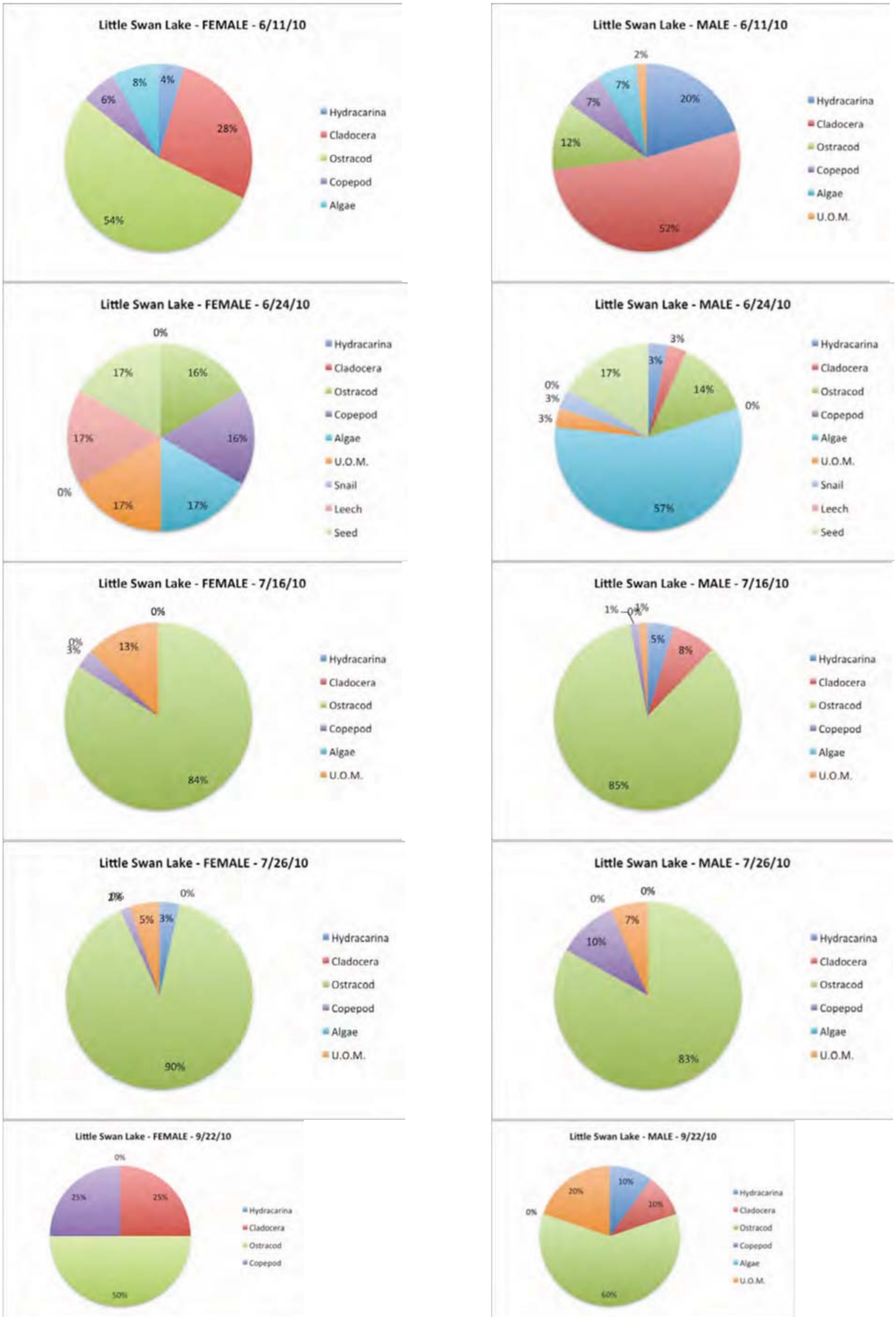
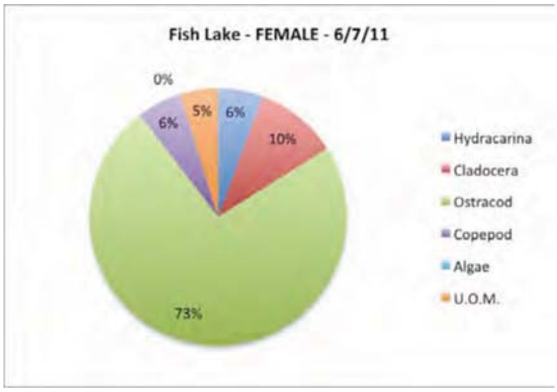
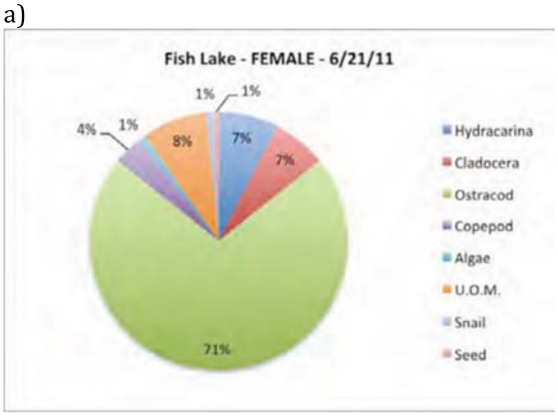


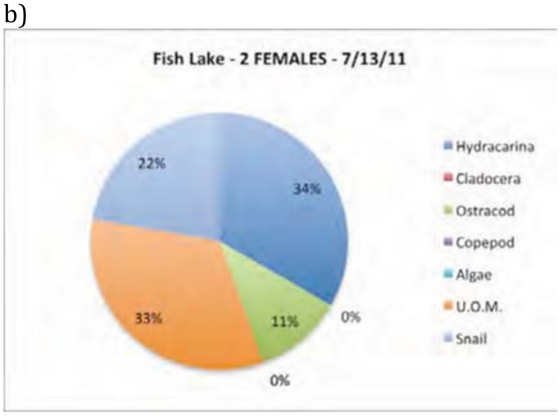
Figure 26. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *N. anogenus*, Little Swan Lake 2010, separated by sex and collecting date.



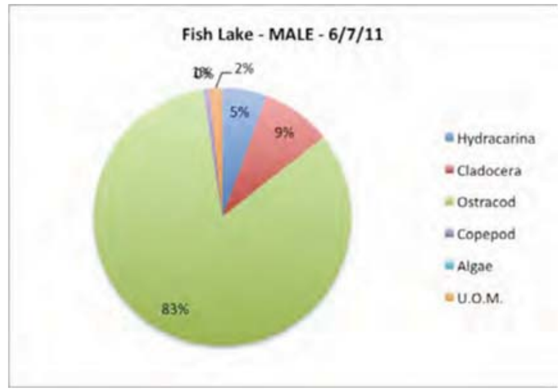
a)



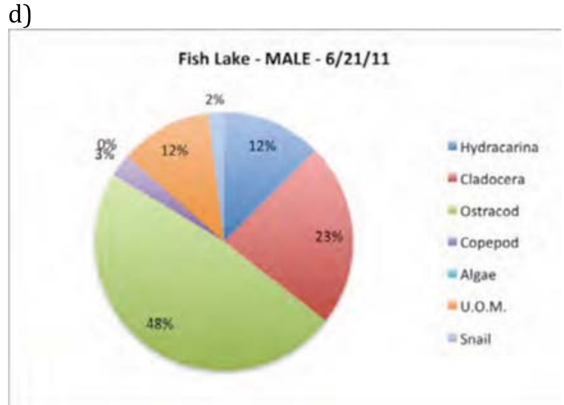
b)



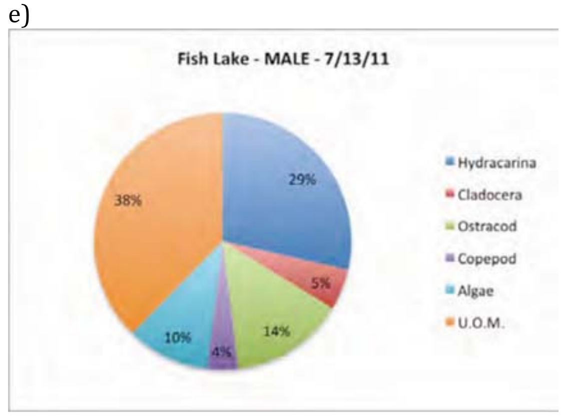
c)



d)



e)



f)

Figure 27. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *N. anogenus*, Fish Lake 2011, separated by sex and collecting date.

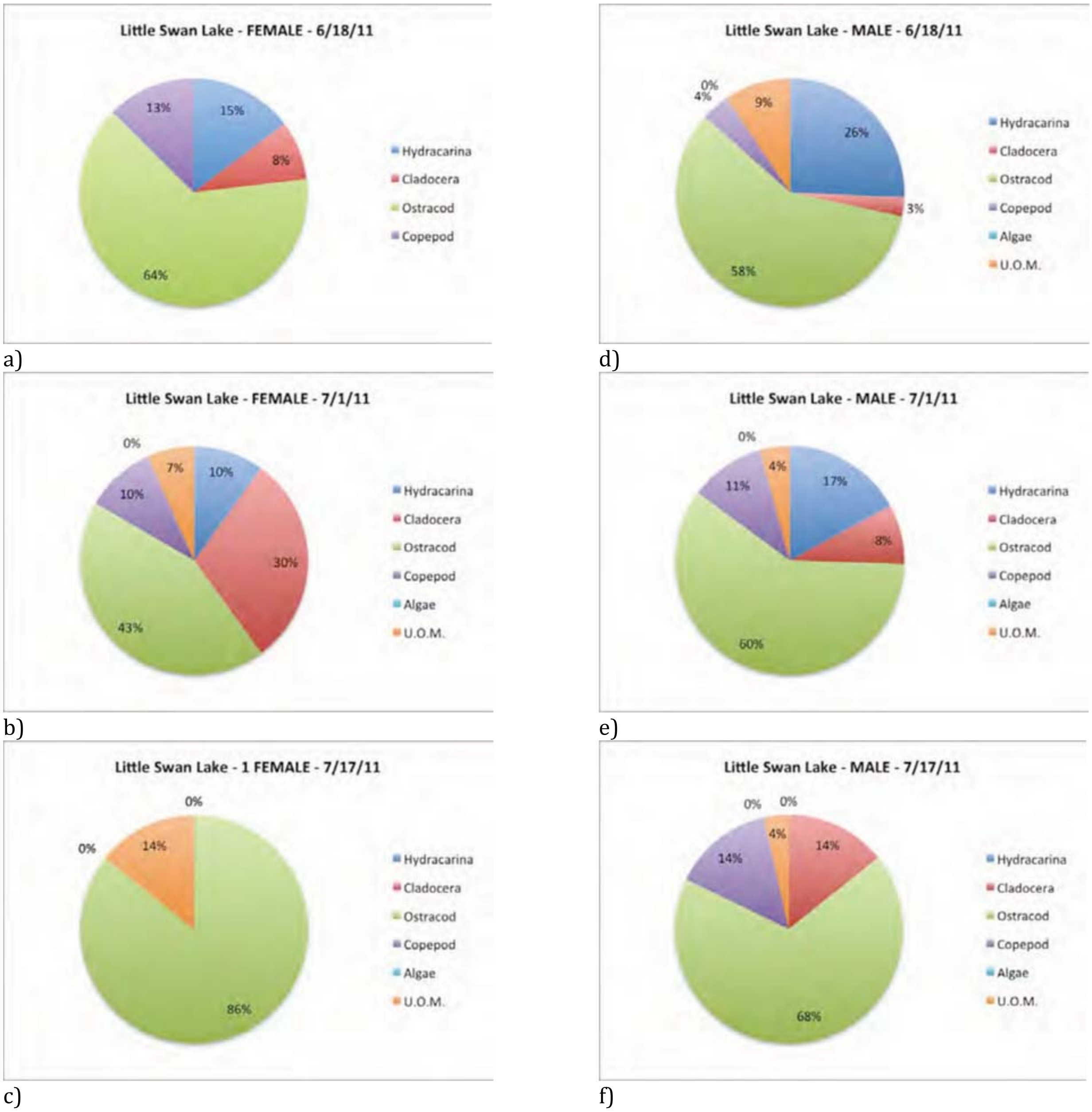
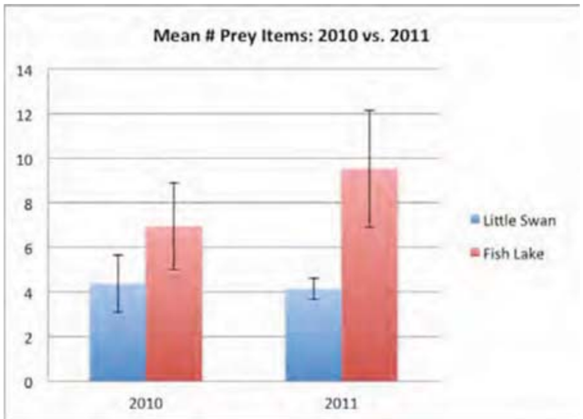
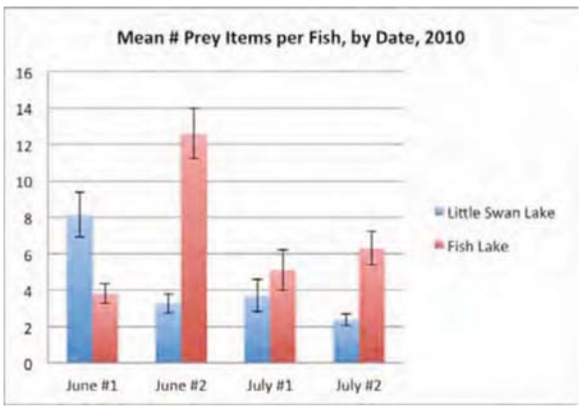


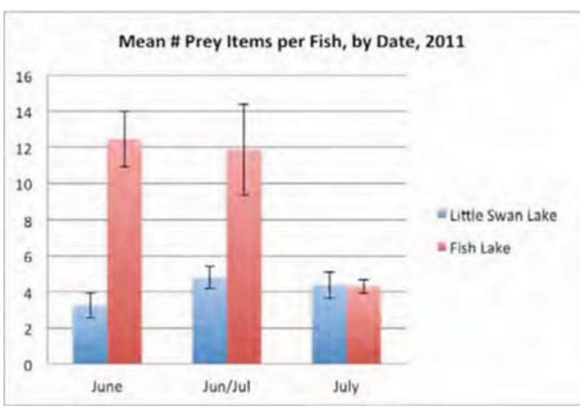
Figure 28. Relative abundances (percent occurrence) of total food items in the stomachs of individuals of *N. anogenus*, Little Swan Lake 2011, separated by sex and collecting date.



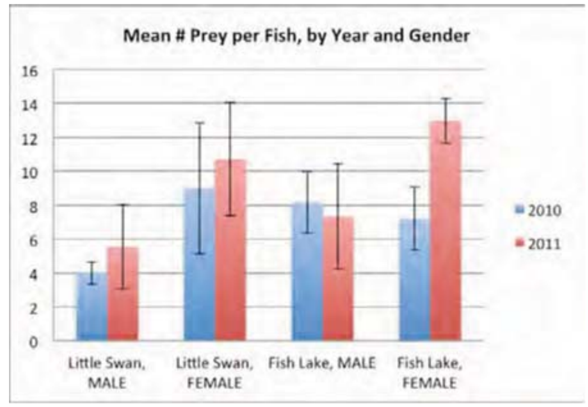
a)



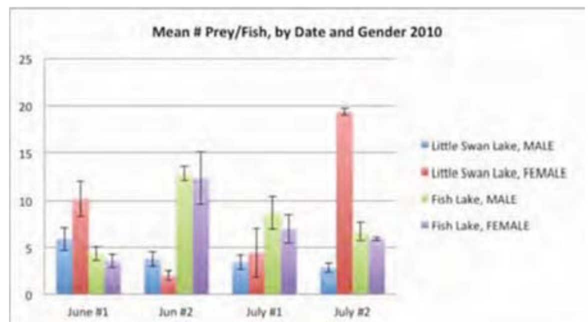
b)



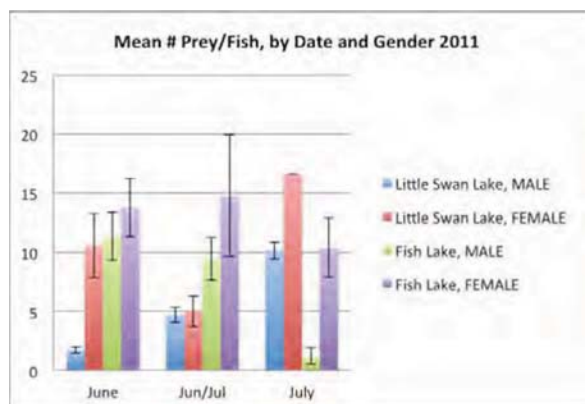
c)



d)



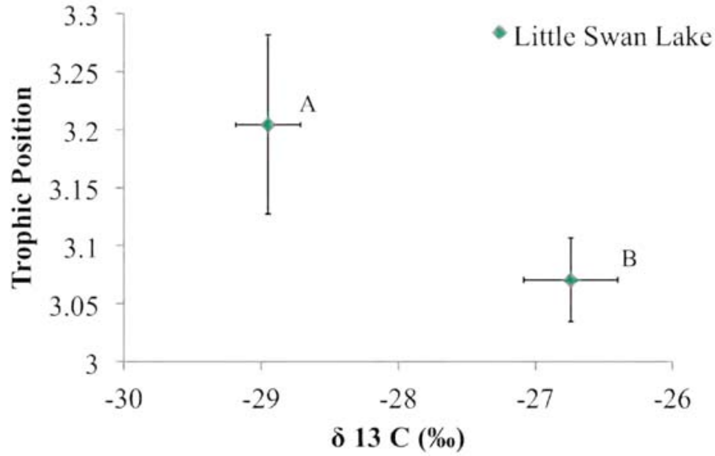
e)



f)

		Fish Lake	Little Swan Lake
2010:	June #1:	June 16	June 11
	June #2:	July 1	June 24
	July #1:	July 20	July 16
	July #2:	Aug 8	July 26
2011:	June:	June 7	June 18
	Jun/July:	June 21	July 1
	July:	July 13	July 17

Figure 29. Mean # of prey items in the stomachs of individuals of *N. anogenus*. Error bars represent +/- one Standard Error.



δ 13 C (‰) vs. Trophic Position of PNS in Little Swan Lake in 2010. Error bars show + / - one standard error.

A= June 24, 2010
B = July 26, 2010

Trophic Position

- Fish fed at significantly different trophic levels between A (6/24) and B (7/26) ($p<0.01$ at $\alpha=0.05$).
- Significant difference in carbon sources between A (6/24) and B (7/26) ($p=0.011$ at $\alpha=0.05$).

Figure 30. Stable Isotope results for *N. anogenus*.

Table 1. Number of LES individuals collected during select dates in June and July, 2011.

		Females										Males							
		Age 1		Age 2		Age 3		Age 4		Age 5		Age 2		Age 3		Age 4		Age 5	
	Site	**30-Jun**	**18-Jul**	**30-Jun**	**18-Jul**	**30-Jun**	**18-Jul**	**30-Jun**	**18-Jul**	**30-Jun**	**18-Jul**	**30-Jun**	**18-Jul**	**30-Jun**	**18-Jul**	**30-Jun**	**18-Jul**	**30-Jun**	**18-Jul**
Turtle Lake	A	4	29	7	20	5	2	3	–	–	–	3	6	3	–	1	–	–	–
	B	5	24	28	15	13	1	1	–	1	–	6	15	2	3	2	–	1	–
	C	–	5	5	1	9	2	2	–	2	–		3	8	–	2	–	–	–
	D	–	3	2	11	3	1	–	–	–	–	1	5	4	–	–	–	–	–
	Total	9	61	42	47	30	6	6	–	3	–	10	29	17	3	5	–	1	–
Dates combined		70		89		36		6		3		39		20		5		1	
Movil Lake	A	–	1	1	12	1	9	–	–	2	–	–	–	5	2	11	–	–	–
	B	–	4	–	9	–	4	1	1	–	–	–	5	3	2	–	–	1	–
	C	–	2	–	–	9	5	2	–	–	–	3	1	1	–	1	–	1	–
	Total	–	7	1	21	10	18	3	1	2	–	3	6	9	4	12	–	2	–
Dates combined		7		22		28		4		2		9		13		12		2	

Table 2. Stomach contents of LES in Movil Lake 2010-2011, by collection date. Numbers in parentheses are numbers of stomachs examined.

	Percent of Stomachs in which Food Items Occurred								**Mean Number of Food Items per Stomach**							
	(13)	(8)	(37)	(23)	(1)	(6)	(18)	(10)	(13)	(8)	(37)	(23)	(1)	(6)	(18)	(10)
Food Item	10-Jun	22-Jun	13-Jul	26-Jul	17-May	14-Jun	1-Jul	18-Jul	10-Jun	22-Jun	13-Jul	26-Jul	17-May	14-Jun	1-Jul	18-Jul
Nematoda																
Nematomorpha	23.1	...	29.7	43.5	...	...	11.1	20.0	0.23	...	0.68	0.82	...	...	0.11	0.40
Annelida																
Hirudinea	7.7	...	...	8.7	...	...	...	...	0.08	...	...	0.09	...	...	...	...
Oligochaeta	30.8	...	...	...	...	...	...	...	0.08	...	...	...	...	...	...	...
Mollusca																
Gastropoda	7.7	...	...	...	...	...	...	...	0.08	...	...	...	...	...	...	...
Arachnida																
Hydracarina	...	...	...	8.7	...	...	...	...	...	...	...	0.09	...	...	...	...
Ixodidae	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Decapoda																
Crayfish (juv)	...	...	...	...	...	...	5.6	...	...	...	...	...	...	...	0.06	...
Amphipoda																
Gammarus	53.8	71.4	59.5	39.1	100.0	50.0	55.6	40.0	3.77	4.13	2.81	0.91	1.00	0.50	4.94	3.50
Hyalella	...	...	...	4.3	...	...	...	...	...	...	...	0.04	...	...	...	...
Branchiopoda																
Cladocera	30.8	...	...	...	...	...	5.6	...	1.69	...	...	...	...	...	0.06	...
Insecta																
Ephemeroptera	30.8	...	...	4.3	...	...	72.2	10.0	0.38	...	...	0.09	...	...	3.61	0.10
Zygoptera	...	...	...	4.3	...	...	5.6	...	...	...	...	0.04	...	...	0.06	...
Anisoptera	15.4	...	...	...	100.0	...	...	10.0	0.23	...	...	...	1.00	...	...	0.10
Plecoptera	7.7	42.9	...	...	...	16.7	27.8	10.0	0.08	...	...	...	...	0.17	0.33	0.10
Megaloptera																
Corydalidae	...	...	...	...	...	...	11.1	...	...	...	...	...	...	...	0.22	...
Trichoptera	30.8	28.6	62.2	73.9	100.0	16.7	22.2	10.0	0.38	0.25	2.41	1.39	1.00	0.17	0.28	0.10
Coleoptera																
Haliplidae	...	...	5.4	8.7	...	16.7	38.9	30.0	...	...	0.05	0.09	...	0.17	0.83	0.60
Hydrophilidae	...	...	...	...	...	...	...	10.0	...	...	...	...	...	...	...	0.10
Diptera																
Diptera Unknown	...	...	...	...	...	...	11.1	...	...	...	...	...	...	...	0.11	...
Chironomidae	23.1	...	...	...	100.0	16.7	72.2	30.0	1.31	...	...	...	3.00	0.17	4.22	0.50
Tabanidae	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Fish Eggs	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Other	...	...	2.7	4.3	...	50.0	16.7	10.0	...	...	0.03	0.04	...	0.50	0.22	0.10
Unidentified Organic Matter	30.8	57.1	...	4.3	...	...	...	20.0	0.31	0.50	...	0.04	...	...	...	0.20
Empty	...	...	2.7	9.1	...	...	...	10.0	...	...	...	...	...	...	...	...

Table 3. Stomach contents of LES in Turtle Lake 2010-2011, by collection date. Numbers in parentheses are numbers of stomachs examined.

	Percent of Stomachs in which Food Items Occurred								Mean Number of Food Items per Stomach							
	(6)	(37)	(16)	(15)	(7)	(23)	(11)	(10)	(6)	(37)	(16)	(15)	(7)	(23)	(11)	(10)
Food Item	10-Jun	22-Jun	13-Jul	26-Jul	17-May	14-Jun	30-Jun	18-Jul	10-Jun	22-Jun	13-Jul	26-Jul	17-May	14-Jun	30-Jun	18-Jul
Nematoda																
Nematomorpha	...	...	18.8	...	...	4.3	18.2	36.4	...	...	0.38	...	...	0.04	0.18	0.60
Annelida																
Hirudinea	...	...	...	...	...	4.3	...	...	...	...	...	...	...	0.09	...	...
Oligochaeta	...	8.1	...	...	...	...	9.1	...	...	0.14	...	...	...	...	0.09	...
Mollusca																
Gastropoda	...	...	...	...	...	4.3	...	...	...	...	...	...	...	0.04	...	...
Arachnida																
Hydracarina	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Ixodidae	...	...	...	...	...	...	9.1	...	...	...	...	...	...	...	0.09	...
Decapoda																
Crayfish (juv)	...	...	...	...	...	...	9.1	...	...	...	...	...	...	...	0.09	...
Amphipoda																
Gammarus	50.0	67.6	75.0	33.3	100.0	73.9	45.5	36.4	1.33	1.59	2.50	1.13	5.00	3.83	3.91	5.10
Hyalella	...	...	...	...	...	4.3	...	...	...	...	...	...	...	0.04	...	...
Branchiopoda																
Cladocera	...	...	...	...	...	...	9.1	...	...	...	...	...	...	...	0.09	...
Insecta																
Ephemeroptera	...	135.0	...	...	57.1	47.8	27.3	...	...	0.24	...	...	1.43	1.48	0.45	...
Zygoptera	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Anisoptera	...	2.7	...	...	14.3	8.7	...	9.1	...	0.03	...	...	0.14	0.09	...	0.10
Plecoptera	...	2.7	...	...	42.9	...	9.1	9.1	...	0.03	...	...	0.86	...	0.09	0.10
Megaloptera																
Corydalidae	...	...	...	...	...	...	...	9.1	...	...	...	...	...	...	...	0.20
Trichoptera	83.3	78.4	81.3	53.3	14.3	26.1	45.5	9.1	2.00	2.41	3.69	2.33	0.14	0.26	1.27	0.40
Coleoptera																
Haliplidae	...	18.9	6.3	...	...	4.3	...	27.3	...	0.22	0.06	...	...	0.04	...	0.60
Hydrophilidae	...	...	...	...	...	...	9.1	18.2	...	...	...	...	...	...	0.09	0.60
Diptera																
Diptera Unknown	...	2.7	...	...	...	4.3	18.2	...	...	0.03	...	...	...	0.04	0.64	...
Chironomidae	...	2.7	...	...	100.0	43.5	45.5	54.5	...	0.05	...	...	7.71	4.43	2.18	3.90
Tabanidae	...	2.7	...	...	...	...	...	...	...	0.03	...	...	...	...	...	...
Fish Eggs	...	...	...	...	...	...	...	18.2	...	...	...	...	...	...	...	1.20
Other	...	...	...	13.3	14.3	34.8	27.3	45.5	...	...	...	0.15	0.14	0.35	0.27	0.50
Unidentified Organic Matter	...	5.4	...	...	...	...	...	9.1	...	0.05	...	...	...	...	...	0.10
Empty	...	...	6.3	20.0	...	4.3	...	9.1	...	...	...	...	...	...	...	...

Table 4. Approximate number of PNS collected in the two study areas on select dates in 2010 and 2011.

	Females															Males									
	Age 1					Age 2					Age 3					Age 1					Age 2				
	Dates*					Dates*					Dates*					Dates*					Dates*				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Fish Lake	20	12	4	16	4	4	–	–	2	–	–	–	–	–	–	48	12	36	22	36	–	–	–	–	–
Dates combined	56					6					–					154					–				
Little Swan Lake	20	20	26	2	2	4	4	38	12	–	–	–	–	6	–	18	18	46	40	10	8	12	4	44	4
Dates combined	70					58					6					132					72				

***Dates:**

Fish Lake 1:	16 June, 2010
Little Swan 1:	11 June, 2010
Fish Lake 2:	1 July, 2010
Little Swan 2:	24 June, 2010
Fish Lake 3:	20 July, 2010
Little Swan 3:	26 July, 2010
Fish Lake 4:	21 June, 2011
Little Swan 4:	18 June, 2011
Fish Lake 5:	13 July, 2011
Little Swan 5:	17 July, 2011

Table 5. Stomach contents of *PNS* in Fish Lake 2010-2011, by collection date. Numbers in parentheses are numbers of stomachs examined.

	Percent of Stomachs in which Food Items Occurred							**Mean Number of Food Items per Stomach**						
	(36)	(12)	(20)	(20)	(20)	(20)	(20)	(36)	(12)	(20)	(20)	(20)	(20)	(20)
Food Item	16-Jun	1-Jul	20-Jul	5-Aug	7-Jun	21-Jun	13-Jul	16-Jun	1-Jul	20-Jul	5-Aug	7-Jun	21-Jun	13-Jul
Filamentous Algae	8.3	41.7	80.0	55.0	...	5.0	90.0	0.08	1.58	6.75	3.45	...	0.05	0.40
Seed	...	...	...	...	...	5.0	...	...	...	...	...	...	0.05	...
Annelida														
Hirudinea	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Mollusca														
Gastropoda	...	8.3	...	...	...	15.0	...	...	0.08	...	...	...	0.15	0.10
Arachnida														
Hydracarina	58.3	50.0	35.0	15.0	30.0	25.0	65.0	2.33	2.25	0.80	0.45	0.70	1.15	1.25
Branchiopoda														
Cladocera	38.9	75.0	...	...	75.0	55.0	15.0	0.92	3.25	...	...	1.20	1.65	0.20
Copepoda	19.4	66.7	15.0	25.0	20.0	40.0	10.0	0.33	1.92	0.30	0.60	0.40	0.40	0.15
Ostracoda	5.6	75.0	...	35.0	95.0	95.0	35.0	0.14	3.50	...	1.80	9.75	7.25	0.60
Unidentified Organic Matter	...	...	...	...	30.0	95.0	5.0	...	...	...	...	0.40	1.15	1.60
Empty	16.7	...	20.0	15.0	...	...	...	...	...	...	...	...	...	...

Table 6. Stomach contents of *PNS* in Little Swan Lake 2010-2011, by collection date. Numbers in parentheses are numbers of stomachs examined.

	Percent of Stomachs in which Food Items Occurred								**Mean Number of Food Items per Stomach**							
	(21)	(25)	(26)	(57)	(20)	(52)	(16)	(8)	(21)	(25)	(26)	(57)	(20)	(52)	(16)	(8)
Food Item	11-Jun	24-Jun	16-Jul	26-Jul	22-Sep	18-Jun	1-Jul	17-Jul	11-Jun	24-Jun	16-Jul	26-Jul	22-Sep	18-Jun	1-Jul	17-Jul
Filamentous Algae	19.0	24.0	...	...	...	...	...	...	0.62	0.08	...	...	...	...	...	...
Seed	...	12.0	...	...	...	...	...	...	...	...	...	...	...	...	...	...
Annelida																
Hirudinea	...	4.0	...	...	...	...	...	...	...	0.24	...	...	...	...	...	...
Mollusca																
Gastropoda	...	4.0	...	...	...	...	...	...	...	0.04	...	...	...	...	...	...
Arachnida																
Hydracarina	38.1	4.0	11.5	3.5	5.0	30.8	37.5	...	0.81	0.04	0.12	0.04	0.05	0.63	0.69	...
Branchiopoda																
Cladocera	61.9	4.0	15.4	...	10.0	9.6	25.0	37.5	2.95	0.04	0.19	...	0.15	0.19	0.81	0.50
Copepoda	23.8	4.0	7.7	10.5	10.0	7.7	37.5	37.5	0.52	0.72	0.08	0.16	0.10	0.29	0.50	0.50
Ostracoda	52.4	16.0	73.1	75.4	30.0	57.7	75.0	87.5	3.19	0.20	3.12	2.04	0.50	2.00	2.56	3.13
Unidentified Organic Matter	4.8	8.0	11.5	14.0	5.0	11.5	18.8	25.0	0.05	0.04	0.19	0.14	0.10	0.13	0.25	0.25
Empty	...	56.0	19.2	17.5	50.0	19.2	...	...	...	...	...	...	...	...	...	...