# CHAPTER ONE

# INTRODUCTION

## *Overview*

Invasive fish species can have a detrimental influence on desirable fish stocks. Nuisance species can disrupt the natural ecological balance and incur a large societal cost (Li and Moyle 1999, Wydoski and Whitney 2003). Invasive species are considered an important variable in 68% of all fish extinctions in the United States and are estimated to negatively impact 70% of fish protected under the Endangered Species Act (Li and Moyle 1999). An estimated 34% of native US fish species are currently at risk of extinction due to the introduction of non-native fish (Wydoski and Wiley 1999). Invasive species constitute one of the most detrimental variables to the extinction of endemic fish species throughout the world (Nagle and Ruhl 2002).

The impact of invasive fish species on desirable fisheries has been well documented (Fausch and White 1986, Johannes and Larkin 1961, Li and Moyle 1981, Schoenherr 1981). For example, redside shiners (*Richardsonius balteatus R.)* were found to displace juvenile rainbow trout (*Oncorhynchus mykiss W.)* in a Canadian lake due to their ability to out-compete trout for food resources in weed beds (Johannes and Larkin 1961). Alewife (*Alosa pseudoharengus W.)* in the Great Lakes have reduced the abundance of large zooplankton causing declines in native whitefish populations (Li and Moyle 1999). Golden shiners (*Notemigonus crysoleucus M.*) in the western US have threatened endemic species (Wydoski and Whitney 2003). Non-native piscivorous species such as pike (*Esox sp.*), walleye (*Stizostedion vitreum M.*), and bass (*Micropterus sp.*) increase predation on native species like salmon and steelhead smolts (Li and Moyle 1999).

However, the introduction of new fish species to an aquatic system does not always produce a nuisance situation (Wydoski and Wiley 1999). Societal, economic, as well as ecological considerations, define a species as problematic. In general, a species is considered a problem if it 1) disrupts the trophic balance of a lake, 2) adversely impacts native fish species, 3) is a potential vector for disease, 4) does not contribute to a commercial or sport fishery, 5) inhibits a sport fishery, or 6) has other impacts on aquatic systems (Wydoski and Wiley 1999). Trophic structure interruptions can potentially impact all species and cause the most harm to desirable fish populations.

Trophic structure represents the relationship of energy movement through organisms at different feeding levels in a food web (Wetzel 2001). Primary producers convert energy from the sun through photosynthesis and form the foundation for the trophic hierarchy. Primary heterotrophs feed on primary producers. Secondary and tertiary heterotrophs represent successive levels; all consuming organisms in the previous groups (Carpenter and Kitchell 1993, Wetzel 2001). Energy is lost as heat when transferred from each trophic level, and progressively higher trophic levels will have substantially fewer individuals (Wetzel 2001). However, secondary and tertiary heterotrophs can apply a controlling influence on lower trophic organisms (Carpenter and Kitchell 1993, Strong 1992, Persson et. al. 1993, Ramcharan et. al. 1996, Romare and

Hansson 2003). Increases in zooplankton density from piscivores consuming planktivorous fish is generally referred to as a trophic cascade (Wetzel 2001).

Because top predators and zooplanktivorous fish can influence prey populations and indirectly impact lower trophic levels, the introduction of non-native fish species can lead to competition for food resources. Competition can disrupt established food webs, inducing behavioral and, in some cases, morphological changes of prey species (Carpenter and Kitchell 1993, Romare and Hansson 2003, Wydoski and Wiley 1999).

Non-native largemouth bass (*Micropterus salmoides L.*) and golden shiners have become established in Twin Lakes, Washington within the last 20 years and these fish pose a threat to more desirable salmonid populations. Although the rainbow and brook trout were also introduced, these later species provide a popular fishery. Population and trophic interaction data are needed to understand potential impacts of invasive bass and shiners, and ultimately to support to sound fishery management decisions to accomplish tribal resource management objectives for Twin Lakes.

Largemouth bass are natural predators on golden shiners in their native ranges (Wydoski and Whitney 2003) and could provide a top-down control mechanism to limit shiner populations. In Twin Lakes, dense aquatic macrophyte beds provide extensive cover for golden shiners to escape bass predation (Bettoli et. al. 1992, Olson et. al. 1998, Petr 2000, Savino and Stein 1982, Savino and Stein 1989, Smith 1995, Trebitz et. al. 1997). Reduction of macrophyte densities and bass fishing regulations are potential tools to enhance bass piscivory on shiners (Novinger 1990, Bettoli et al. 1992). Therefore, a central goal of this study was to develop data to evaluate the potential for macrophyte removal and bass slot limits for control of golden shiners. Population dynamics and trophic relationships between shiners, bass, rainbow trout and brook trout in the lakes were evaluated by examining seasonal aspects of diet structure for these fish.

### ***Lake History and Site Description***

North and South Twin Lakes are located about 8.5 miles west of Inchelium in Ferry County on the Colville Confederated Tribes Reservation in north central Washington. Basic physical data for both lakes are presented in Table 1. North Twin Lake is fed by 5 small tributaries; Granite and Beaverdam Creeks entering from the west, Carsen Creek and two unnamed tributaries entering from the north. Outflow from the lakes is into Stranger Creek and Cornstalk Creek. These two streams are controlled by artificial outlets located in the channel between North and South Twin Lakes and along the east bank of South Twin Lake (Figure 1). The Twin Lakes watershed area is approximately 11,580 ha.

**Table 1. Physical data for North and South Twin Lakes (Halfmoon 1978).**

| | Elevation (m) | Surface Area (hectares) | Volume ($10^7$ $m^3$) | Maximum Depth (m) | Mean Depth (m) | Trophic State |
|---|---|---|---|---|---|---|
| North Twin Lake | 784 | 371 | 3.6 | 15.2 | 9.7 | Mesotrophic |
| South Twin Lake | 784 | 413 | 4.2 | 17.4 | 10.4 | Mesotrophic |

South Twin Lake receives inflow from two unnamed tributaries entering from the south shore, from Sucker Creek, and from three intermittent tributaries entering from the west (Figure 1). Lake outflow is into Stranger Creek through an artificial spillway on the east bank. Stranger Creeks eventually drains into Lake Roosevelt on the Upper Columbia River southeast of Inchelium, WA. The lakes are connected by a small channel that allows for the free movement of water and biota. The channel opening into South Twin Lake is approximately 6 m wide, 1 m deep and cut through an earthen embankment constructed in the 1980's.

There are two resorts on the lakes; a tribal-owned establishment on North Twin Lake and a private resort on South Twin. Extensive developments of trailers, cabins and homes are located along the eastern shores of both lakes. Public access to the lakes is excellent and waterskiing, fishing, swimming, and other recreational uses take place from May through September. The lakes are important water resources for northeastern Washington communities and for the Collville tribes.

Timber harvest and livestock grazing represent approximately 80-90% of land use in the Twin Lakes watershed. The entire watershed is densely forested with conifers. Ponderosa pine (*Pinus ponderosa)*, and Douglas fir (*Pseudotsuga menziesii)* are the most abundant tree species.

Watershed nutrient sources include logging, grazing, and domestic and commercial developments. Nutrient inputs are assumed to have supplied the sediments that permit extensive macrophyte colonization of Twin Lakes littoral zones. Approximately 80% of surface area of the channel and adjacent cove are covered by watershield. Watershield covers approximately 6.3% (23.5 ha) of North Twin surface area and 1.9% (7.7 ha) of South Twin (Figure 1). Heavy macrophyte density has been a concern to managers due to fisheries implications, angler accessibility, recreation, and aesthetics.

Twin Lakes support populations of hatchery-raised rainbow trout, hatchery and naturalized brook trout, (*Salvelinus fontinalis M.),* largemouth bass, and golden shiners, all of which are non-native. Native redband rainbow trout (*Oncorhynchus mykiss gardneri M.)* were historically present in the lake but it is not known if any remnant populations survive. Brook and rainbow trout were introduced into Twin Lakes in the 1950's by the Washington Department of Fish and Wildlife. Their populations are currently supplemented by fish raised at the tribal hatchery. Largemouth bass were introduced into Twin Lakes illegally within the last 20 years (Dan Fairbank,

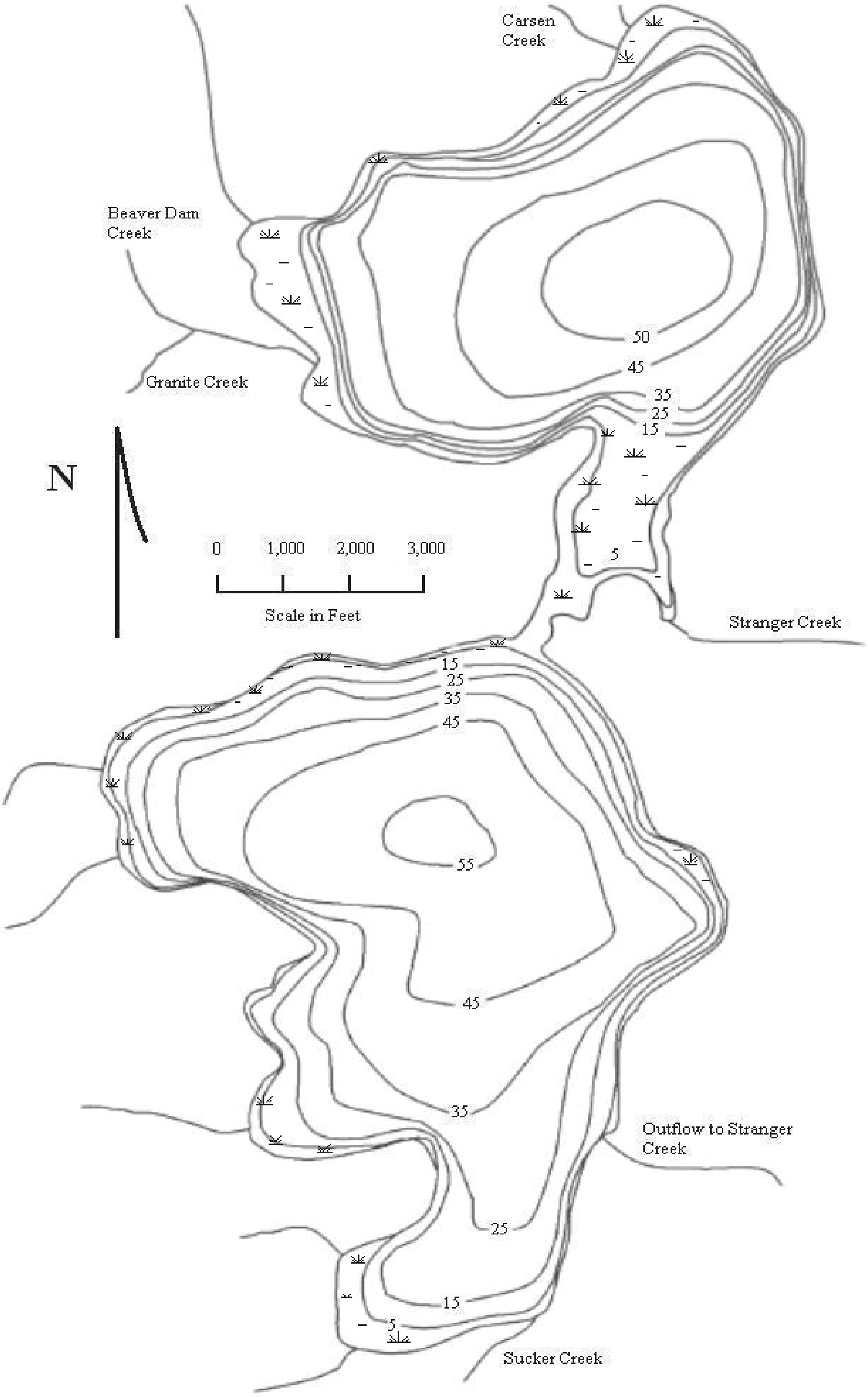


**Figure 1. Bathymetric map of North and South Twin Lakes showing tributaries, and general areas of macrophyte development during the sampling period. Contour intervals = 5 ft.**

personal communication). Golden shiners were also introduced illegally and were first documented in Twin Lakes in the 1990's (Dan Fairbank, personal communication).

### *Purpose and Goals of Fishery Assessment*

The goal of this study is to determine prey selectivity, population dynamics and trophic relationships of largemouth bass, golden shiners, brook trout, and rainbow trout in North and South Twin Lakes, Washington. Specifically, we desire to know if salmonid growth and health condition has been negatively impacted by bass and shiner populations. Our goal is to determine if macrophyte removal and bass slot-limits could be used to enhance piscivory of bass on shiners. We also seek to understand if chemical and physical habitat parameters may be influencing fish populations in the lakes.

### *Objectives*

**Obj. 1** Determine prey selectivity of largemouth bass, rainbow and brook trout throughout the growing season.

**Obj. 2** Determine age structure and growth rates of largemouth bass, golden shiner, rainbow trout, and brook trout.

**Obj. 3** Determine population size, size class distribution and relative weights (Wr) of largemouth bass, golden shiners, rainbow trout and brook trout.

**Obj. 4** Determine aquatic plant distribution and density.

**Obj. 5** Determine oxygen-temperature profiles and zooplankton density throughout the summer growing season.

# CHAPTER 2

## SPECIES AUTECOLOGY

### *Largemouth Bass*

Largemouth bass is a robust member of the *Centrarchidae* (sunfish family) characterized by its large mouth in which the maxillary bones extend past the center of the eyes (Wydoski and Whitney 2003). The species has a dark lateral band and the dorsal fin has two sections, visibly separate but connected at the base. There are 10 spines on the anterior dorsal fin and none on the posterior dorsal fin (Simpson and Wallace 1982).

Native range of largemouth bass in North America is from southern Quebec and Manitoba, southward through the Great Lakes and Mississippi River drainage, into southern Florida and northern Mexico (Wydoski and Whitney 2003, Simpson and Wallace 1982). Largemouth bass have been extensively introduced throughout the world and are now abundant outside their native range (Scott and Crossman 1973). In Washington, bass were first introduced in the 1890's by the US Bureau of Fisheries (Wydoski and Whitney 2003). They are well established in waters within the Columbia River drainage (Wydoski and Whitney 2003). They were first noticed in Twin Lakes in the late 1970's or early 1980's, where they are currently well established and provide a recreational fishery (Dan Fairbank, personal communication). However, bass impact on the more desirable salmonid populations in the lakes is not known.

Largemouth bass are a warm-water species, preferring water temperatures >20˚C and demonstrating good growth at temperatures up to about 27˚C (Wydoski and Whitney 2003). Below 10˚C and above 27˚C, bass become less active and feeding is reduced. Mortality may be expressed when temperatures rise near 35˚C (Wydoski and Whitney 2003). They generally require at least 3 mg/l of dissolved oxygen (DO) for regular metabolic functioning.

Largemouth bass prefer littoral areas of lakes and ponds with established macrophyte beds. They also populate backwaters areas of larger rivers (Wydoski and Whitney 2003). Bass are ambush predators, using macrophyte beds or other structure as cover to attack prey such as golden shiners and bluegill, (*Lepomis macrochirus R.)*. Bettoli et al. (1992) defined largemouth bass as piscivorous when 60% of sampled bass stomachs contained fish remains. This can vary depending on the type of prey and their availability (Bettoli et. al. 1992).

Whole-lake perturbation experiments have included macrophyte removal to expose prey species to largemouth bass (Olson et. al. 1998, Trebitz et. al. 1997, Bettoli et. al. 1992). This research has shown that largemouth bass and their prey can become stunted when extensive macrophyte beds exist. Stunting may be related to feeding reduction as dense macrophytes interfere with prey capture efficiency (Bettoli et. al. 1992, Olson et. al. 1998, Petr 2000, Savino and Stein 1982, Savino and Stein 1989, Smith 1995, Trebitz et. al. 1997). The inverse is true: when macrophyte density is reduced; feeding and prey capture efficiency increase, leading to elevated growth for both bass and the prey. Other common prey species for largemouth bass, such as fathead minnows (*Pimephales promelas R.*) and golden shiners, have been shown to respond in a similar manner due to macrophyte density reduction (Wydoski and Whitney 2003, Savino and Stein 1989).

Largemouth bass begin spawning in the spring as temperatures reach 15˚C and cease as temperatures surpass 24˚C (Scott and Crossman 1973). Males dig nests by fanning out a plate-sized depression in sand, gravel, or over other solid substrate. They tend to locate nests adjacent to cover in calm water less than 2 m deep (Simpson and Wallace 1982, Scott and Crossman 1973). Females may deposit 5,000 to 43,000 eggs in one nest. The eggs are 0.5 to 2 mm and usually hatch in 3 to 7 days. Newly hatched fry are black in color. Males protect embryos from predation and provide circulation by fanning their fins. If temperatures drop rapidly or fall below 10˚C, bass will abandon nests and embryo mortality may reach 100 percent through predation and/or suffocation (Scott and Crossman 1973, Wydoski and Whitney 2003). Males remain with the juveniles until they are large enough to swim off, which usually occurs after the air bladders have fully developed (Scott and Crossman 1973). Females may spawn in several nests and the male bass may allow other species, such as golden shiners or pumpkinseed (*Lepomis gibbosus L.*), to deposit eggs in the nest (Scott and Crossman 1973, Shao 1997).

In Washington, female bass generally reach sexual maturity in 3 to 4 years and may deposit 5,000 to 43,000 eggs in one nest. Adult bass can live up to 14 years and reach 10 to 15 pounds (Scott and Crossman 1973, Wydoski and Whitney 2003). Largemouth bass recruitment may be suppressed and over-winter mortality of young fish can be high in northern latitudes (Modde and Scalet 1985).

### *Golden Shiners*

Golden shiners are a member of the *Cyprinidae* (minnow) family, identified by laterally-compressed morphology. They have a scaleless, “fleshy” keel extending from the anus to the pelvic fins that serves as a unique species identifier (Page and Burr 1991, Wydoski and Whitney 2003). The lateral line is strongly decurved downward towards the anterior end. The leading edge of the anal fin is sickle-shaped with 11 to 13 rays (Page and Burr 1991, Wydoski and Whitney 2003). Golden shiners juveniles are most often silver, then develop bright orange/golden coloration on the sides as they mature (Wydoski and Whitney 2003). Maturity may be reached in 1 year depending on growth rates. Golden shiners may live to 10 years and reach lengths over 10 inches (Wydoski and Whitney 2003). In Twin Lakes, the oldest shiner sampled during 2004 was only 4 years old and approximately 9 inches in length (personal observation).

Native range of the golden shiner is east of the Continental Divide from southern Canada to southern Florida and Texas, the Missouri and Mississippi River Drainages, the Great Lake Drainage, and parts of the Hudson Bay region (Page and Burr 1991). Golden shiners have been introduced throughout the western US (Wydoski and Whitney 2003). They were first sampled in Washington in the early 1990’s from Long Lake, near Spokane (Wydoski and Whitney 2003). Currently, reported populations of golden shiners in Washington are in Long Lake, Fazon Lake, and Twin Lakes (personal observation, Wydoski and Whitney 2003). Most likely, Twin Lake shiners originated from Long or Fazon Lakes.

Golden shiners do best in calm, clear waters of lakes, ponds and slow rivers that have extensive macrophyte development (Wydoski and Whitney 2003, Shao 1997).

Optimum growth occurs between 17˚ to 24˚C (Wydoski and Whitney 2003). Shiners are omnivorous and feed on algae, plants, macroinvertebrates, zooplankton, crustaceans, and juvenile fish (Stone and Thomforde 2001, Wydoski and Whitney 2003). Plants and algae may represent up to 50% of golden shiner diets, while zooplankton makes up most of the remainder (Stone and Thomforde 2001, Wydoski and Whitney 2003).

Golden shiners are natural prey for largemouth bass in their native range. Shiners often congregate in schools and enter pelagic waters in the evenings and night while utilizing dense macrophyte beds during the day. The presence of golden shiners in several western US waterways has had negative impacts on native, endemic species (Wydoski and Whitney 2003). This is because of their ability to feed on a wide array of organisms, their high reproductive and growth rates, and their tolerance of diverse habitat conditions.

Golden shiner spawning occurs when temperatures approach 20˚C and terminates near 27˚C (Wydoski and Whitney 2003). Under optimum conditions, golden shiners may spawn 4 or 5 times in a single year. In Twin Lakes, adequate temperatures for spawning may extend from spring to early fall. Shiners are broadcast spawners, spreading adhesive eggs over vegetation in shallow littoral waters. Adult females may contain as many as 200,000 eggs (Wydoski and Whitney 2003). In temperatures over 20˚C, the eggs will hatch in 2 to 4 days (Wydoski and Whitney 2003). During spawning, shiners show dimorphisism, in which intense orange coloration on the lateral and ventral sides and black edges on the anal fin are expressed. Golden shiners do not build spawning beds but may utilize the protection of male *Centrarchids* by depositing eggs in their nests (Shao 1997).

### ***Brook Trout***

Brook trout are a member of the *Salmonidae* (trout) family and are closely related to bull trout (*Salvelinus confluentus S.*) and lake trout (*Salvelinus namaycush W.*). They have a fusiform morphology, adipose fin development, cycloid scales and abdominal placement of the pelvic fins. Brook trout have distinct dark green vermiculations on their backs and dorsal fins that differentiate them from other species. Their sides contain red spots surrounded by blue halos, while the pelvic and anal fins have distinct white leading edges with a black inner line. The lateral line contains 210 to 230 scales while the dorsal fin is composed of 10 to 14 rays (Simpson and Wallace 1982). Like other members of the charr genus, their spotting is lighter than the dark background epidermis. This is a key distinguishing feature between charr and other trout species such as rainbow and cutthroat (*Oncorhynchus clarki R.*) that have dark spots on lighter background coloration.

Brook trout are native to the eastern US and Canada , from Labrador and the western side of Hudson Bay, south to Georgia along the Appalachians, northward to Maine and the Great Lakes region (Page and Burr 1991, Scott and Crossman 1973). They were extensively introduced to water bodies outside of their range from 1950 to 1960. Many streams and lakes in the western US contain naturalized brook trout populations. Brook trout are known to be anadromous in some drainages on the east coast of Canada (Scott and Crossman 1973). In Washington, they are most abundant in the northeast (Wydoski and Whitney 2003) and were introduced into Twin Lakes in the 1950's (Dan Fairbank, personal communication).

These fish reproduce along the shoreline of Twin Lakes as well as in small tributary streams. Levels of natural recruitment are unknown. The tribe plants an average of 78,324 brook trout into North and South Twin Lakes each year (Dan Fairbank, personal communication). Brook trout are a highly favored game fish in Twin Lakes.

Brook trout require clean, cold water <20˚C and DO >5mg/l for optimum growth and survival. Temperatures near 25˚C and DO <5mg/l may become lethal after prolonged exposure (Wydoski and Whitney 2003). Brook trout are relatively short-lived (5-7 years) and reach a maximum size of 6 to 7 lbs (Scott and Crossman 1973, Wydoski and Whitney 2003). They often become overpopulated and stunted in oligotrophic waters, in which mature fish may only be 6 to 10 in. in length.

Brook trout spawn in the fall when temperatures are dropping. Spawning is initiated at about 10˚C to 8˚C. They require clean gravel for spawning, but tend to be more plastic than other salmonids (Scott and Crossman 1973). Their eggs are semi-adhesive, allowing them to spawn in more diverse areas than other trout species. Brook trout can successfully spawn along lake shorelines with moderate currents in areas of upwelling that aerate and flush the eggs of nitrogenous waste products (Scott and Crossman 1973). Females dig the redds in gravel and cover the eggs when finished. Only one male spawns with the female, and both fish will defend the redd (Wydoski and Whitney 2003).

Sexual dimorphism between brook trout males and females becomes apparent as they reach sexual maturity. The male will develop a protruding lower jaw (kype) and intense darkening and enhancement of skin coloration. Depending on the size, an adult female may carry 100 to 6,000 eggs (Simpson and Wallace 1982, Wydoski and Whitney 2003). Predation can be high on newly hatched fry because of their vulnerability. Because the eggs tend to be large (3 to 5 mm) and the water cold, incubation may take as long as 50 days (Scott and Crossman 1973). Sexual maturity is usually reached in 3 to 5 years, depending on conditions of the water body, regardless of size (Scott and Crossman 1973, Wydoski and Whitney 2003).

Brook trout in lentic environments feed primarily on zooplankton, dipteran larvae, amphipods, and zygopterans. In lotic systems, their food items are primarily macroinvetebrates. Brook trout may be piscivorous under certain circumstances. They are detrimental to native cutthroat and juvenile salmon in tributary streams because of predation and competition (Scott and Crossman 1973, Wydoski and Whitney 2003). Brook trout can hybridize with native bull trout, producing infertile offspring, negatively impacting bull trout populations.

### ***Rainbow Trout***

Rainbow trout are members of the *Salmonidae* family and are closely related to cutthroat trout and Chinook salmon (*Oncorhynchus tshawytscha W.*). They have a fusiform morphology, adipose fin development, cycloid scales and abdominal placement of the pelvic fins. Rainbow trout contain 120 to 160 scales in the lateral line, and 11 to 12 rays in the dorsal and anal fins (Simpson and Wallace 1982, Wydoski and Whitney 2003). Coloration of rainbow trout can vary greatly depending on habitat. However, they are often characterized by a silver/white belly and sides, olive-green back, and a lateral red band. They are visibly distinguished from charr by their dark spotting on a lighter background. Rainbow trout have two major life history traits, anadromous (steelhead) or resident forms such as redbands, that represent separate subspecies (Behnke 1992).

Rainbow trout are native to lakes and streams that have unhindered access to the ocean. Their range extends from the west side of the continental divide from northern Mexico to southern Alaska (Page and Burr 1991,Simpson and Wallace 1982, Wydoski and Whitney 2003) Steelhead are also native to the east coast of Russia (Scott and Crossman 1973). Steelhead inhabit coastal streams and rivers such as the Columbia, Fraser, and Snake (Wydoski and Whitney 2003) that extend inland almost a thousand miles. Dam construction on the Columbia and Snake Rivers has prevented migration, limiting steelhead range by nearly 50% by (Wydoski and Whitney 2003). Resident redband rainbow trout inhabit inland waters and do not migrate to the ocean (Behnke 1992). Rainbow trout have been extensively transplanted and are considered the most widely distributed salmonid in the world (Scott and Crossman 1973).

Verbal accounts from Colville tribal elders suggest that Twin Lakes historically contained redband rainbow trout (Dan Fairbank, personal communication). Coastal strains of hatchery rainbows have been extensively stocked there each year since the 1950's. It is unknown if these fish have naturalized and are reproducing successfully in Twin Lake tributaries. It is also not clear if rainbows in the tributaries are native redbands or an introgressed product of native and hatchery strains. Rainbow trout in Twin Lakes are supplemented by fish raised at the tribal hatchery in Bridgeport, WA. An average of 90,127 rainbow trout are planted each year into North and South Twin Lakes (Dan Fairbank, personal communication).

In general, rainbow trout prefer cold, clean water. They do best in temperatures <20˚C, but the redband form has been documented to tolerate water temperatures as high as 26˚C in desert streams (Behnke 1992). Typically, rainbow trout require at least 5 mg/l of DO for adequate growth and survival. These fish usually live 5 to 7 years, but some may live up to 10 years, and reach weights of 19 lbs, depending on the productivity of the water body (Simpson and Wallace 1982, Wydoski and Whitney 2003). Lake-type strains of rainbow trout, such as the Kamloops-Gerrard, are piscivorous and mature at a later age, allowing them to reach weights near 30 lbs (Simpson and Wallace 1982). Steelhead may reach 30 lbs after returning from a 1 to 3 years in the ocean. Age and length of stay in ocean waters influence the size of a steelhead. In cold oligotrophic streams, steelhead may remain in fresh water for 7 years before reaching adequate size to migrate. In more productive streams they may go to the ocean after only1 year (Wydoski and Whitney 2003).

Rainbow trout spawn in the spring in clean gravel of streams and small rivers. This usually occurs when water temperatures increase above 10˚C from March to June. Sexual maturity is reached at 2 to 3 years for inland resident populations (Simpson and Wallace 1982, Wydoski and Whitney 2003). Females dig redds and deposit eggs that are 3 to 5mm in size. Steelhead and Kamloops rainbows may have as many as 1,300 to 5,000 eggs per female while redbands may have <1,000 (Scott and Crossman 2003).

Rainbow trout in lentic environments feed primarily on zooplankton, dipteran (true flies) larvae, amphipods (scuds,) and zygopterans (damselflies). In lotic systems, their food items are primarily macroinvetebrates. Kamloops-Gerrard strains may feed primarily on other fish.

# CHAPTER 3

# METHODS

## *Physical and Chemical Sampling*

DO and temperature profiles were determined in June, July, August, and September of 2004, and 2005 from the deepest portion of each lake using a combination meter (Handy Gamma Oxy-guard). Physical and chemical sampling sites are represented by WQ in (Figure 2). Readings were taken at 1 m intervals from the surface to the bottom. The DO probe was recalibrated at each sampling location on each date. DO was measured in mg/l and temperature in degrees Celsius. Water transparency was estimated with a standard 20 cm Secchi disc with black and white alternating quadrants. Secchi disc measurements were recorded in m by averaging the depth of disappearance and reappearance.

## *Macrophyte and Zooplankton Sampling*

Macrophytes were sampled from each lake from ten 10 m transects on July 17th, 2004 (Figure 2). Macrophytes were cut at the base within a metal hoop of known area (0.338 $m^2$ ). Samples were taken every m along each transect and were placed in plastic bags. Macrophytes were identified to species then dried in a large oven at 350˚F for 6 hrs. Total dry weight $kg/m^2$ for all transects in each lake was determined. Macrophyte data from each transect were used to determine relative distribution and occurrence of each plant species. In each plot, macrophyte coverage was classified as high density, medium density, low density, scattered, widely scattered, or bare substrate. Substrate was determined as organic muck, peat, rocky, gravelly, or sandy. Watershield coverage was calculated by comparing digital aerial photos to maps of Twin Lakes. Planimetry was used to confirm the watershield surface coverage determined from the aerial photos.

Zooplankton were sampled using a standard zooplankton trawl with a 31.75 cm gape and 75 µm mesh net. Samples were taken from the deepest location in each lake, once a month from June to September in 2004 and 2005 (Figure 2). Samples were preserved in 5% formaldehyde. Zooplankton density was determined from the mean of 4 replicate tows made at each location. Density ($n/m^3$) was determined for each species by the following formula (Wetzel and Likens 2000):

$$n = (NV_s/V_f) * 1000$$

where:

$n$ = number of zooplankton/$m^3$
$N$ = the mean number of a species across all sub-samples
$V_s$ = volume of the sample (ml)
$V_f$ = volume of lake water filtered (l) during the zooplankton tow.

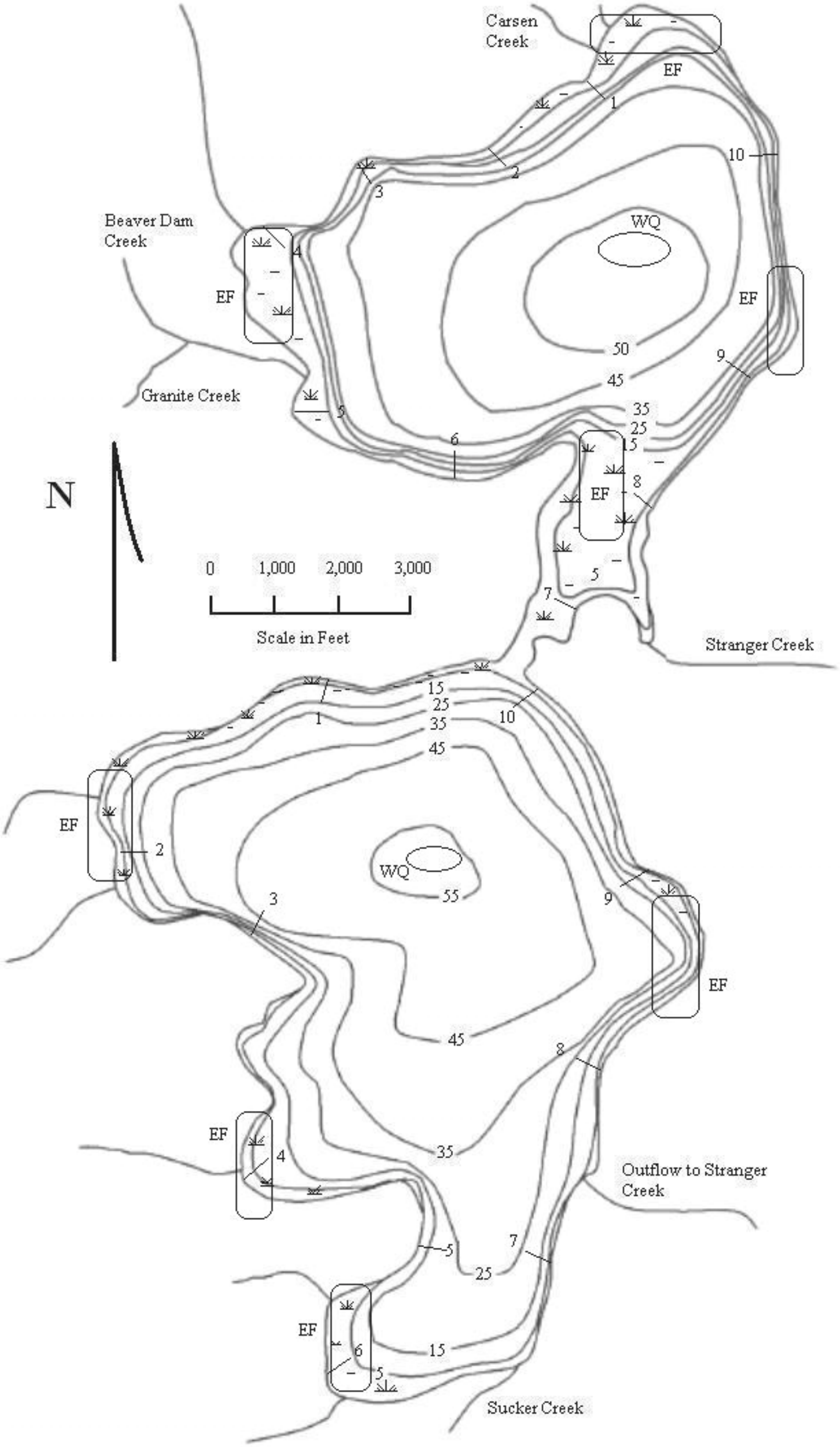


**Figure 2. Map of North and South Twin Lakes showing electrofishing transects (EF), water quality and zooplankton sampling locations (WQ), and macrophyte transects labeled 1-10 for each lake.**

### *Fish Sampling and Enumeration*

Largemouth bass and golden shiners were sampled from four different locations in North and South Twin Lakes on each sampling excursion. Sampling dates were June 8-9th, June 29-30th, August 2-3rd and September 9-10th in 2004 and June 3-4th, July 11th-12th, August 8th-9th, and September 13th-14th in 2005. A stratified random sample design was used. Four transects were identified around the lakes in vegetated and non-vegetated areas and recorded on a GPS unit (Fish Eagle) (Figure 2). We used a Smith-Root GPP 5.0, DC electrofishing boat with umbrella boom-shaped anodes and hanging-cable cathodes. Duty cycle and range were adjusted appropriately to induce proper fish taxis (positive response to electrical current). Transects from the east shore of North and South Twin Lakes were located in areas of low to absent macrophyte coverage (Figure 2). Sampling was discontinued after the first night due to the absence of fish in our samples. All transects were sampled at night for approximately 30 minutes of generator time.

At the end of each sampling period, fish were weighed to the nearest 0.01 g and measured to the nearest 5 mm in total length. A minimum of 10 largemouth bass stomachs were sampled using two 60 cc syringes as a modified gastric lavage (Aceituno and Vanicek 1976, Kamler 2001). The 10 stomachs were taken from each size class, designated as extra-small (<100 mm), small (100 to 199 mm), medium (200 to 299 mm) and large ( >300 mm), for a total of 40 stomachs sampled from each lake for each sampling period. All fish were released unharmed after measurements and samples were taken.

Stomach contents were preserved in 10% formalin, labeled, and stored on ice until identification. Prey fish were identified to species using a dichotomous key (Wydoski and Whitney 2003). Macroinvertebrates removed were identified to the order or family level using a dichotomous key (Merritt and Cummins 1996). Wet weights for golden shiners in bass stomachs were estimated by a length-weight regression formula developed from lake sampling ( Figure 3). Invertebrate prey lengths were measured to the nearest 1 mm and 0.01 g. If 10 or more of the same specimen were sampled, a subset of 10 individuals was measured for that species (Liao et al. 2002). We assumed the wet weight of invertebrate prey species to be 5 times greater than the dry weight (Morin and Dumont 1994, Pelham et al. 2001) and the dry weight of zooplankton to be 7% that of the wet weight (Pelham et al. 2001, Lawrence et al. 1987). Wet weights were estimated for macroinvertebrates and zooplankton from length-weight regressions developed by Smock (1980) and Dumont (1975) (Table 2). Percent by weight was determined for each prey species found in stomach samples of bass and trout. Frequency of occurrence for all prey fish sampled from bass stomachs was also determined.

The Student's T-test was used to determine p-values to compare mean stomach contents by weight between North and South Twin Lake. Significance was determined at $\alpha = 0.05$ for each prey species per feeding group of largemouth bass sampled using statistical software(Minitab 2003).

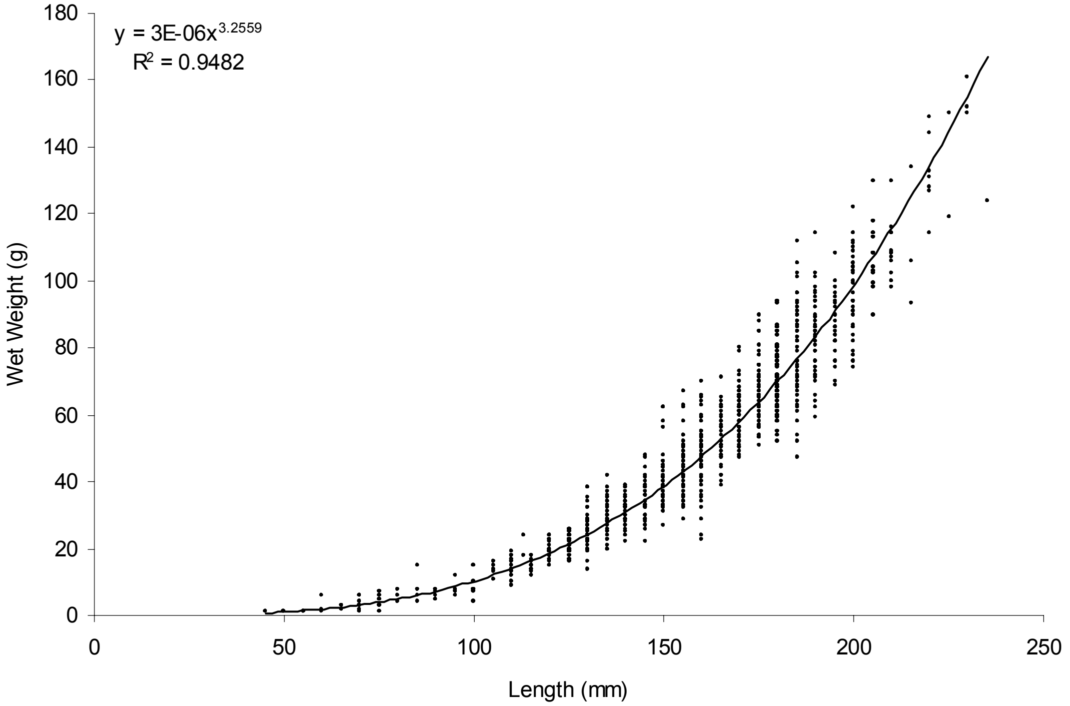


**Figure 3. Regression curve depicting relationship between length (mm) and weight (g) of golden shiners (n=1,025) sampled from Twin Lakes, WA 2004. The equation of the line, $R^2$-value, and data points are shown. A total of 1,004 data points are represented in the regression. Twenty-one data outliers (≥20 mm deviation from the mean) were removed. Outliers were assumed to be caused by sampling error.**

Scales were taken from a random sample of 10 largemouth bass and 10 golden shiners from each 10 mm size class (Aceituno and Vanicek 1976) on the last night of sampling from both lakes in 2004. Scales were removed from the midpoint of an imaginary line connecting the top of the operculum and the anterior base of the dorsal fin. Scales were projected on an electronic microfiche reader, and age was determined by counting the number of annuli surrounding the focus of the scale. The mean length at each age for sampled fish was determined for North and South Twin Lakes and compared to other regional waters.

Each largemouth bass and golden shiner collected received a hole punch in the anal fin before release. These marks were used as identifiers for the Schnabel multiple-census mark-recapture population estimation in 2004 (Anderson and Neumann 1996):

$$N=\sum(C*M)/\sum R$$

Where:

N = population size
C = number of fish captured
M = number of fish marked
R = number of fish recaptured.

Two 250 ft. by 6 ft. sinking, experimental monofilament gill nets were set in both lakes for 1 night for approximately 8 hours on each sampling date in 2004 and 2005. Nets were placed to specifically catch rainbow and brook trout in deeper and more open water. Nets were extended perpendicular from shore in areas where the contours of the lake rapidly dropped to >20 ft (Figure 2). Salmonid length was measured to the nearest 5 mm and weight to the nearest 0.01 g. Stomach samples were collected and analyzed as described for largemouth bass. Largemouth bass stomachs were not sampled from fish captured in gill nets since nets usually captured larger fish and stomach contents were often emitted from the fish.

Elastomer tags inserted and adipose fins were identified on each salmonid sampled. Green tags represented fish planted in 2003 and orange tags represented fish planted in 2002 (Dan Fairbank, personal communication). Fish without tags and with an adipose fin were assumed to be wild.

Length class histograms were created for each species sampled to assess population size distribution. Relative weights (Wr) values were calculated for all species to assess fish health. Relative weights were determined using two calculations. The power function is used to determine the expected weight (Ws) of the fish and the relative weight (Wr). The equation calculates the proportion of the actual weight to the expected weight.

1. Power Function $Ws=a*(L^b)$
2. Relative Weight $Wr=100*(W/Ws)$

Where:

Ws = expected weight (g) of fish based on regression
L = actual length of fish sampled
Wr = relative weight
W = actual weight of fish

Values of y-intercept (a) and slope (b) are determined by log-regression line of length (mm) and weight (g) for each species. Predetermined values for (a) and (b) are recorded in Table 3 (Anderson and Neumann 1996). Slope is represented by (b), and (a) is the y-intercept.

Volume of available trout habitat was determined by identifying the boundaries of preferred trout habitat (≥5 mg/l DO, ≤20˚C). The space was calculated by using a planimeter to determine volume at these depths. The equation;

$$V=(\pi*r^2)*h$$

was used, where V=volume, r=radius, and h=height.

**Table 2. Length-weight regression determinates for y-intercept (a) and slope (b) for macroinvertebrate dry weight (mg) and length (mm) adapted from (Smock 1980) and regression equations for zooplankton dry weight (µg) and length (mm) (Downing 1984, Pelham et al. 2001).**

| Macroinvertebrates | ln a | b |
|---|---|---|
| Coleoptera | -1.878 | 2.18 |
| Diptera | -5.221 | 2.43 |
| Ephemeroptera | -5.021 | 2.88 |
| Hemiptera | -3.461 | 2.40 |
| Megaloptera | -5.843 | 2.75 |
| Odonata | -4.269 | 2.78 |
| Plecoptera | -6.075 | 3.39 |
| Trichoptera | -6.266 | 3.12 |
| Gomphidae | -5.426 | 3.12 |

Power equation $W=a*L^b$

Where:

$W$ = estimated weight of species
$a$ = y-intercept of line
$b$ = slope of line
$L$ = Length of the prey item (mm)

| Zooplankton | |
|---|---|
| Cladocerans | |
| *Daphnia pulex* | $W = (2.4 * 10^{-8})*L^{2.77}$ |
| *Holopedium sp.* | $W = (4.5 * 10^{-3})*L^{2.77}$ |
| Copepods | |
| *Diaptomus sp.* | $W =( 7.9 * 10^{-7})*L^{2.33}$ |

**Table 3. Contains the log length (mm)-weight (g) regression determinates of y-intercept (a) and slope (b) for selected fish species found in the literature (Anderson and Neumann 1996) These values can be used in the power equation to determine expected weights of fish species sampled in bass and trout stomachs.**

| Species | a | b |
|---|---|---|
| Largemouth Bass | $10^{-5.316}$ | 3.191 |
| Golden Shiner | $10^{-5.593}$ | 3.302 |
| Brook Trout | $10^{-5.085}$ | 3.043 |
| Rainbow Trout | $10^{-5.023}$ | 3.024 |

# CHAPTER FOUR

# RESULTS

## *Physical and Chemical Data*

Clinograde DO profiles were observed on all dates for each lake in 2004 except on August 3, 2004 positive heterograde profiles were found for each lake (Figure 4 and 6). In 2005, positive heterograde profiles were recorded for each sampling date (Figure 5 and 7). Clinograde profiles are characterized by higher surface DO that declines with depth. This profile results from biological control of DO, with photosynthesis dominating at the surface and respiration dominating in the hypolimnion or deeper waters (Wetzel 2001). Positive heterograde distribution result from algae photosynthesis and release of oxygen at a particular depth (Figure 4,5,6 and7). Both the DO distributions are typical of eutrophic lakes.

DO and temperature profiles were similar between North and South Twin Lake on each sampling date in 2004 and 2005 (Figure 4,5,6 and 7). Because brook and rainbow trout require DO levels of at least 5mg/l and temperatures less than 20˚C for adequate growth and survival, the amount of preferred habitat decreased substantially in Twin Lakes from June to August, and then began to increase in September (Table 4).

**Table 4. Decline in volume ($m^3$) and depth (m) of suitable trout habitat (DO≥5 mg/l, temperature ≤20˚C) available in North and South Twin Lakes in 2004. This is a habitat reduction of 81% for each lake.**

| | June 8th, 2004 | | August 3rd, 2004 | |
|---|---|---|---|---|
| | Depth (m) | Volume ($m^3$) | Depth (m) | Volume ($m^3$) |
| North Twin | 9 | 13,604,000 | 3 | 2,603,000 |
| South Twin | 9 | 19,258,000 | 3 | 3,681,000 |

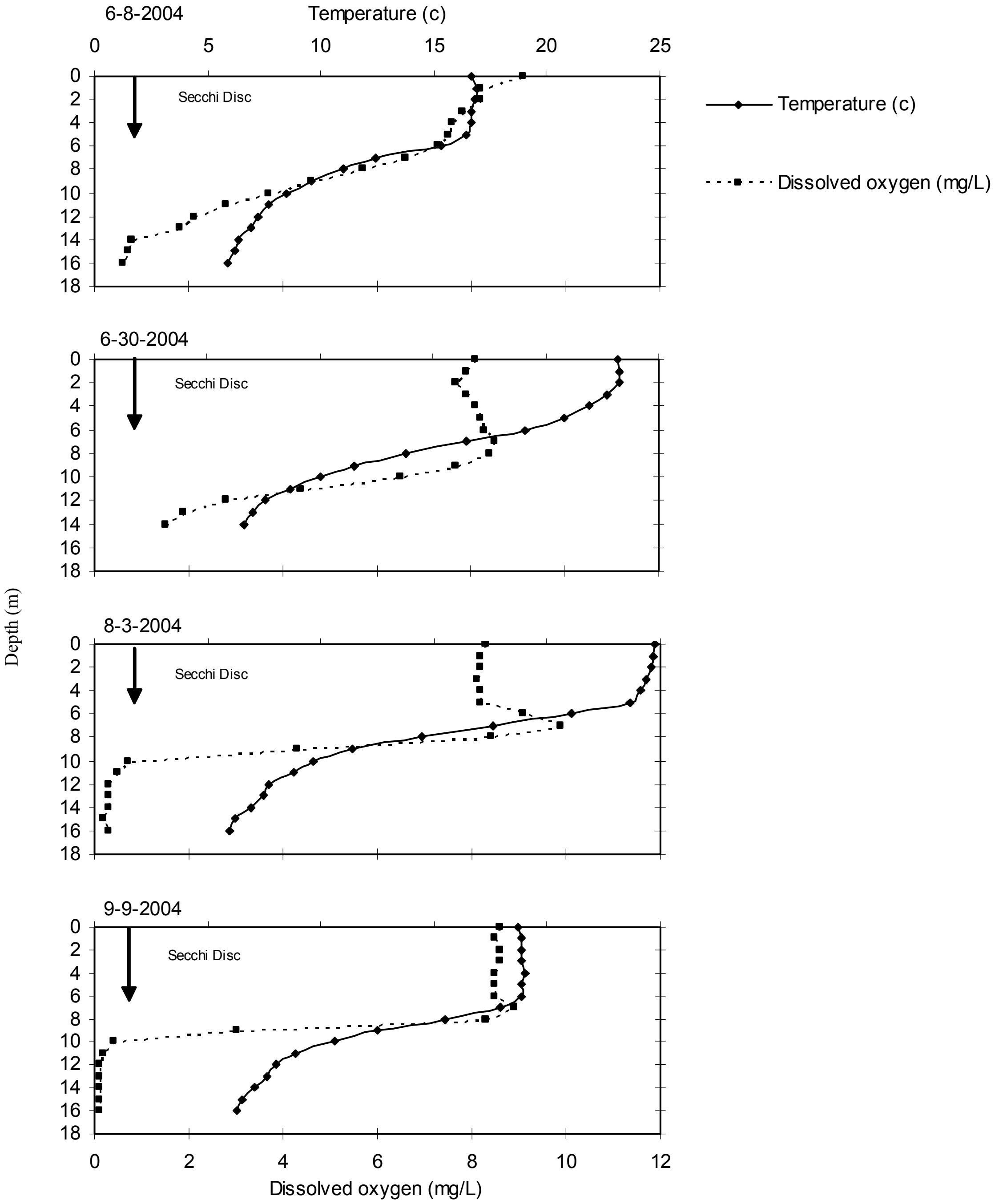


**Figure 4. Dissolved oxygen and temperature profiles with Secchi depths for North Twin Lake during the summer of 2004. Disolved oxygen values are in (mg/l) and temperature is measured in (C°). Measurements were taken at one meter (m) increments from the surface to the bottom.**

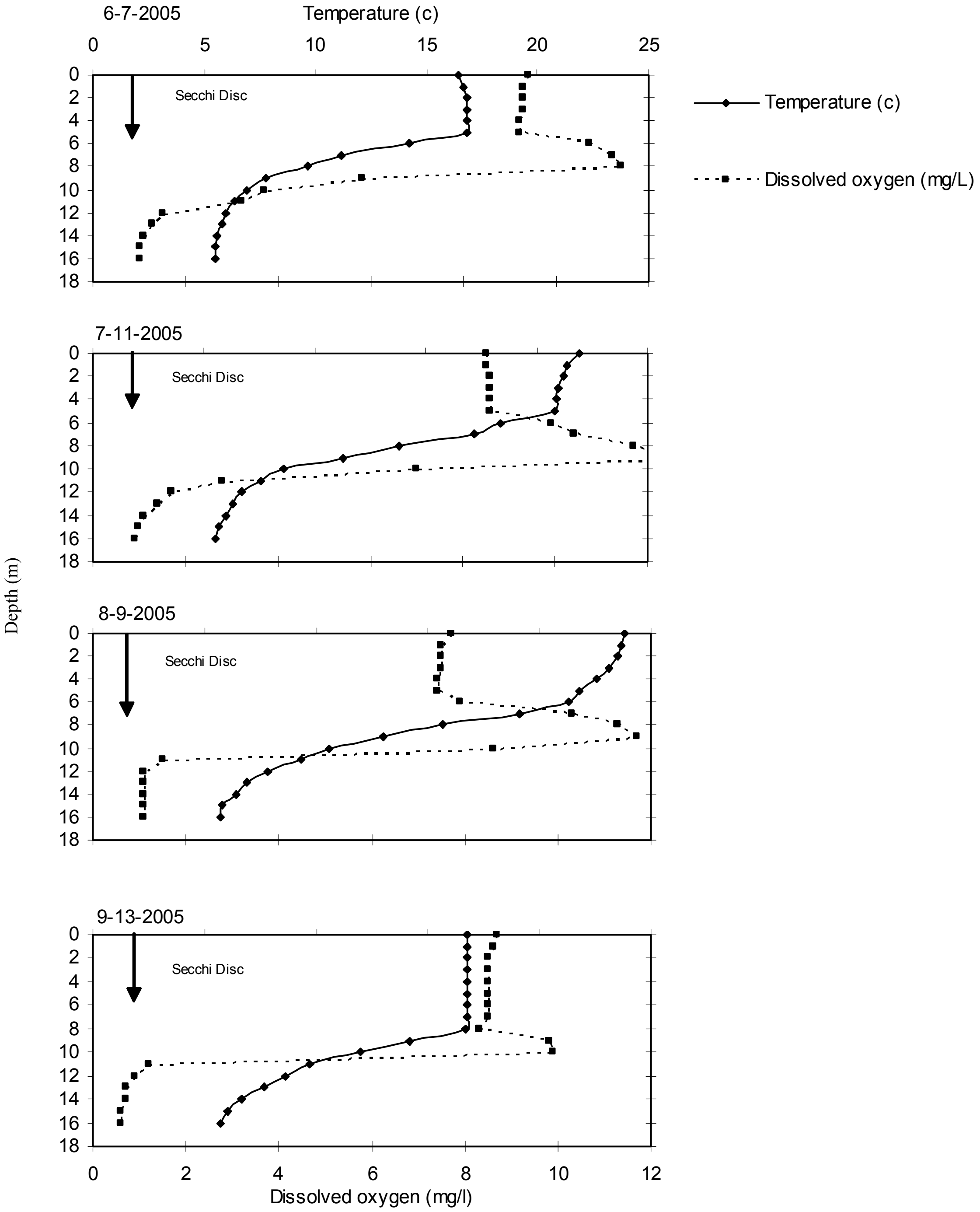


**Figure 5. Dissolved oxygen and temperature profiles with Secchi depths for North Twin Lake during the summer of 2005. Disolved oxygen values are in (mg/l) and temperature is measured in (C°). Measurements were taken at one meter (m) increments from the surface to the bottom.**

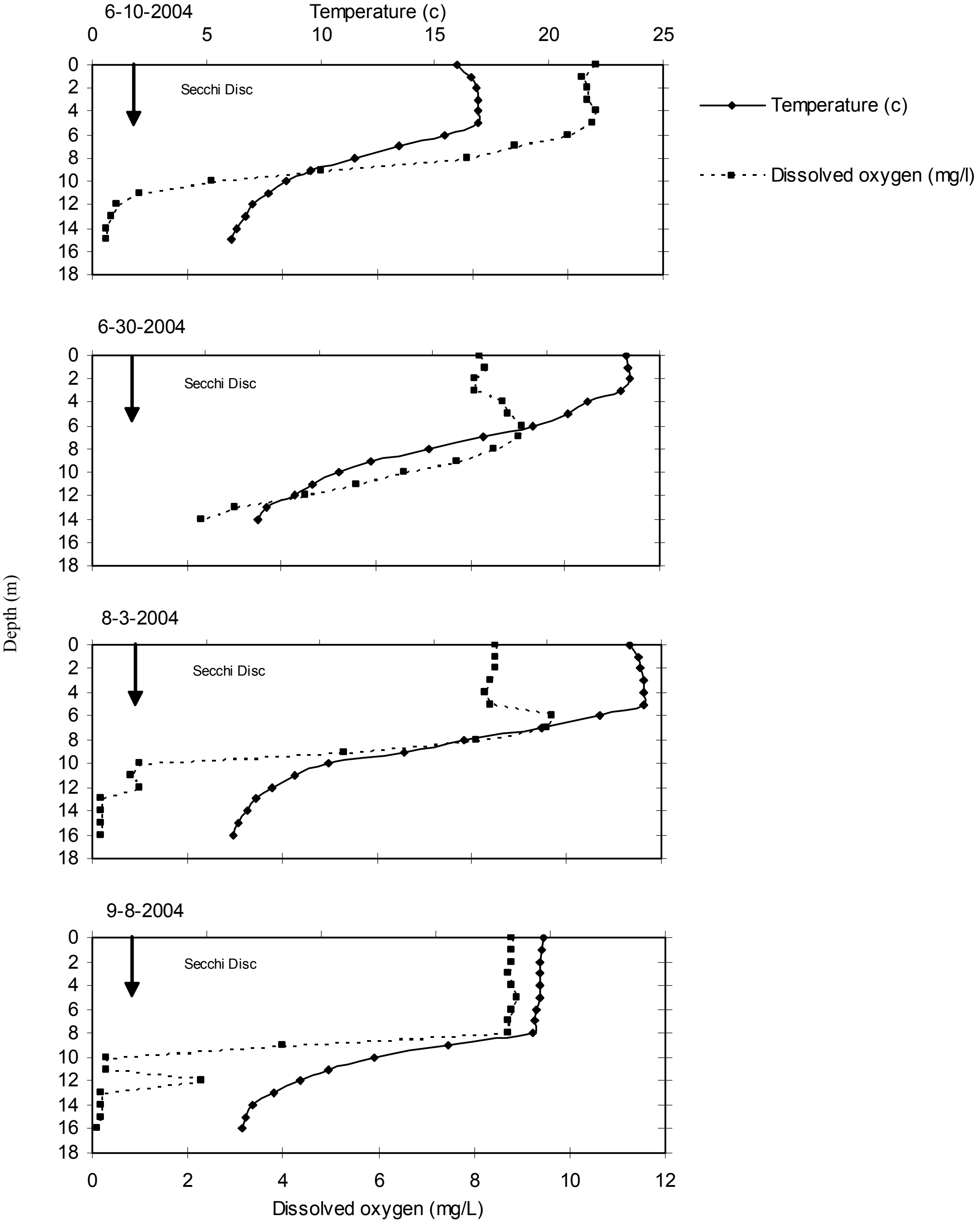


**Figure 6. Dissolved oxygen and temperature profiles with Secchi depths for South Twin Lake during the summer of 2004. Disolved oxygen values are in (mg/l) and temperature is measured in (C°). Measurements were taken at one meter (m) increments from the surface to the bottom.**

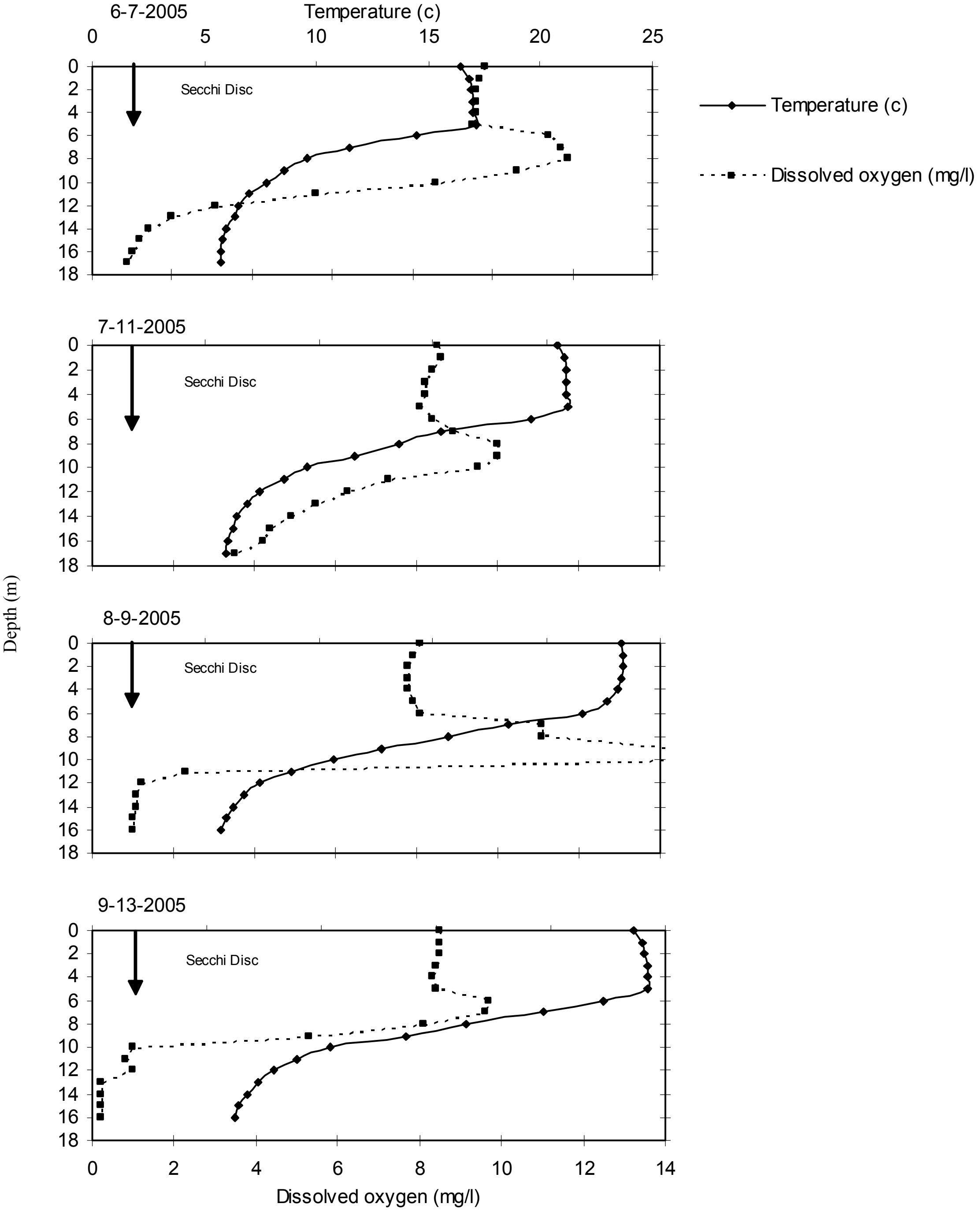


Figure 7. Dissolved oxygen and temperature profiles with Secchi depths for South Twin Lake during the summer of 2005. Disolved oxygen values are in (mg/l) and temperature is measured in (C°). Measurements were taken at one meter (m) increments from the surface to the bottom.

### *Macrophytes*

Total macrophyte biomass for each transect in North Twin Lake was .04 kg/m$^2$ and South Twin Lake was .04 kg/m$^2$. *Brasenia shreberi* beds covered approximately 6.3% (58 acres) of the surface area of North Twin Lake and 1.9% (19 acres) of South Twin Lake. There were 16 different species of macrophytes identified from North Twin Lake and 14 species from South Twin Lake (Table 5 and 6).

*Brasenia schreberi*, *Elodea canadensis*, and *Isoetes sp.* were present in 7 of the 10 transects in North Twin Lake. *B. schreberi* and *E. canadensis* were found in the highest densities in North Twin. Although the transect data in Table 5 and 6 does not accurately depict *B. schreberi* coverage because of small transect lengths, aerial photographs shows that this plant dominates coverage and species abundance. The cove and channel connecting Twin Lakes, the north and south coves, and the north shore of North Twin Lake are dominated by *B. schreberi* (Figure 1).

*E. canadensis*, *Myriophylum sibiricum, Potamogeton robbinsii, Ceratophyllum demersum,* and *Chara sp.* occurred in scattered patches in 6 to 8 of the South Twin Lake transects. All other species were found in 5 or fewer transects. *B. schreberi* was relatively abundant in transects 1, 2, 4, and 6 (Table 5 and 6). The greatest concentration of *B. schreberi* and other macrophytes in both lakes were in coves fed by tributary streams (Figure 1). This is most likely due to deposition of nutrient-rich sediments from inlet streams.

**Table 5. Macrophyte distribution and abundance in North Twin Lake during July 2004. Density is described as high density (H), medium density (M), low density (L), scattered (S), widely scattered (WS), and bare substrate (B). Substrate types are organic muck (OM), peat (P), rocky (R), gravelly (G), and sandy (S).**

| North Twin Lake | | Transect | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | Common Name | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *Brasenia schreberi* | Watershield | H, OM | M, OM | H, OM | | | S, OM | S, OM | H, OM | | WS, S |
| *Ceratophyllum demersum* | Coontail | WS, OM | | | | | | L, OM | B, OM | | |
| *Chara sp.* | Stonewort | | | | WS, OM | | | B, OM | | | WS, B |
| *Eleocharis sp.* | Spike Rush | | | | | | | WS, OM | | | |
| *Elodea canadensis* | Waterweed | S, OM | WS, OM | S, OM | M, OM | | S, OM | L, OM | L, OM | | |
| *Gramineae sp.* | Grass | | WS, S | | WS, OM | | | WS, S | B, OM | | |
| *Isoetes sp.* | Quilwort Fern | | WS, OM | WS, S | L, OM | WS, S | WS, OM | L, OM | WS, S | | WS, S |
| *Myriophyllum sibiricum* | Northern Water Milfoil | WS, OM | WS, OM | | WS, OM | | | S, OM | B, OM | | |
| *Najas flexilis* | Bushy Pondweed | | | | | | | | | | |
| *Nuphar polysepala* | Yellow Water Lily | WS, OM | | | WS, OM | | | | | | |
| *Potamogeton praelongus* | Pondweed | | | | | | | | | | |
| *Potamogeton robbinsii* | Pondweed | | | WS, OM | | | | WS, OM | | | |
| *Potamogeton sp.* | Pondweed | | | | | | | | | | |
| *Tofieldia glutinosa* | Sticky Tofieldia | | WS, OM | S, S | | | | WS, OM | | | |
| *Typha sp.* | Cattail | WS, OM | | | | | | | WS, OM | | |
| *Utricularia minor* | Bladderwort | S, OM | S, OM | | | | | | WS, OM | | |
| *Utricularia vulgaris* | Bladderwort | WS, OM | L, OM | | S, OM | | | L, OM | WS, OM | | |
| Other | | | WS, OM | | | | | | B,S | | |

**Table 6. Macrophyte distribution and abundance in South Twin Lake during July 2004. Density is described as high density (H), medium density (M), low density (L), scattered (S), widely scattered (WS), and bare substrate (B). Substrate types are organic muck (OM), peat (P), rocky (R), gravelly (G), and sandy (S).**

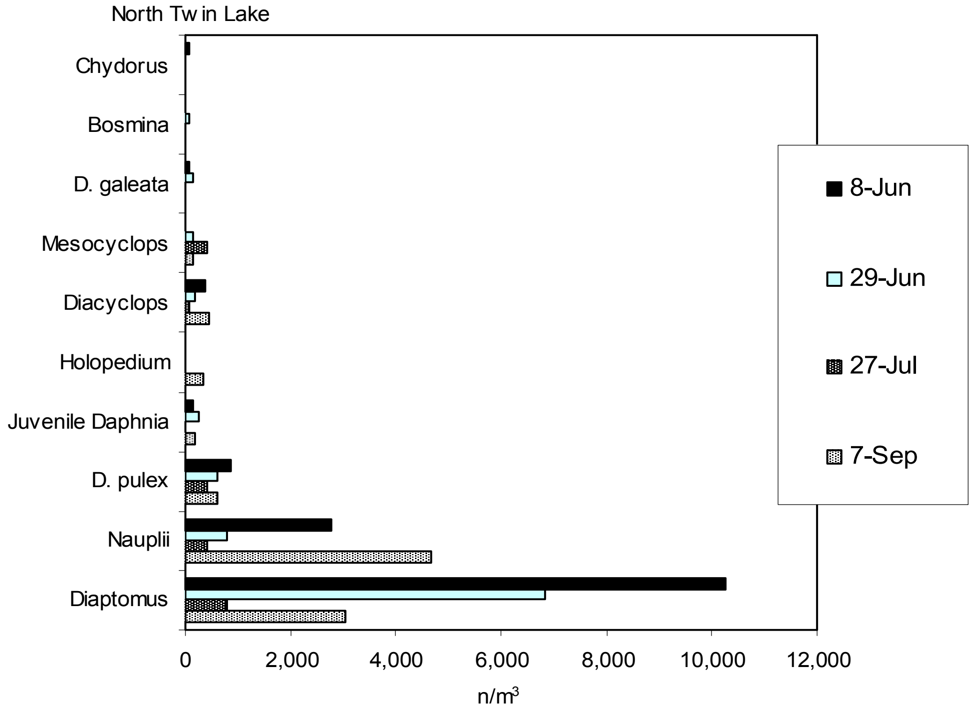
| South Twin Lake | | Transect | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Species | Common Name | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *Brasenia schreberi* | Watershield | M, OM | M, OM | L, OM | L, OM | | B, OM | | | | |
| *Ceratophyllum demersum* | Coontail | | | WS, S | WS, S | S, S | S, S | S, OM | | | S, S |
| *Chara sp.* | Stonewort | | | S, OM | S, OM | WS, OM | WS, OM | S, OM | | | S, S |
| *Eleocharis sp.* | Spike Rush | | | | | | | | | | |
| *Elodea canadensis* | Waterweed | WS, S | WS, S | S, OM | S, OM | WS, S | WS, S | L, OM | | | S, OM |
| *Gramineae sp.* | Grass | | | | | | | | | | |
| *Isoetes sp.* | Quilwort Fern | | | | | L, S | L, S | | B, G | B, G | |
| *Myriophyllum sibiricum* | Northern Water Milfoil | B, OM | B, OM | | | WS, S | WS, S | | S, G | S, G | WS, S |
| *Najas flexilis* | Bushy Pondweed | | | S, OM | S, OM | | | L, OM | | | |
| *Nuphar polysepala* | Yellow Water Lily | | | | | | | M, OM | | | |
| *Potamogeton praelongus* | Pondweed | | | | | | | L, OM | | | |
| *Potamogeton robbinsii* | Pondweed | | | S, S | S, S | WS, S | WS, S | S, OM | | | WS, OM |
| *Potamogeton sp.* | Pondweed | B, OM | B, OM | | | | | B, OM | | | WS, OM |
| *Tofieldia glutinosa* | Sticky Tofieldia | | | | | | | | | | |
| *Typha sp.* | Cattail | | | WS, OM | WS, OM | | | L, OM | | | |
| *Utricularia minor* | Bladderwort | | | | | | | WS, OM | | | WS, OM |
| *Utricularia vulgaris* | Bladderwort | | | | | | | H, OM | | | |
| Other | | | | | | | | | | | |

### *Zooplankton*

Ten species of zooplankton were found in Twin Lakes in 2004 and 2005 (Figure 8 and 9). North Twin Lake had a greater density of the copepod, *Diaptomus,* in June while *Diaptomus* density in South Twin Lake was greater during August and September, 2004. *Daphnia pulex*, the largest of the zooplankton (0.9 to 2.5 mm), was most abundant during the September in South Twin Lake and for June in North Twin Lake, and was present in all 2004 samples. *Holopedium* only appeared in the September samples and was most abundant in South Twin Lake in 2004. Juvenile daphnia were more abundant in South Twin Lake than North Twin on all sampling dates in 2004 (Figure 8). Zooplankton such as *Daphnia pulex* and *Holopedium* are important as fish food because of their large size (Table 7). In 2005, the largest zooplankton species dominated by number. *Daphnia pulex* was the most abundant with *Diaptomus* and *Holopedium* with high densities (Figure 9). *Holopedium* appeared in the North Twin Lake samples in June, August, and September in 2005 as apposed to only the September samples in 2004.

**Table 7. Mean length (mm) of large zooplankton sampled from North and South Twin Lakes. Sample size for each species is approximately 10 to 30 individuals.**

| Species | North Twin (mm) | South Twin (mm) |
|---|---|---|
| *Daphnia pulex* | 1.4 | 1.3 |
| *Holopedium sp.* | 0.8 | 0.9 |
| *Daphnia galeata* | 0.8 | 0.8 |
| *Diaptomus sp.* | 0.8 | 0.8 |
| *Diacyclops sp.* | 0.7 | 0.6 |
| *Mesocyclops sp.* | 0.6 | 0.5 |



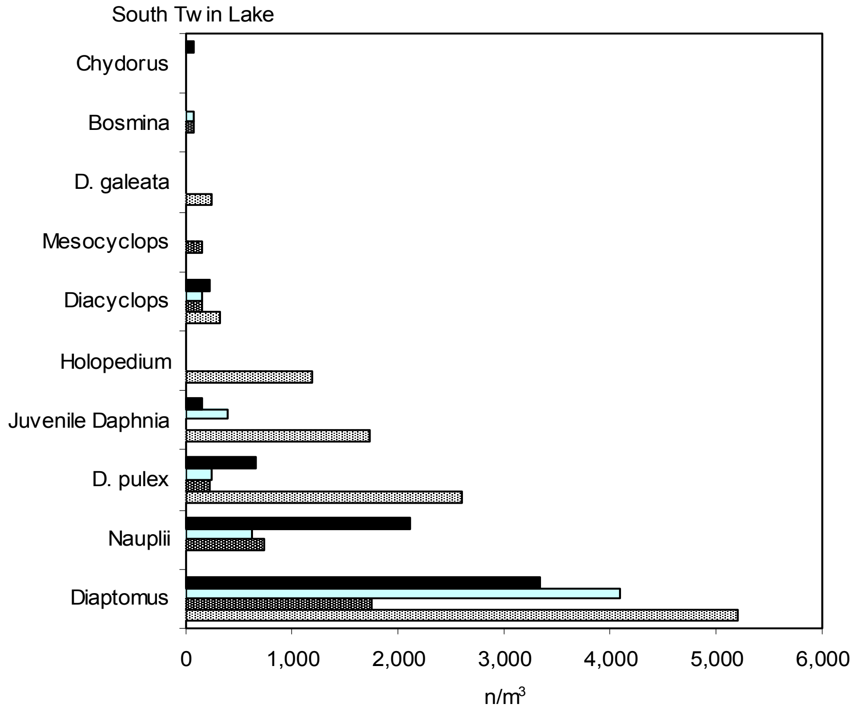


**Figure 8. Zooplankton density (n/m$^3$) sampled from North and South Twin Lakes during the June-Sept. 2004 sampling periods. Species are arranged from lowest to highest density.**

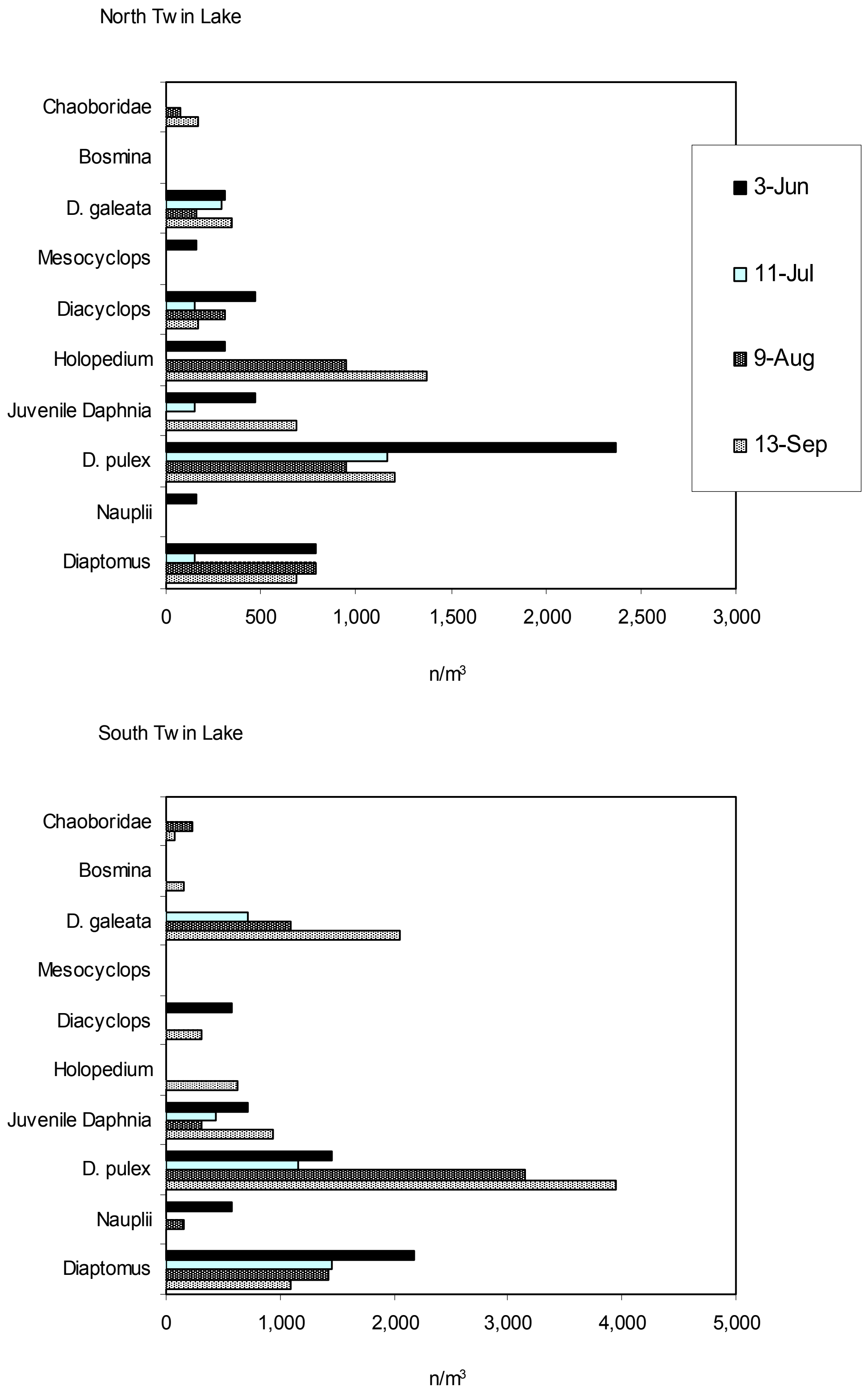


**Figure 9. Zooplankton density (n/m$^3$) sampled from North and South Twin Lakes during the June-Sept. 2005 sampling periods. Species are arranged from lowest to highest density.**

### *Largemouth Bass and Golden Shiners*

In 2004, 494 largemouth bass and 383 golden shiners were sampled from North Twin and 900 largemouth bass and 730 golden shiners were sampled from South Twin Lake. Table 8 contains population estimates, relative abundance and total sampling size of bass and shiners taken from North and South Twin Lakes from June through September, 2004. Table 8 also shows sample size and relative abundance of largemouth bass feeding groups. Although sample sizes were much larger in South Twin Lake, relative abundance of bass and shiners is similar (Table 8). Largemouth bass comprise 48% in North Twin and 54% of the sampled population in South Twin Lake. Golden shiners represent 37% in North Twin and 44% of the fish sampled in South Twin Lake. The remainder of the sampled fish community is represented by brook and rainbow trout (Table 8). Population estimates of bass and shiners are considerably higher for South Twin Lake (Table 8). Recaptures of bass and shiners were never greater than 2 individuals, resulting in large variance in population estimates. Larger sample sizes are needed to increase probability of recaptured fish. The actual population size of both species may be much greater in both lakes.

Seventy-four percent of largemouth bass sampled from North Twin and 83% from South Twin Lake were small (100 to 199 mm) (Table 8). Mean length of all largemouth bass was 186 mm for North Twin and 180 mm for South Twin Lakes. These differences were not significance at $\alpha = 0.05$. Mean length of golden shiners was 148 mm from North Twin and 164 mm in South Twin Lake. This showed significance at $\alpha = 0.05$. Length-class histograms of largemouth bass from both lakes are skewed to the large size-classes while golden shiners express a more normal distribution (Figure 10). Only bass >100 mm and shiners >50 mm were considered in this analysis due to an electrofishing bias towards larger fish.

Relative weight (Wr) values for largemouth bass and golden shiners in North and South Twin Lake in 2004 are presented in Figure 11. Wr values for largemouth bass and golden shiners are illustrated with a line at 100. This line represents the 75th percentile weight at a given length for fish sampled by other agencies and recorded in the literature (Anderson and Neumann 1996). Each data point represents a mean Wr value of fish in each 10 mm length class sampled. Most points for largemouth bass in both lakes are near or above 100, representing good condition (Figure 11). Wr values for golden shiners are all near 100, but decline with increasing fish size (Figure 11). Lower Wr estimates at increased length is a common pattern and could be a result of post-spawning fish. In general, Wr estimates of sampled bass and shiners from Twin Lakes represent healthy individuals (Figure 11).

Length-at-age is the mean length of a cohort of fish of the same age. Largemouth bass length-at-age values from September 7 and 8, 2004 demonstrate good health and environmental conditions (Figure 12). Bass length-at-each age estimates are similar for each lake (Figure 12). Young-of-the-year bass for each lake had a mean length just under 100 mm. Length-at-age for bass in both lakes were similar until ages 4 to 6. North Twin Lake bass had a larger mean length expressed at age 4, and smaller lengths for fish at age 5 and 6 (Figure 12). Lengths were similar at age 7 for both lakes. The oldest bass sampled from North Twin Lake was 12 years old. The oldest from South Twin Lake was 7 years old (Figure 12). Length-at-age of bass from both lakes was nearly identical to the

**Table 8. Sample size, relative abundance, and population estimates for fish species sampled from Twin Lakes during the June-September 2004 sampling period.**

| Common name | Scientific name | Total sampled | Relative abundance (%) | Population estimate |
|---|---|---|---|---|
| ***North Twin Lake*** | | | | |
| Largemouth bass | *Micropterus salmoides* | 494 | 48.1 | 19,231 |
| Golden shiner | *Notemigonus crysoleucus* | 383 | 37.3 | 26,230 |
| Brook Trout | *Salvelinus fontinalis* | 35 | 3.4 | _ |
| Rainbow Trout | *Oncorhynchus mykiss* | 116 | 11.3 | _ |
| ***South Twin Lake*** | | | | |
| Largemouth bass | | 900 | 54.0 | 82,642 |
| Golden shiner | | 730 | 43.8 | 48,028 |
| Brook Trout | | 28 | 1.7 | _ |
| Rainbow Trout | | 10 | 0.6 | _ |

| ***North Twin Lake*** | **Size classes:** | **100-199mm** | **200-299mm** | **>300mm** |
|---|---|---|---|---|
| Total sampled | **Largemouth bass** | 370 | 94 | 30 |
| Relative abundance (%) | | 74.9 | 19.0 | 6.1 |
| ***South Twin Lake*** | | | | |
| Total sampled | | 753 | 99 | 48 |
| Relative abundance (%) | | 83.7 | 11.0 | 5.3 |

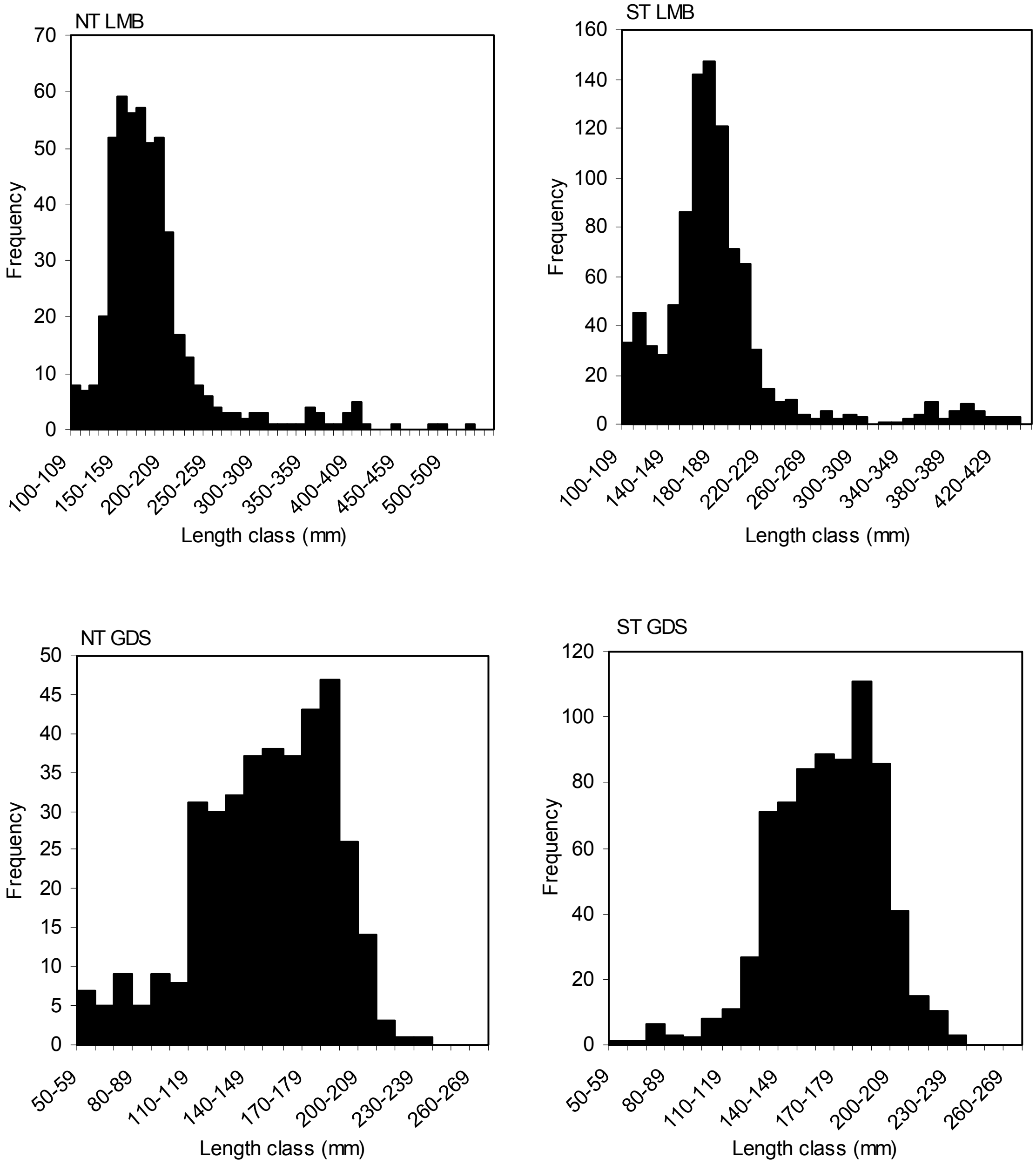


**Figure 10. Length class histograms (mm) for largemouth bass (LMB) (n=494) and golden shiners (GDS) (n=383) sampled from North Twin Lake (NT) and largemouth bass (n=900) and golden shiners (n=730) from South Twin Lake (ST), 2004.**

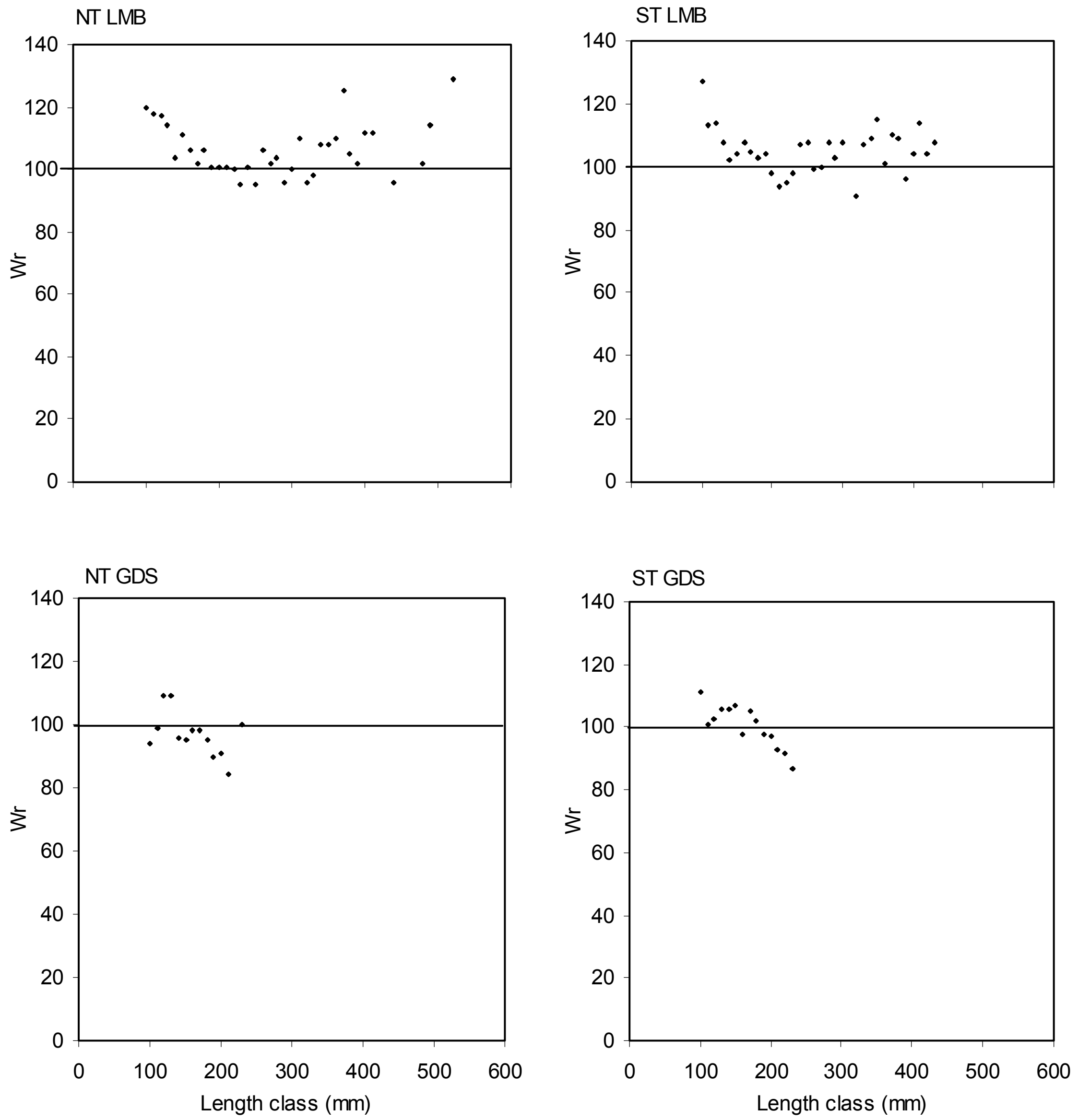
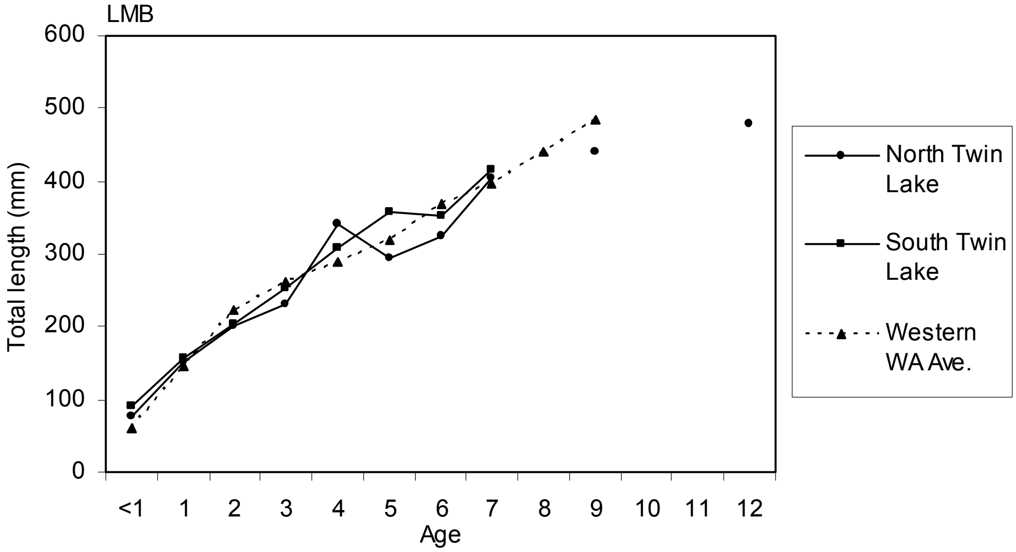


**Figure 11. Mean relative weights (Wr) for largemouth bass (LMB) (n=494) and golden shiners (GDS) (n=383) sampled from North Twin Lake (NT) and largemouth bass (n=900) and golden shiners (n=730) from South Twin Lake (ST), 2004. The line at 100 represents the 75th percentile of observed fish weights recorded in the literature (Anderson and Neumann 1996).**



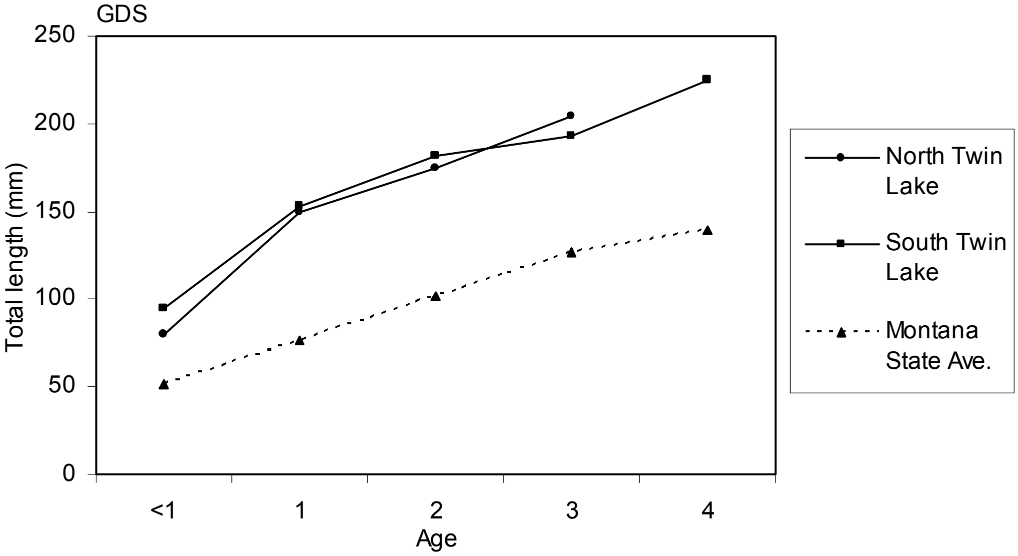


**Figure 12. Length (mm)-at-age for largemouth bass (LMB) (N. Twin n= 52, S. Twin n=46), and golden shiners (GDS) (N. Twin n=31, S. Twin n=30) and comparison across waters. Mean lengths taken from fish sampled in 2004.**

Western Washington average bass length-at-age compiled from 17 similar lakes in the state (Wydoski and Whitney 2003).

Golden shiners sampled ranged from 3 to 4 years in age and from 200 to 225 mm in length (Figure 12). Young-of-the-year shiners were between 75-90 mm in length. Length-at-age curves for both lakes are similar, but mean lengths for each age class (Figure 12) are much greater than the Montana average obtained from Wydoski and Whitney (2003). Such a comparison demonstrates good health of Twin Lake shiners and favorable environmental conditions.

Since largemouth bass have relatively small territories (0.18 to 2.07 hectares) (Fish and Savitz 1983), we assumed that food contents of stomachs sampled represented feeding within the transect habitat (Bettoli et al. 1992). In 2004, extra small bass (<100 mm) consumed zooplankton, midge larvae, and scuds throughout the growing season in North Twin Lake (Figure 13). In South Twin Lake, these fish consumed zooplankton, scuds, damselflies, and midges (Figure 15). Damselflies made up 84% of their diet during late July in South Twin Lake (Figure 15) bur were not present in the extra small bass obtained from North Twin (Figure 13). Scuds were also absent from stomach samples during June in North Twin Lake (Figure 13) but present in stomach samples taken from South Twin Lake (Figure 15).

Damselflies comprised between 30% and 85% of diets for small bass throughout the summer for both lakes (Figures 13 and 15). Golden shiners became more prevalent in stomach samples at 5 to 15% of the bass total diet for both lakes near the end of the sampling periods (Figures 13 and 15).

Medium bass (200 to 299 mm) in North Twin Lake consumed golden shiners during early June (up to 45%) and September (up to 42%) while diet proportions of shiners were near zero during July and early August (Figure 13). In South Twin Lake, golden shiners comprised up to 75% of medium bass diet during July and early August, 25% in early June, and 2% in September (Figure 15). During September, crawfish increased to 90% of South Twin medium bass diets (Figure 15) while no crawfish were sampled from bass stomachs in North Twin Lake for that size group (Figure 13).

Large bass (>300 mm) consumed nearly 100% golden shiners during late July and early August for both lakes (Figures 13 and 15). North Twin Lake bass diet proportions were nearly 20% crawfish in early June. These largest fish began to cannibalize other largemouth bass from August to September (Figure 13). However, in South Twin Lake, brook trout comprised nearly 50% of the bass diet from August to September. Crawfish were nearly 50% of the bass diet in early June and 25% in September (Figure 15).

Although diet proportions of golden shiners are considerably different between medium and large bass in North Twin (Figure 13) and South Twin (Figure 15), mean shiner weights sampled from largemouth bass stomachs were not significantly different between the two lakes ($\alpha$=.05). There were significant differences (p-value <.05) between some macroinvertebrate and amphipoda sonsumption on certain dates between the two lakes. This may have caused the offset in proportions as noted Figures 13 and 15.

In 2005, extra small bass (<100 mm) diets consisted of mostly damselflies, scuds, and midges in North Twin Lake (Figure 14). In August and September, a small portion of the bass diets consisted of small young-of-the-year golden shiners (Figure 14), in contrast to 2004. Small bass mm diets were mostly damselflies (Figure 14). Shiners became a greater portion of the bass diet in August and September (Figure 14) compared to 2004. The high presence of young-of-the-year shiners in dense weed beds may be the reason for the increase in shiner consumption for extra small and small bass in North Twin Lake. Damselflies, crawfish, and scuds were abundant in medium bass diets (Figure 14). However, shiner proportion by weight was much less than in 2004. Large bass primarily consumed shiners and a small proportion of crawfish (Figure 14).

In 2005 for South Twin Lake, scuds and midges comprised the majority of extra small bass diets (Figure 16). Much fewer zooplankton were observed in 2005 than in 2004 in both lakes. Small bass diets contained primarily damselflies with very little shiners present (Figure 16). Crawfish consumption was much higher than in North Twin Lake. For medium bass, diets contained mostly crawfish with only a small proportion of shiners (Figure 16). Large bass ate mostly shiners, crawfish, and rainbow trout (Figure 16). Rainbow trout were stocked in the lake just before our September 2005 sampling. When available, bass feed heavily on recently stocked trout, which are often very vulnerable. Crawfish consumption was also higher for all bass size classes indicating greater crawfish abundance or vulnerability.

Table 9 represents the frequency of occurrence and 2004 aggregated mean weight of largemouth bass prey. Frequency of occurrence and mean weight were similar for golden shiners for each bass feeding group for both lakes. Bass were found inside bass stomachs, but only in North Twin Lake (Table 9). Brook trout were found only in bass from South Twin (Table 9). Frequency and mean weight of crawfish was generally higher in South Twin except for small bass (Table 9).

As a subjective definition of piscivory, Bettoli et al. (1992) stated that bass were considered piscivores when at least 60% of sampled stomachs contained fish. As a summer aggregate, this definition was only partially achieved with the large bass size class (Table 9). However, piscivory was expressed during individual sampling dates. On June 29-30, 77% of stomachs from both lakes contained fish remains. On July 27-28, 100% of sampled bass stomachs from both lakes contained fish remains. During the last sampling period on September 9, 63% of South Twin bass stomachs and 50% in North Twin contained fish remains. No bass feeding groups <300mm had fish remains in more than 60% of the sampled stomachs. Figure 12 shows the total estimated number of shiners consumed by bass of all length classes. Figures 13 and 15 show rawfish frequency of occurrence, mean weights, and diet proportions in bass stomachs increased during the first and last sampling period when consumption of fish declined in both lakes.

Table 10 presents the frequency of occurrence and mean weight of largemouth bass prey species as a seasonal aggregate for 2005. Frequency of shiners in extra small bass was 9% in 2005 and none in 2004 for North Twin Lake (Table 9 and 10). For small bass, the frequency of occurrence of shiners increased from 8.9% in 2004 to 28% in 2005 in North Twin Lake (Table 9 and 10). The frequency of occurrence of South Twin remained similar to that in 2004. For medium bass, the frequency of shiners was less than observed in 2004 but crawfish frequency increased from 5.1% to 20.8% in South Twin Lake (Table 9 and 10). Shiner frequency was similar for North Twin Lake in both years. However, shiner frequency dropped from 54% in 2004 to 34.8% in 2005 (Table 9 and10). Crawfish frequency increased from 14.3% in 2004 to 34.9% in 2005 (Table 9 and 10). Frequency of trout consumption increased from 3.6% in 2004 to 17.4% in 2005 in South Twin Lake (Table 9 and 10).

Figures 18 and 19 represent the estimated number of shiners consumed by largemouth bass over the 90 day sampling period. The majority of shiners are consumed by small bass. This is because 75-84% of the bass population is comprised of 100-199 mm fish. Although frequency of shiners in small bass stomachs was low, the estimated overall consumption is high (Figures 18 and 19).

Golden shiner stomachs sampled in 2004 showed primarily plant/algae and scuds for both lakes (Figure 17). Snails, clams, midges, caddis, and zooplankton were also sampled from shiners in both lakes. Zooplankton were prevalent in fish stomachs but, by weight, made up only a small proportion of the shiner diet (Figure 17).

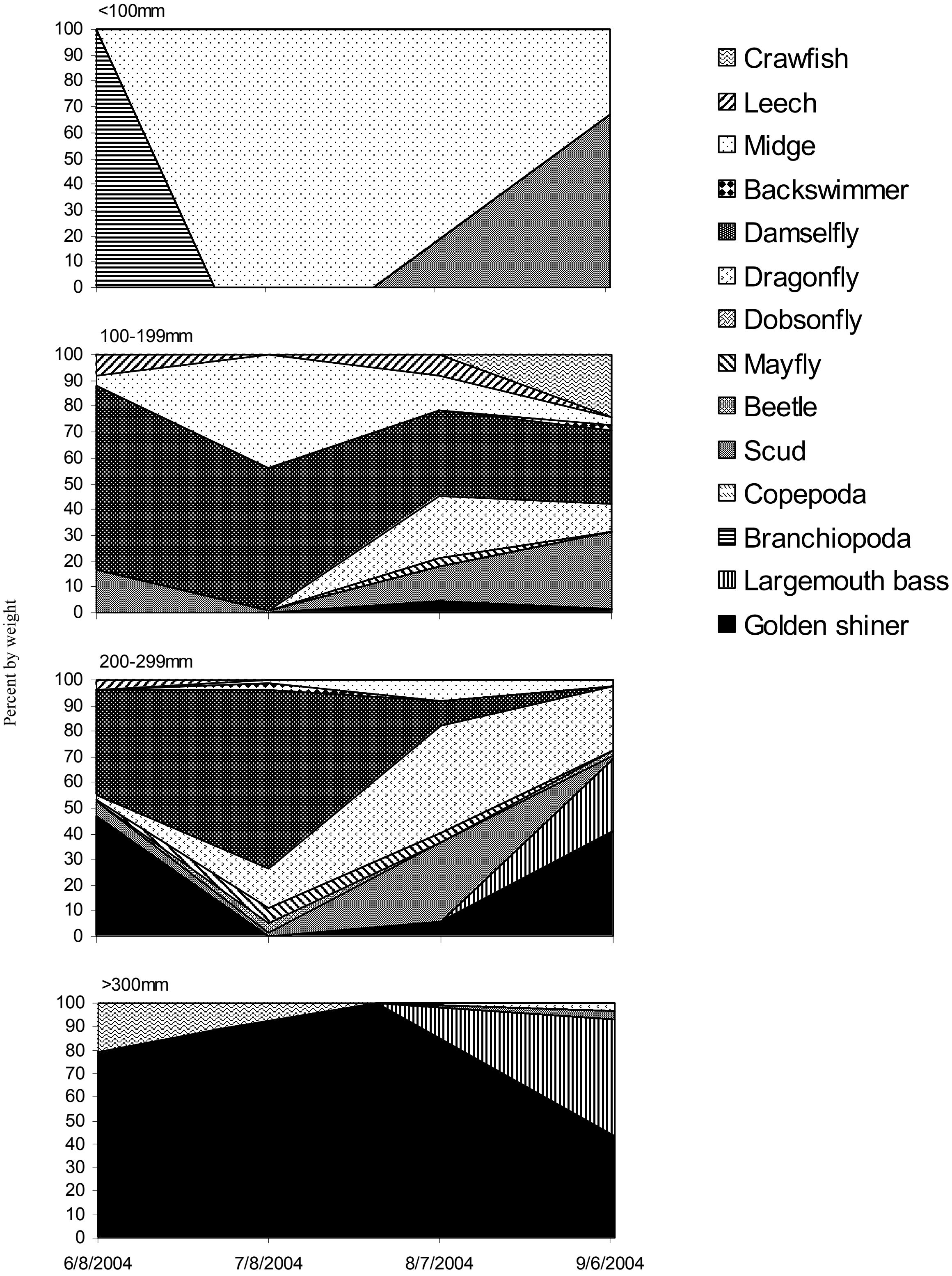


**Figure 13. Diet proportions of largemouth bass (n=127) of total length <100mm, 100-199mm, 200-299mm, and >300mm sampled from North Twin Lake, 2004.**

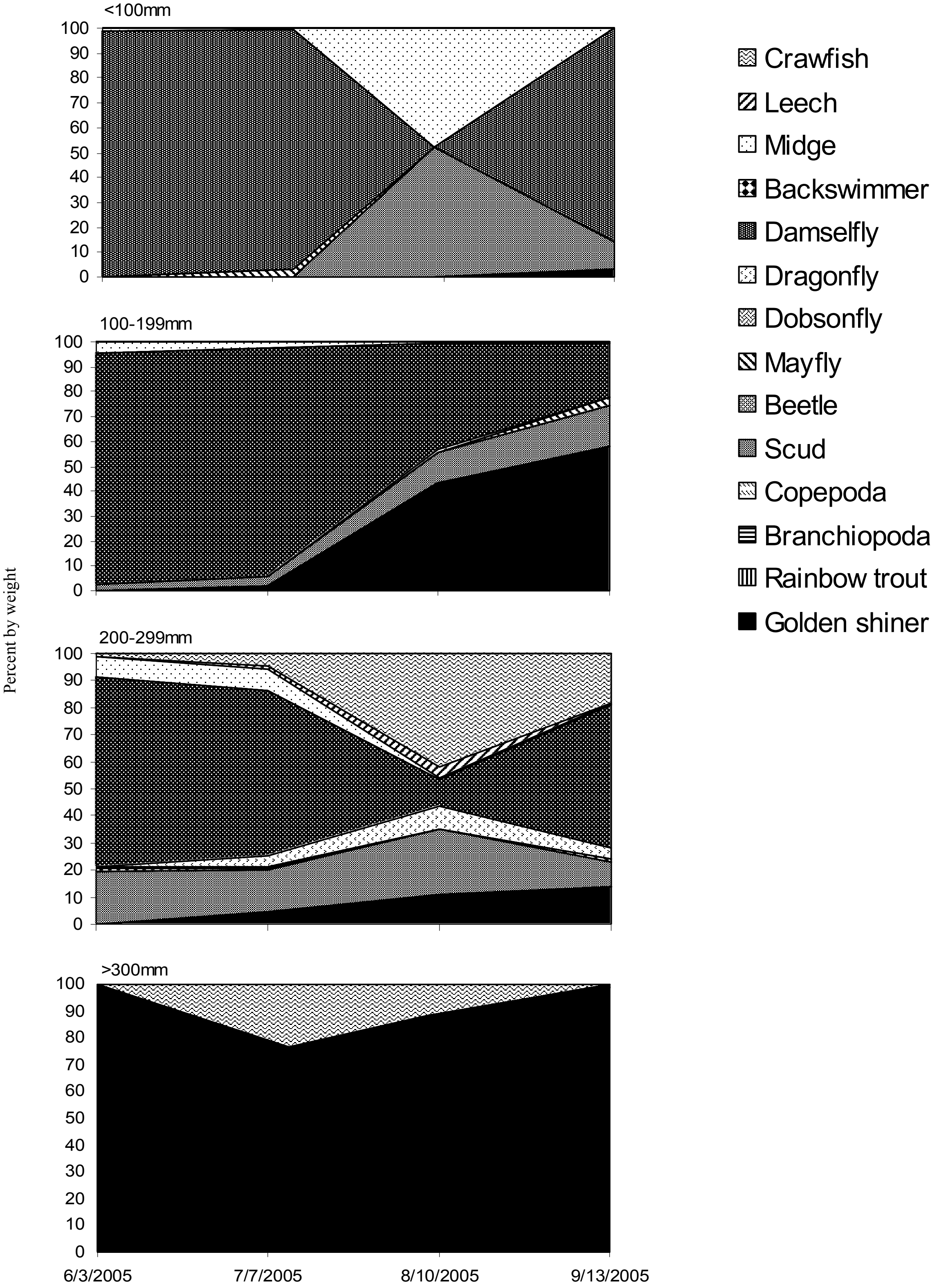


**Figure 14. Diet proportions of largemouth bass (n=172) of total length <100mm, 100-199mm, 200-299mm, and >300mm sampled from North Twin Lake, 2005.**

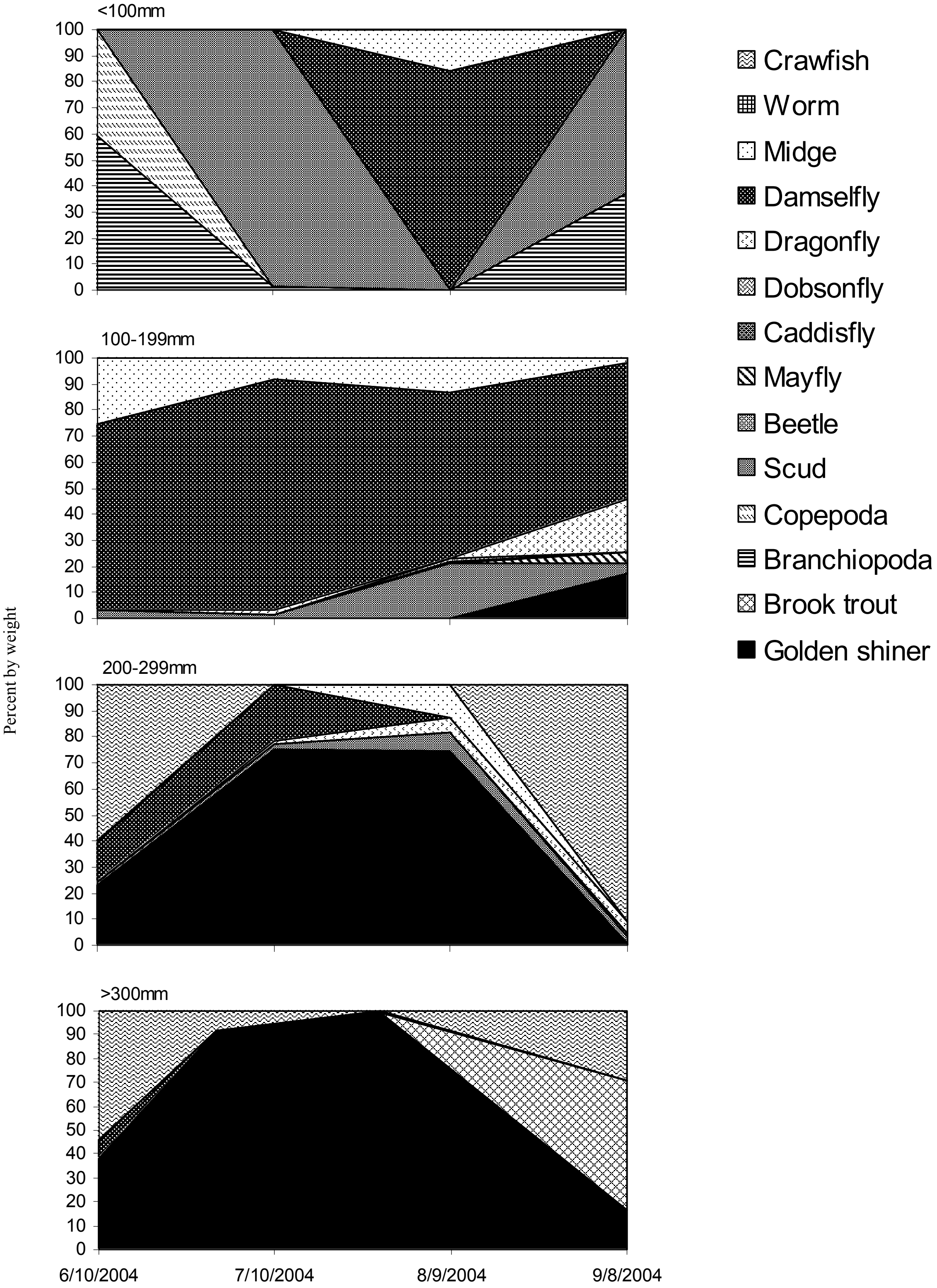


**Figure 15. Diet proportions of largemouth bass (n=134) of total length <100mm, 100-199mm, 200-299mm, and >300mm sampled from South Twin Lake, 2004.**

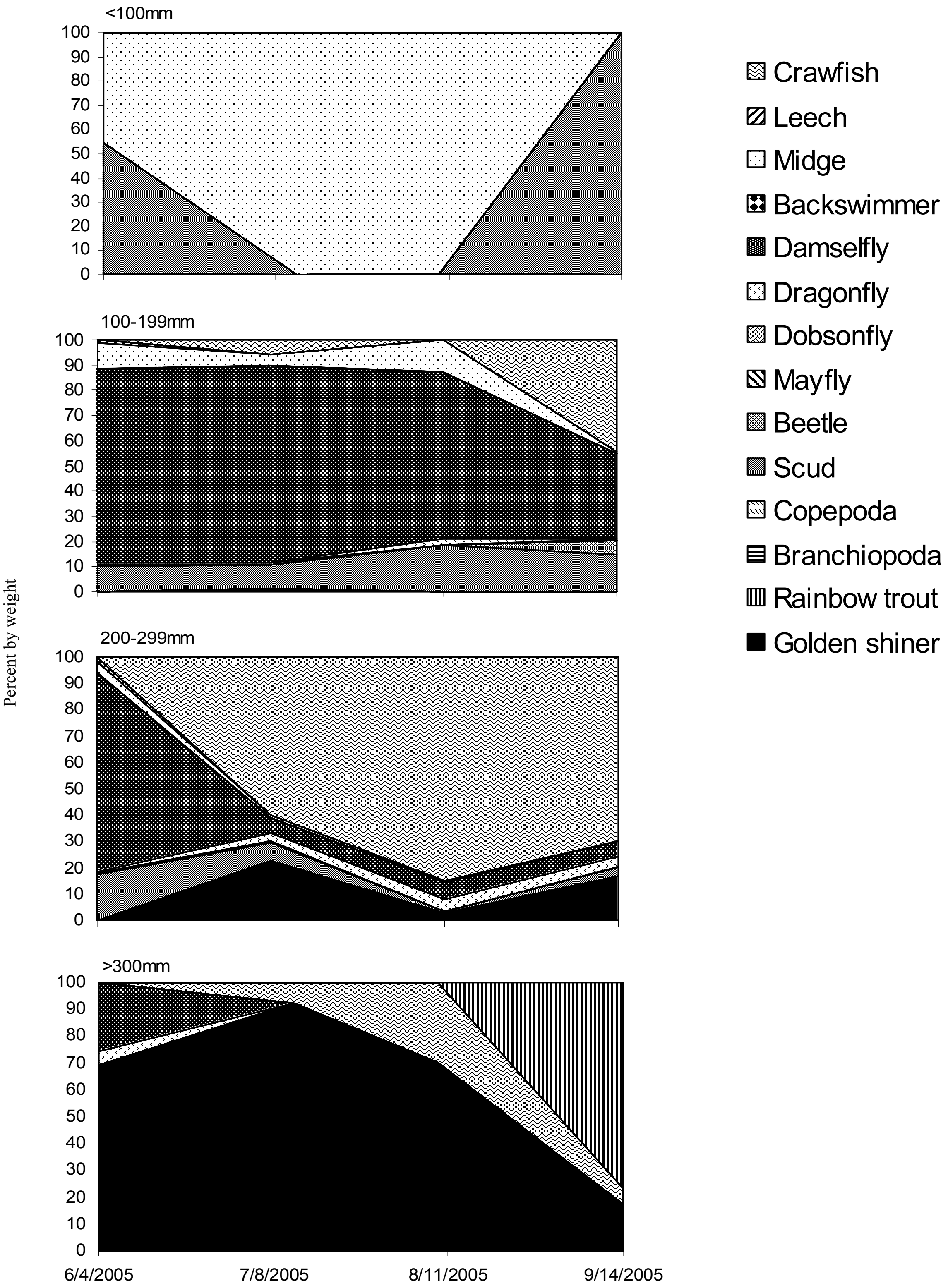


**Figure 16. Diet proportions of largemouth bass (n=189) of total length <100mm, 100-199mm, 200-299mm, and >300mm sampled from South Twin Lake, 2005.**

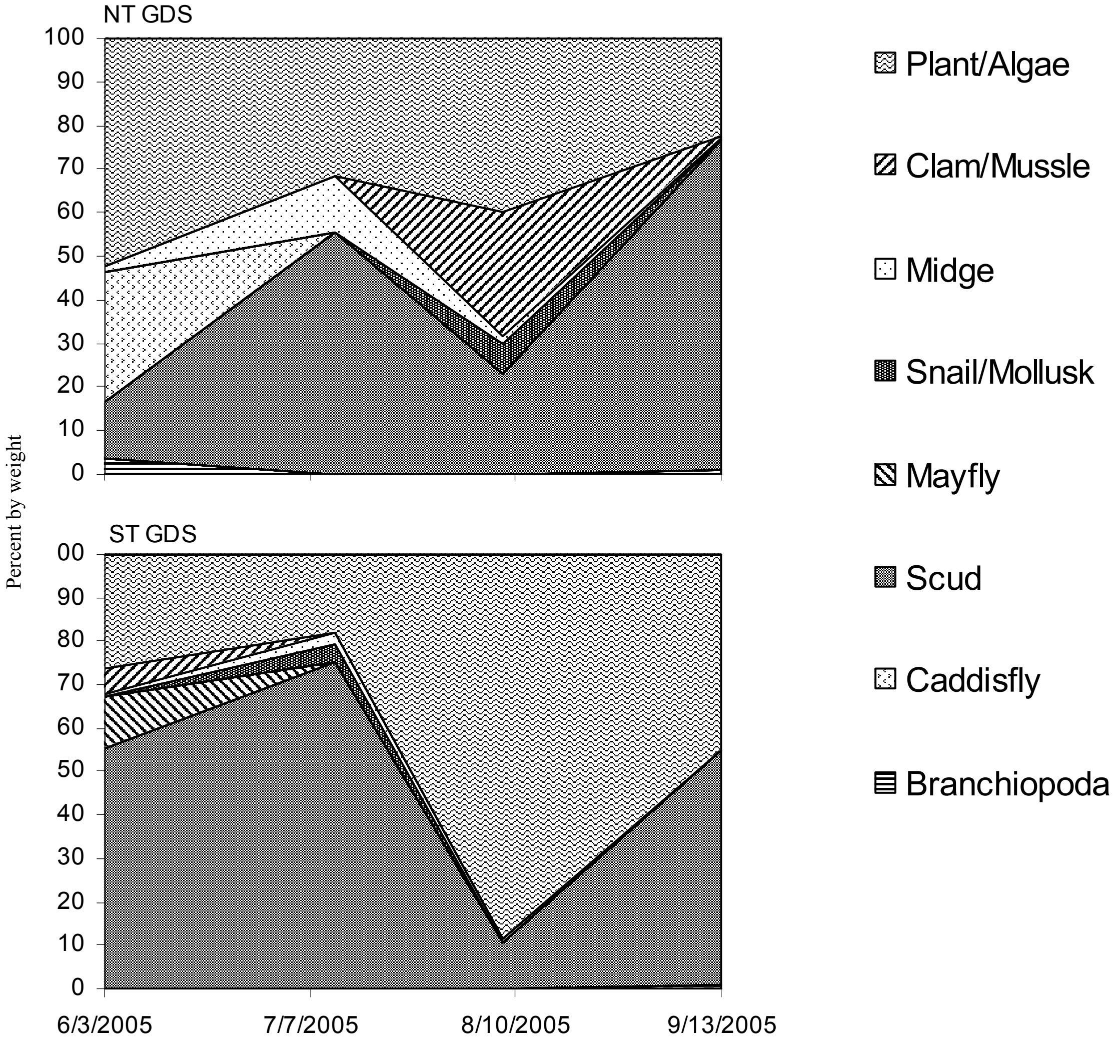


**Figure 17. Diet proportions of golden shiners (GDS) (n=32) from North Twin Lake (NT) and South Twin Lake (n=37) (ST), 2005.**

**Table 9. Frequency of occurrence and mean biomass of prey fish species per largemouth bass stomach sampled from North and South Twin Lakes, 2004. Frequency of occurrence is the percentage of bass stomachs containing the prey species. Largemouth bass <100mm was N=17, 100-199mm (N=45), 200-299mm (N=40) and >300mm (N=26) for North Twin Lake. Largemouth bass <100mm was N=26, 100-199mm (N=40), 200-299mm (N=39) and >300mm (N=28) for South Twin Lake.**

| Prey Taxa | Scientific Name | North Twin Lake | | South Twin Lake | |
|---|---|---|---|---|---|
| | | Frequency | Mean Biomass (g) | Frequency | Mean Biomass (g) |
| | | | <100mm | | |
| Golden shiner | *Notemigonus crysoleucus* | 0 | 0 | 0 | 0 |
| Largemouth bass | *Micropterus salmoides* | 0 | 0 | 0 | 0 |
| Brook trout | *Salvelinus fontinalis* | 0 | 0 | 0 | 0 |
| Crawfish | *Decapoda* | 0 | 0 | 0 | 0 |
| | | | 100-199mm | | |
| Golden shiner | | 8.89 | 0.013 | 7.50 | 0.016 |
| Largemouth bass | | 0 | 0 | 0 | 0 |
| Brook trout | | 0 | 0 | 0 | 0 |
| Crawfish | | 2.22 | 0.12 | 0 | 0 |
| | | | 200-299mm | | |
| Golden shiner | | 17.50 | 0.612 | 12.82 | 0.53 |
| Largemouth bass | | 2.50 | 0.0525 | 0 | 0 |
| Brook trout | | 0 | 0 | 0 | 0 |
| Crawfish | | 2.50 | 0.0001 | 5.13 | 1.01 |
| | | | >300mm | | |
| Golden shiner | | 53.85 | 9.51 | 53.57 | 6.11 |
| Largemouth bass | | 3.85 | 0.08 | 0 | 0 |
| Brook trout | | 0 | 0 | 3.57 | 3.21 |
| Crawfish | | 7.69 | 1.61 | 14.29 | 2.8 |

**Table 10. Frequency of occurrence and mean biomass of prey fish species per largemouth bass stomach sampled from North and South Twin Lakes, 2005. Frequency of occurrence is the percentage of bass stomachs containing the prey species. Largemouth bass <100mm was N=11, 100-199mm (N=64), 200-299mm (N=81) and >300mm (N=16) for North Twin Lake. Largemouth bass <100mm was N=5, 100-199mm (N=84), 200-299mm (N=77) and >300mm (N=23) for South Twin Lake.**

| Prey Taxa | Scientific Name | North Twin Lake | | South Twin Lake | |
|---|---|---|---|---|---|
| | | Frequency | Mean Biomass (g) | Frequency | Mean Biomass (g) |
| | | | | <100mm | |
| Golden shiner | *Notemigonus crysoleucus* | 9.09 | 0.005 | 0 | 0 |
| Rainbow trout | *Oncorhynchus mykiss* | 0 | 0 | 0 | 0 |
| Crawfish | *Decapoda* | 0 | 0 | 0 | 0 |
| | | | | 100-199mm | |
| Golden shiner | | 28.13 | 0.134 | 8.33 | 0.003 |
| Rainbow trout | | 0 | 0 | 0 | 0 |
| Crawfish | | 0.00 | 0 | 2.38 | 0.071 |
| | | | | 200-299mm | |
| Golden shiner | | 13.58 | 0.104 | 6.49 | 0.176 |
| Rainbow trout | | 0 | 0 | 0 | 0 |
| Crawfish | | 7.41 | 0.206 | 20.78 | 0.934 |
| | | | | >300mm | |
| Golden shiner | | 50.00 | 13.32 | 34.78 | 9.94 |
| Rainbow trout | | 0 | 0 | 17.39 | 6.95 |
| Crawfish | | 12.50 | 1.06 | 34.78 | 1.89 |

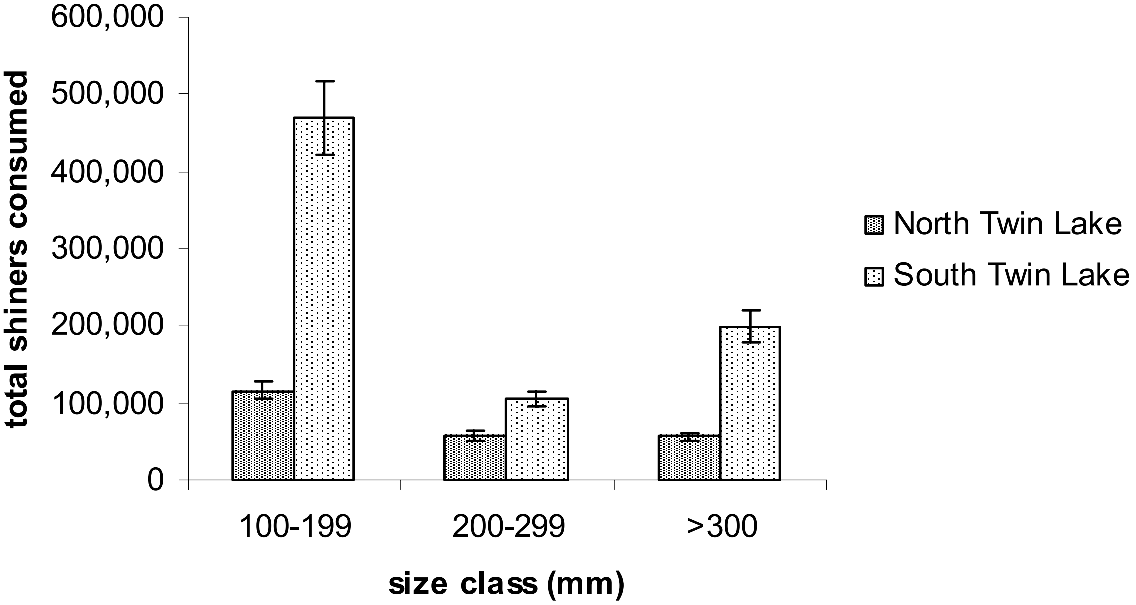


**Figure 18. Estimated number of golden shiners consumed by largemouth bass in Twin Lakes from June 6, 2004 to Sept. 7, 2004. Estimates were extrapolated from frequency of occurrence of shiners in bass stomachs and bass population estimates. Brackets are ten-percent error bars.**

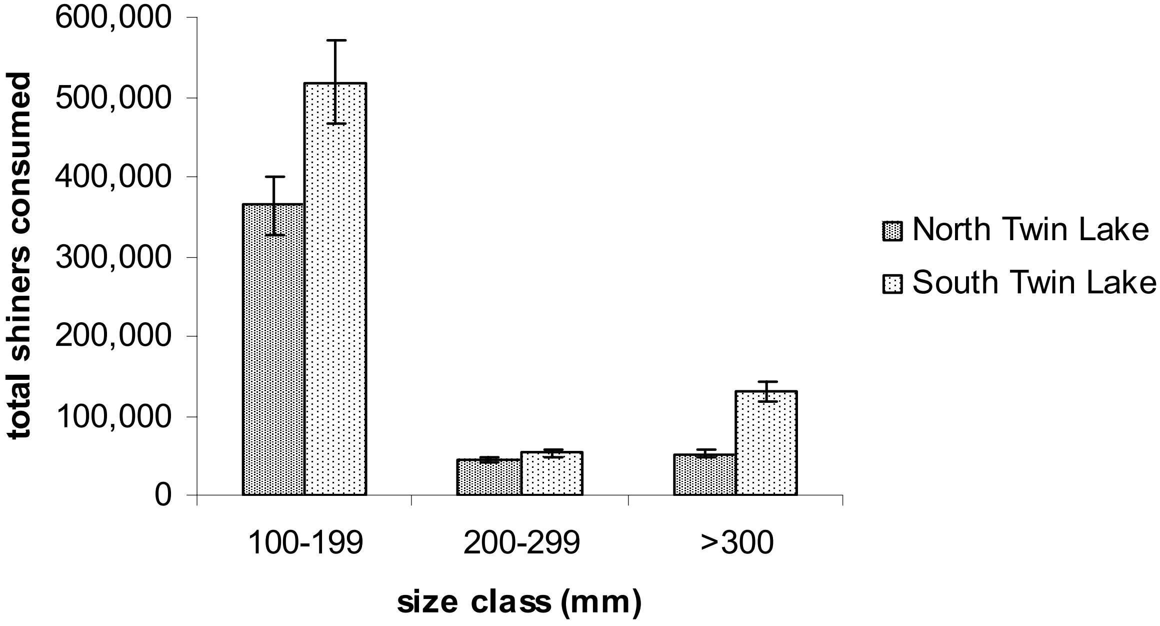


**Figure 19. Estimated number of golden shiners consumed by largemouth bass in Twin Lakes from June 6, 2005 to Sept. 7, 2005. Estimates were extrapolated from frequency of occurrence of shiners in bass stomachs and bass population estimates for 100-199 mm, 200-299 mm, and >300 mm length classes. Brackets are ten-percent error bars.**

### *Brook and Rainbow Trout*

During the summer of 2004, 116 rainbow and 35 brook trout were sampled from North Twin and 10 rainbow and 28 brook trout were sampled from South Twin (Table 8). Salmonid relative abundance was much lower than bass and shiners (Table 8). species Diet proportions for trout may have been higher, but sample sizes were relatively small. Population estimates could not be obtained due to the method of sampling. Sample sizes of rainbow and brook trout for 2005 are presented in Table 11.

Length class histogram for rainbow trout in North Twin Lake in 2004 shows a bell-shaped curve, slightly skewed to the right (Figure 20). The mean length of rainbow trout sampled is 293 mm. Seventy-six of 88 rainbow trout sampled (86%) had adipose fin clips from the tribal fish hatchery. Of these fish, 63 had green elastomer tags (72%) indicating 2003 plants, and 13 had orange tags (15%) indicating 2002 plants. Twenty-nine fish with adipose fin clips had similar lengths to fish with green elastomer tags, and were assumed to be of the same cohort as tagged fish. Likewise, 5 adipose-clipped fish had similar lengths to orange marked fish. They were assumed to be in the 2002 cohort.

The 2004 rainbow trout sample from South Twin Lake was only 10 fish, too small to create a meaningful histogram. Four fish had adipose fin clips, and only one had a green elastomer tag. Average rainbow length from South Twin was 335 mm. Inadequate sample size precluded statistical comparison of mean length was made between the lakes for rainbow trout.

Mean length of brook trout from North Twin was 323 mm, and 358 mm for South Twin. These values were not statistically significant was found ($\alpha = 0.05$, Figure 20). Of 35 fish sampled from North Twin, five had adipose fin clips (14%), one with a green elastomer tag, and one with an orange tag. All remaining fish (86%) were assumed to be naturalized wild fish. In South Twin, none of the 28 sampled fish had adipose fin clips or elastomer tags, and were assumed to be naturalized wild fish.

Relative weight (Wr) values for sampled rainbow trout in both lakes were mostly below 100 (Figure 21). This represents fish health lower than the 75th percentile of Wr estimates published in the literature (Anderson and Neumann 1996), suggesting that environmental conditions are less than optimal for this species. Wr values for brook trout in North Twin are near 100 but mostly below 100 in South Twin (Figure 14).

Figure 21 shows length-at-age of rainbow and brook trout in Twin Lakes compared to other Eastern Washington Lakes. Maximum sampled rainbow trout age was 4 years for both lakes. Rainbow trout age was determined by elastomer tags inserted into the epidermis of the anal fin. Green tags represent fish planted in 2004 and orange represents fish planted in 2003. Other fish were 4-year-old, unmarked individuals. Rainbow trout in North and South Twin Lakes represented similar lengths at each age, but South Twin Lake fish were slightly longer (Figure 22). Lengths were comparable to rainbow trout sampled from Ross Lake, WA, except fish in Ross Lake were smaller at age 1 (Figure 22). Rainbow trout in Deer Lake, WA had similar lengths at ages 1 and 2 but declined below Twin Lake fish lengths at ages 3 and 4.

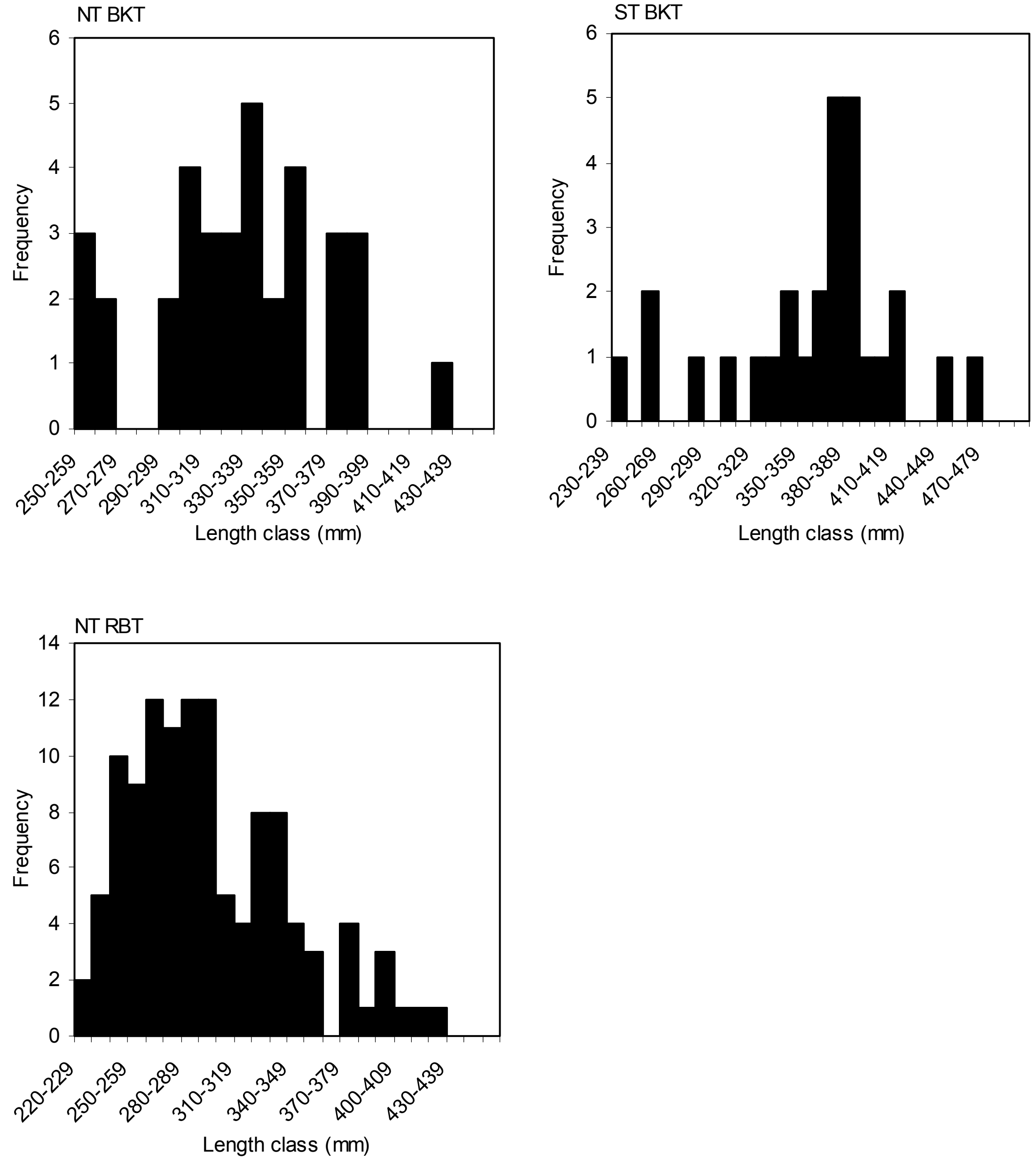


**Figure 20. Length class histograms for brook trout (BKT) (n=35) and rainbow trout (RBT) (n=116) sampled from North Twin Lake (NT) and brook trout (n=28) from South Twin Lake (ST), 2004.**

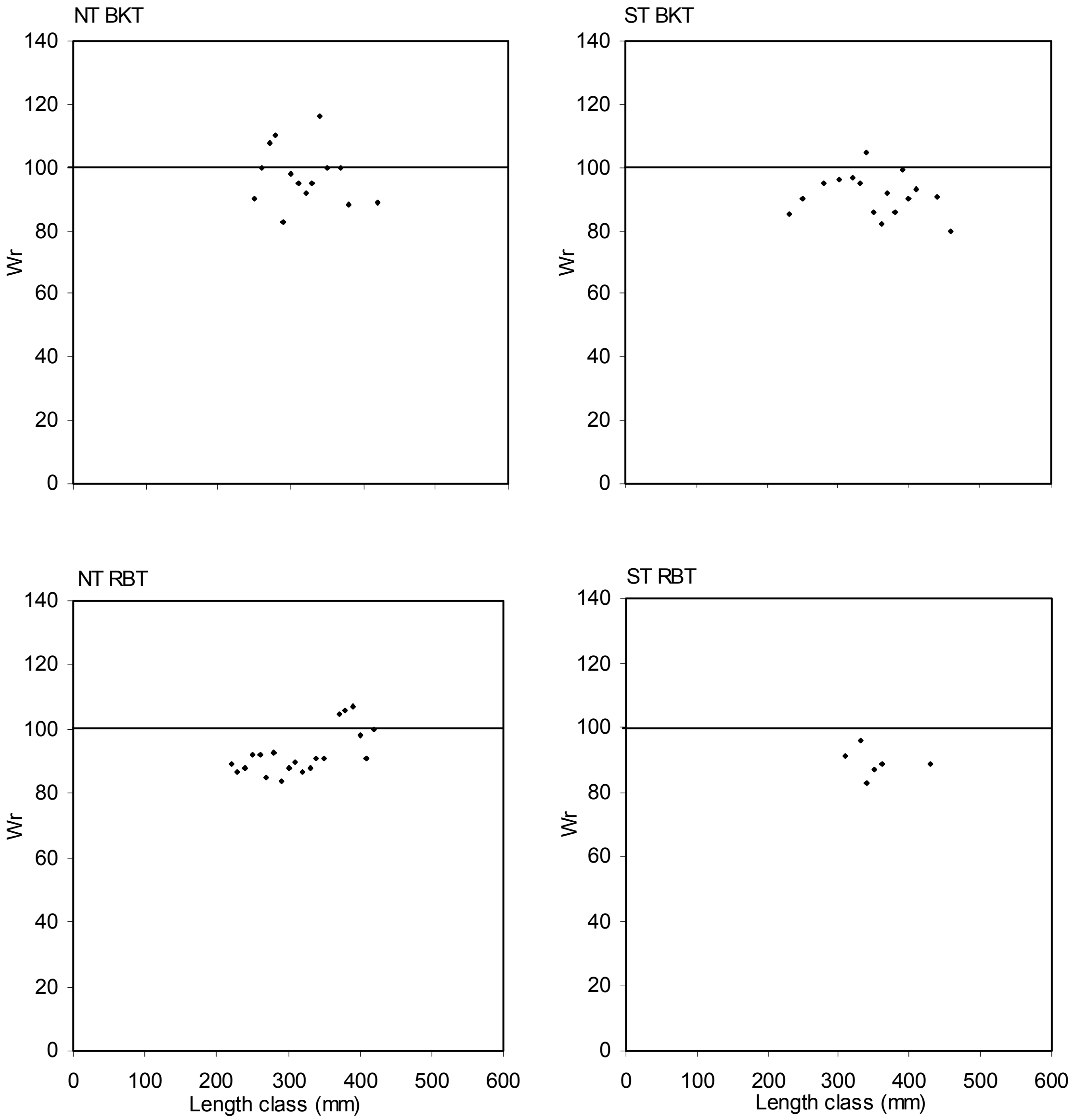
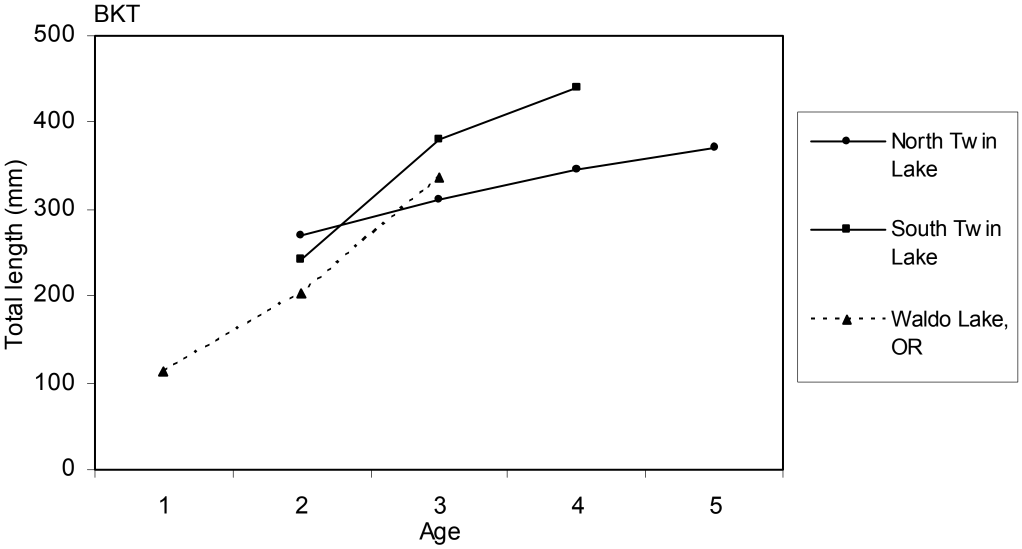


Figure 21. Mean relative weights (Wr) for brook trout (BKT) (n=35) and rainbow trout (RBT) (n=116) sampled from North Twin Lake (NT) and brook trout (n=28) and rainbow trout (n=9) from South Twin Lake (ST), 2004. The line at 100 represents the 75th percentile of observed fish weights recorded in the literature (Anderson and Neumann 1996).

Maximum age for brook trout were 4 years in South Twin and 5 years in North Twin Lake (Figure 22). Brook trout length-at-age relationships were more variable between the lakes compared to rainbow trout. This could be the result of a smaller sample sizes (Figure 22). Length-at-age 2 was similar for Twin Lakes. However, lengths of fish sampled from North Twin dropped well below South Twin Lake fish for ages 3 to 5 (Figure 22). Lengths of brook trout from Waldo Lake, WA were slightly lower at age 2 and were intermediate at age 3 compared to Twin Lake fish.

Rainbow and brook trout diets consisted almost entirely of midges and scuds in both lakes in 2004 (Figures 23 and 25). Unknown fish species appeared in almost 90% of the large rainbow trout from North Twin Lake in September (Figure 23). By number, zooplankton was the most prevalent and abundant food source for rainbow and brook trout. However, by weight, zooplankton comprised very little of the trout diet. Due to small sample sizes, significance at $\alpha = 0.05$ was not reached between North and South Twin Lakes. Larger samples are needed to make truly evaluate the statistical significance of these differences.

In 2005, midges were the primary food source by weight for all sampling dates for both rainbow and brook trout (Figures 24 and 26). Scuds also made up a significant portion of trout diets. As many and 3,500 zooplankton were sampled from a single stomach. Zooplankton was the most abundant prey species in the trout stomachs but, by weight, made up only a small portion of the trout diets (Figures 24 and 26). In July, golden shiners were a significant portion of North Twin rainbow trout diets (Figure 24).



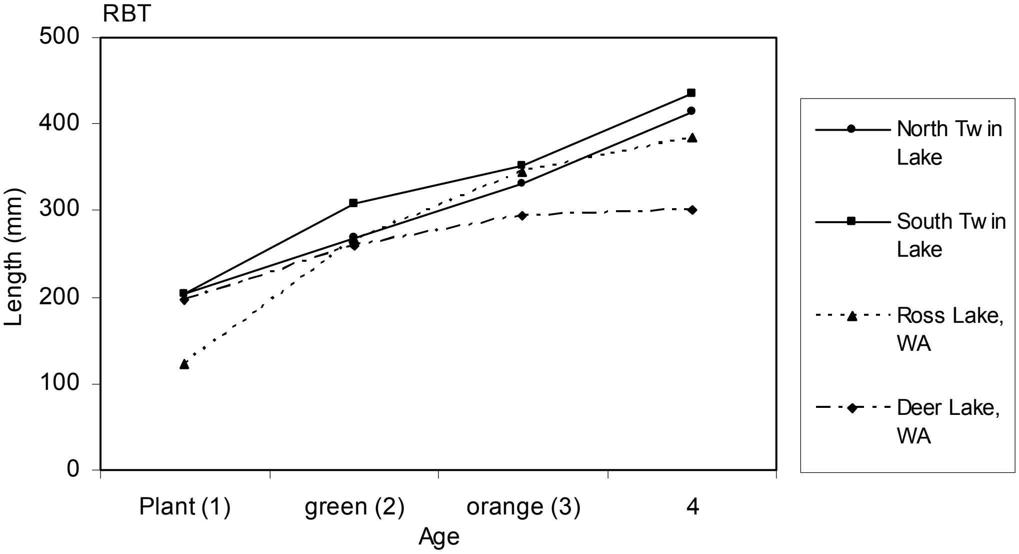


**Figure 22. Length (mm)-at-age for brook trout BKT (N. Twin n=10, S. Twin n=6), and rainbow trout RBT (N. Twin n=87, S. Twin n=10) and comparison across waters. Mean lengths taken from fish sampled in 2004.**

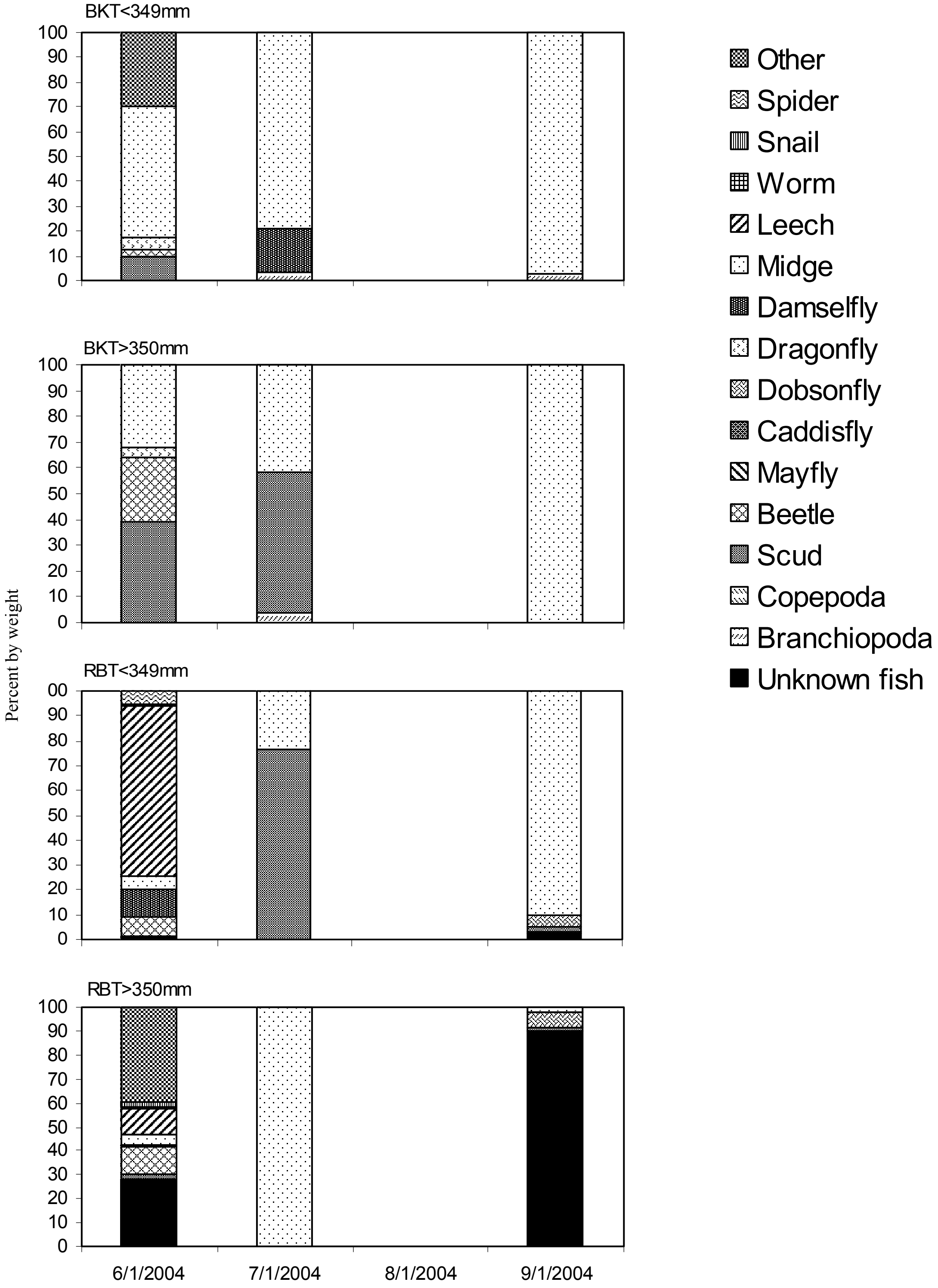


**Figure 23. Diet proportion of brook trout (BKT) (n=31) and rainbow trout (RBT) (n=44) <349mm and >350mm sampled from North Twin Lake from June to September 2004.**

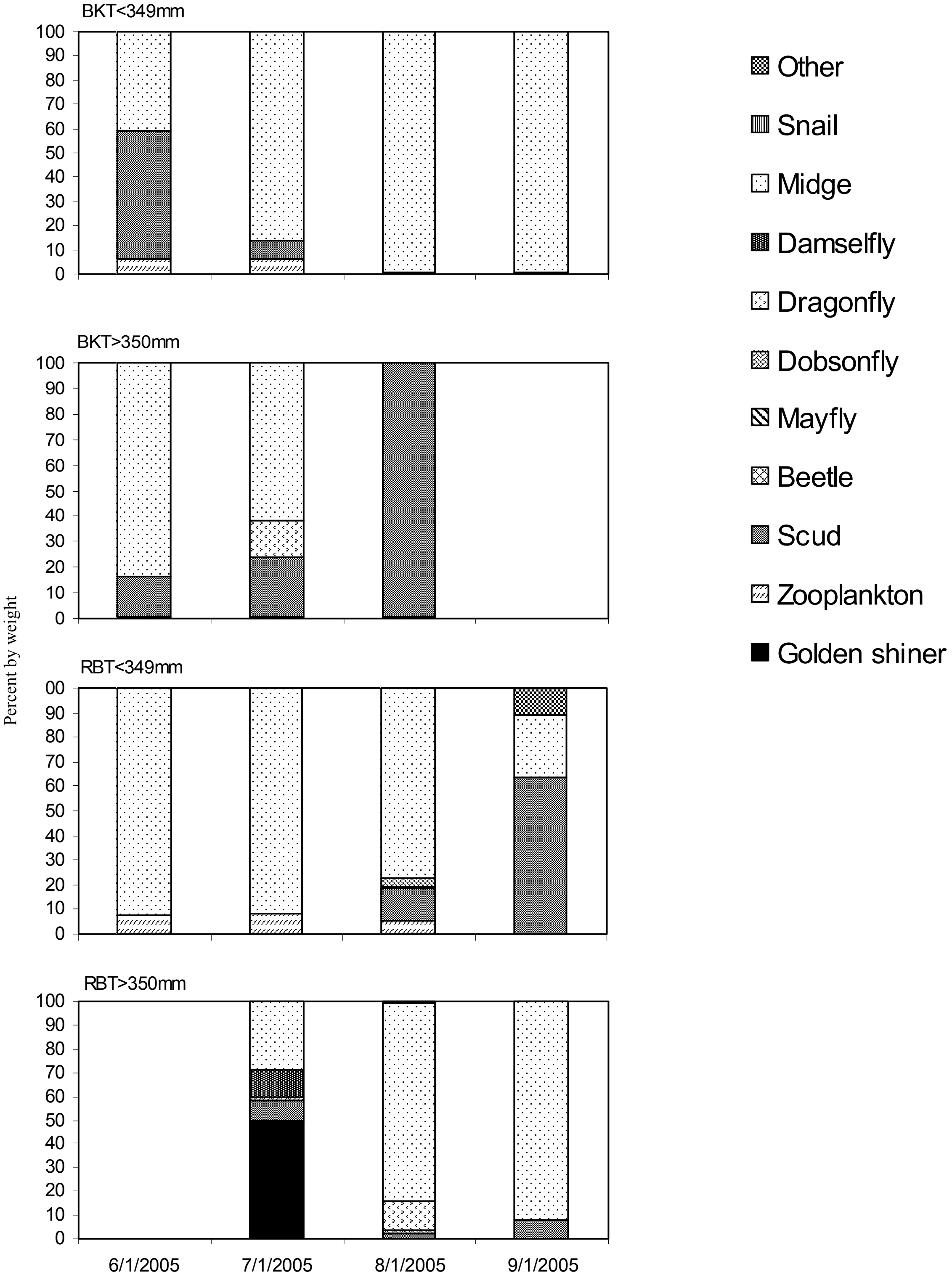


**Figure 24. Diet proportion of brook trout (BKT) (n=25) and rainbow trout (RBT) (n=62) <349mm and >350mm sampled from North Twin Lake from June to September 2005.**

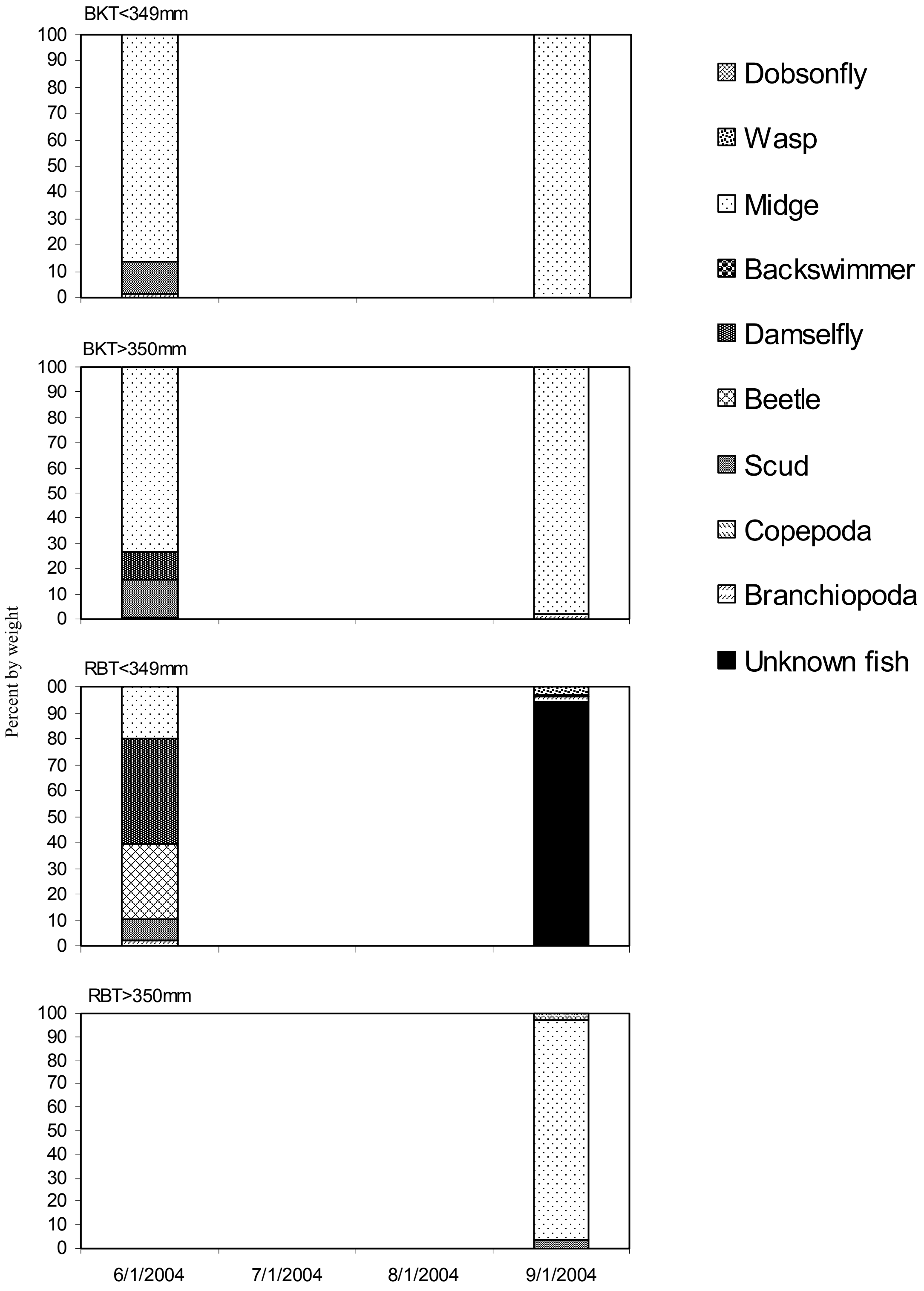


**Figure 25. Diet proportion of brook trout (BKT) (n=21) and rainbow trout (RBT) (n=8) <349mm and >350mm sampled from South Twin Lake from June to September 2004.**

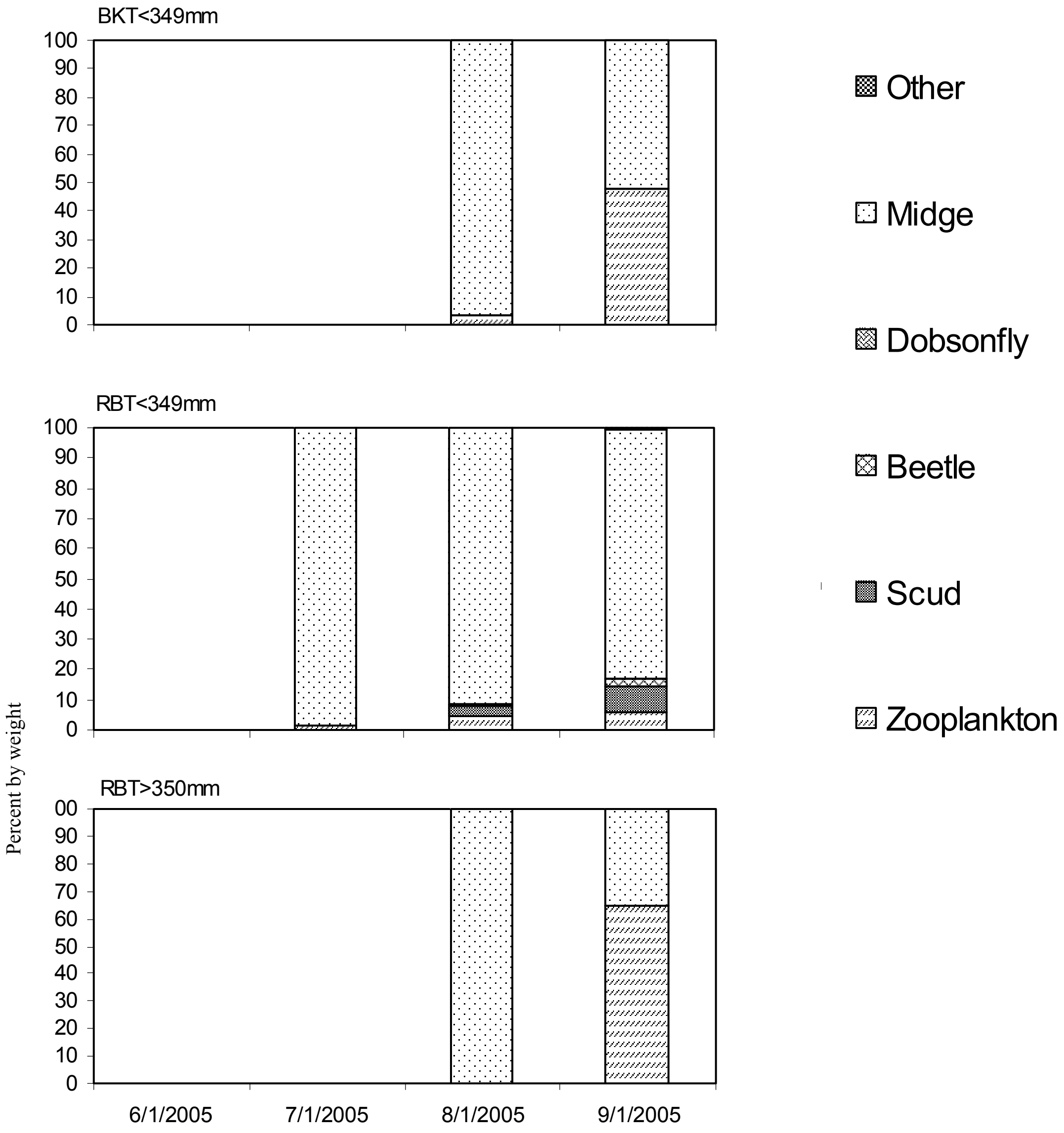


**Figure 26. Diet proportion of brook trout (BKT) (n=6) and rainbow trout (RBT) (n=32) <349mm and >350mm sampled from South Twin Lake from June to September 2005.**

**Table 11. Sample size of rainbow trout and brook trout taken from North and South Twin Lakes in 2005. Marked fish are those with adipose fins clipped, elastomer tags, or both. Unmarked fish contained neither mark. Proportions are represented for marked, unmarked, and each color of elastomer marked fish and for total sample size by species. Pink marked fish were planted in the fall of 2005, green marked fish were planted in the spring of 2005, and orange marked fish were planted in the spring of 2004.**

| North Twin Lake 2005 | | | | | Tag Color | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | Scientific name | N marked | N unmarked | Total Sample | N Pink | Length | N Green | Length | N Orange | Length |
| Rainbow trout | *Oncorhynchus mykiss* | 133 | 6 | 139 | 3 | 160 | 121 | 265 | 9 | 375 |
| | | 96% | 4% | 85% | 2% | | 91% | | 7% | |
| Brook trout | *Salvelinus fontinalis* | 3 | 22 | 25 | 0 | | 0 | | 3 | 319 |
| | | 12% | 88% | 15% | | | | | 100% | |

| South Twin Lake 2005 | | | | | Tag Color | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Species | Scientific name | N marked | N unmarked | Total Sample | N Pink | Length | N Green | Length | N Orange | Length |
| Rainbow trout | *Oncorhynchus mykiss* | 43 | 1 | 44 | 0 | | 39 | 275 | 4 | 370 |
| | | 98% | 2% | 88% | | | | | | |
| Brook trout | *Salvelinus fontinalis* | 1 | 5 | 6 | 0 | | 0 | | 1 | 340 |
| | | 17% | 83% | 12% | | | | | 100% | |

# CHAPTER 5

# DISCUSSION

### *Largemouth Bass and Golden Shiners*

Golden shiners are critical prey for largemouth bass >300 mm (large size class) in Twin Lakes. Although shiners are present in less than 60% of large bass stomachs, they appear to be a central part of the bass diet. September declines in shiner abundance and bass predation rates suggest bass are impacting the population. Extrapolated predation rates in both lakes suggest strong top-down influence on shiner populations. Despite lower watershield coverage in South Twin Lake, there is no significant difference in predation on shiners between the lakes. However, watershield is so dense that bass are limited to utilizing edge habitats for feeding. This was apparent in our sampling when most bass were recovered from peripheral or less dense areas of vegetation. Increasing the edge habitat in North Twin Lake could provide more feeding lanes and opportunities for bass.

Small bass (100 to199 mm) appear to be consuming the most shiners in Twin Lakes. Small bass contain only a small portion of shiners in their diet, but this size class makes up 75 to 84% of the entire bass population. Because high mortality exists when fish reach 200 mm, enhancing survival of larger fish using slot-limits could maximize their top-down control on shiners.

Macrophyte reduction could also be used to enhanced bass piscivory on shiners. However, it should be cautioned that this study found no difference in shiner consumption by smaller bass between the two lakes, despite differences in watershield coverage. Therefore, results of macrophyte reduction might be minimal for this size class.

Another objective of this study was to determine prey selectivity of largemouth bass throughout the growing season. Small bass fed primarily on zooplankton, midges, damselflies, and scuds, results similar to other studies (Berthou 2002, Liao et al. 2002, Ward and Neumann 1998). Extra small bass principally consumed zooplankton, and the peak utilization correlates to those periods of high zooplankton density. Copepods (*Diaptomus sp.*) and Cladocerans (*Daphnia pulex, Holopedium sp.*) were identified in individual <100 mm bass stomachs. Due to their size and abundance, *Diaptomus sp.*, *Daphnia pulex*, and *Holopedium sp.* appear to be the most significant zooplankton in the fish diets.

Zooplankton utilization of weed beds to escape predation may explain reduced consumption by extra small bass in July and August. Watershield beds were the most extensive in North Twin. Zooplankton have been documented to enter weed beds to escape predation by fish (Romare and Hansson 2003). This may explain why no zooplankton were sampled from bass stomachs in North Twin during August and September.

Zooplankton also move pelagically as warmer water forces salmonids deeper into the lake (Romare and Hansson 2003). Golden shiners migrate diurnally between littoral and pelagic waters. Increased shiner predation on zooplankton may reduce zooplankton abundance, which could also explain the changes in bass consumption (Stone and Thomforde 2001, Wydoski and Whitney 2003). Reduced bass consumption of zooplankton can also be explained by increased availability of other prey species such as damselflies and midges. When available, damselflies and midges may be preferred over zooplankton as noted in 2005 bass stomach samples.

Diversity of prey items increased dramatically with the small bass. Small bass diets overlapped with diets of the extra small bass, but the number of prey species consumed increased from 3 to 4 items to 9 to 10 items in the larger fish. Increase in fish mouth gape and greater mobility improves prey capture efficiency (Liao et al. 2002).

Small bass consumed golden shiners, but these represented only a small portion of their diet. Mean length of golden shiners consumed was 28 to 30 mm but only appeared in 7.5% to 8.89% of the sampled stomachs. This suggests prey fish were not a significant food item for small bass in 2004. This is most likely due to the small gape size of the bass and the inability to capture other fish (Liao et al. 2002). However, in 2005, shiners appeared in 28% of small bass stomachs. This result is most likely due to the increased availability of young-of-the-year shiners for 2005, and does indicate the potential importance of shiners in the bass diet when they are available and small enough.

Dense weed beds offer shelter and rearing conditions for young largemouth bass (Hoyer and Canfield 1996). However, dense canopy-forming weed beds like those prevalent in Twin Lakes may reduce piscivory and increase reliance on macroinvertebrates (Bettoli et al. 1992, Dibble and Harrel 1997, Savino and Stein 1982, Valley and Bremigan 2002). Dibble and Harrel (1997) found that at varying macrophyte densities, juvenile largemouth bass diets in non canopy-forming pondweed to be 71% macroinvertebrates and 29% prey fish. In canopy-forming Eurasian water milfoil beds, bass diets were 67% prey fish and 33% macroinvertebrates. This suggests an optimum plant density exists at which fish predation is the highest.

Importance of golden shiners increased significantly for medium bass (200-299 mm). The wet weight proportion of shiners in medium bass diets in South Twin Lake reached nearly 75% during July and August but <10% in North Twin Lake in 2004. Although diet proportions are different, actual wet weights are not significantly different. Extensive watershield beds in North Twin Lake can provide an increase in diversity and density of macroinvertebrates, potentially altering the diet proportion. Because prey abundance and species availability vary considerably, the age at which bass switch primarily to piscivory varies (Bettoli et al. 1992). This was observed in the different predation rates between 2004 and 2005. Studies by various authors show that an increase in macrophyte density and canopy coverage reduces piscivory of largemouth bass (Bettoli et al. 1992, Dibble and Harrel 1997, Savino and Stein 1982, Smith 2000, Trebitz et al. 1997, Valley and Bremigan 2002). However, none of these studies examined predator/prey relationships between largemouth bass and golden shiners. Golden shiner diurnal pelagic/littoral habitat occupation presents a unique relationship in which shiners may be able to escape bass predation during the day in dense macrophyte beds and avoid predators at night by migrating to pelagic waters. This behavior of shiners is not extensively reviewed in the literature and should be researched further.

Crawfish are the most significant prey item, second only to shiners, for medium bass sampled in South Twin Lake. However, crawfish consumption was insignificant in North Twin Lake bass. The absence of crawfish in medium bass stomachs in North Twin Lake could also be influenced by weed bed abundance and density. Dense weed beds could prevent bass from adequately feeding on crawfish. Higher crawfish consumption in

South Twin Lake could indicate crawfish are a preferred prey item when available for consumption.

Large bass were almost entirely piscivorous over the course of the summer in both lakes. Combined frequency of prey fish occurrence from June to September for large bass was just under 60% for both lakes. During July and August, 75% to 100% of all large bass stomachs sampled contained fish. Golden shiners were the most abundant fish species in all stomachs. Crawfish and bass comprised most of the remaining proportion of the largemouth bass diet in North Twin Lake, and crawfish and brook trout in South Twin Lake. Crawfish frequency of occurrence in South Twin Lake was significantly higher (p-value <.05) than in North Twin Lake. This suggests an increase in crawfish density, availability or both in South Twin Lake. Crawfish may be preferred over shiners when they are available. Shiner consumption by large bass in both lakes was the lowest during June and September while crawfish, bass and brook trout consumption were the highest during those months.

Several factors may influence this relationship. First, crawfish may be more abundant during the early and late sampling periods and may be the preferred prey species for bass. Second, ambush techniques used by bass may not be effective before macrophyte establishment. Third, new YOY shiners were more abundant during July and August sampling periods. YOY shiners reached larger sizes that were too big for consumption by September. Also, fewer shiners were available by September because of bass consumption earlier in the year.

Macrophyte density was the highest in September when shiner consumption declined. Ambush techniques used by bass are not as successful in dense macrophyte beds. This may support the second explanation. Cannibalism in North Twin Lake in September suggests insufficient access to shiners (Pelham et al. 2001). YOY shiners may have obtained larger sizes, increasing their ability to escape bass in weed beds and/or in pelagic habitats (Savino and Stein 1989, Jacobsen et al. 1997). It may also be because fewer shiners are left to be consumed. Predation of brook trout and rainbow trout in September in South Twin Lake also supports this third explanation that shiners have become less available. This is because YOY fish are absent in the spring and larger in the fall forcing largemouth bass to consume alternative prey sources. Brook trout and rainbow trout were not available for bass consumption during July and August due to excessive water temperatures in the littoral areas. Most likely, a combination of each explanation is correct.

Despite differences in diet proportions between North and South Twin Lakes, length class distribution, length-at-age, and relative conditions are similar for largemouth bass. Wr values indicate the fish are in good to excellent condition. Length-at-age values illustrate good bass growth and are similar to bass lengths in 17 other western Washington waters. Regardless of moderate piscivory by large bass, the consumption of macroinvertebrates, amphipods, and crustaceans seems adequate to maintain good condition and comparable size with bass in other waters. It is important to note the variability that exists in prey consumption for each group of bass from year to year.

Length class distribution, length-at-age, and relative conditions indicate good health for golden shiners in Twin Lakes. Golden shiners are reported to reach 10 inches and 10 years in age in other waters (Wydoski and Whitney 2003). In Twin Lakes, some sampled shiners were 9 in long at only 4 years old. Length-at-age comparisons are much greater than the Montana average (Wydoski and Whitney 2003), indicating excellent conditions for shiners. Diets of golden shiners are consistent with the literature that suggests plant/algae are near 50% of the diet while macroinvertebrates make up the rest. Larger shiners indicate top down control by bass. As bass predate on shiners the population becomes cropped, allowing the remaining fish to reach larger sizes because of increased food availability (Modde and Scalet 1985).

The mean length of golden shiners in South Twin was 164 mm, while only 148 mm in North Twin. This difference is significant (p <.05) and may indicate better conditions for shiners in South Twin. One explanation is that increased pelagic habitat and reduced macrophyte densities create more optimum conditions compared to North Twin. Hypoxic conditions under dense macrophyte canopies may prevent shiners from utilizing the more extensive weed beds of North Twin (Frodge et. al. 1990, Moore et al. 1994). Another explanation could be that the greater macrophyte densities in North Twin provide better nursery conditions and better survival rates for smaller fish, leading to a smaller mean length. Golden shiners utilize littoral macrophyte beds and pelagic habitat, unlike other prey fish such as bluegill and pumpkinseeds. Higher piscivory on shiners in South Twin could produce greater mean length in golden shiners compared to North Twin (Modde and Scalet 1985). Although our research showed similar predation rates on shiners in both lakes, it is possible that variation exists, as observed between 2004 and 2005. Larger sample sizes and longer study periods are needed to increase statistical power and better clarify these relationships.

### *Brook and Rainbow Trout*

Clinograde DO profiles may potentially be more detrimental to trout populations in the long-term than are bass and shiners (Doke et. al. 1995, Scott and Crossman 1973, Wydoski and Whitney 2003). Preferred habitat availability for salmonids decreased by 81% from June to August, 2004. Such drastic habitat declines concentrate fish into small volumes, often leading to stress, disease, reduced growth and recruitment, and greater mortality rates (Plumb 1999).

The frequency and intensity of clinograde DO profiles should be expected to increase in Twin Lakes due to enhanced internal and external phosphorus loading. Sediment phosphorus recycling back to the water column is accelerated by low to no-DO in summer hypolimnetic waters. This recycled phosphorus in turns increases algae growth and organic loading to the sediments. Increased sediment organics increases sediment oxygen demand (SOD) due to decomposition, lowering or depleting the available oxygen. Thus, the internal phosphorus loading process becomes a self-reinforcing cycle that accelerates in intensity over time. The presence of macrophytes may enhance this internal loading as well (Moore et al. 1994). Measures to increase the oxygen content in summer hypolimnion and to reduce algae productivity should receive significant attention for fishery, as well as water quality improvement in Twin Lakes. Reductions of external nutrient loading from streams should also be considered in long term management of Twin Lakes.

Despite oxygen depletion in the hypolimnion and increased temperatures in the epilimnion, relative weight condition factors (Wr) for brook trout are just below 100. Values near 100 represent fair to good condition and overall health of the fish. It is hypothesized that (Wr) values would be higher if better thermal and DO regimens existed, especially in summer. Length-at-age of brook trout is comparable to Waldo Lake but more comparisons need to be made for a better assessment. South Twin brook trout growth rates and mean length were significantly greater ($p < .05$) compared to North Twin, but this difference may be due to small sample sizes.

Rainbow trout Wr values are near 90 and represent fair health of sampled fish. Only 9 fish were sampled in South Twin Lake, so data are not robust. Estimates of Wr in North Twin Lake are a better representation of the population with 116 fish sampled. However, recently-released hatchery fish tend to have lower condition factors than do acclimated fish. Most of the brook trout sampled were wild naturalized fish expressing greater Wr values than the rainbows, which were predominantly hatchery-reared.

Rainbow and brook trout consumed mostly midges (*Chaoborus*), scuds, and zooplankton in 2004 and 2005. Although zooplankton comprised very little of the fish diet by weight, they were found in 100% of sampled stomachs. The average trout stomach contained over 200 zooplankton. One brook trout in North Twin Lake contained 3,700 zooplankton. Phantom midges (*Chaoborus*) were also taken from 100% of sampled stomachs, averaging about 62 indiviudals per stomach in 2004. Phantom midges comprised the majority of the fish diet by weight. After stratification, these pelagic and benthic prey species represented the majority of salmonid diets. Midges and zooplankton may have limited availability to trout because of habitat loss during stratification (Doke et. al. 1995). Doke et. al. (1995) found that chaoborids avoid predation by remaining in hypoxic/anoxic waters during stratification.

The general health of rainbow and brook trout suggest that presence of largemouth bass and golden shiners currently has had only a minor impact on the species. However, the status of the trout fishery may change if bass and shiner populations continue to grow. Golden shiners introduced into western waters have had significant negative impacts on native fish species because of competition (Wydoski and Whitney 2003). The diet overlap observed for the Twin Lakes fish community suggests that available food resources for trout are reduced by bass and shiners. Competition could eventually limit salmonid prey availability during portions of the year.

Prey limitations often reduce fish growth and survival (Li and Moyle 1999). For example, redside shiners, similar to golden shiners, were found to displace juvenile rainbow trout in a Canadian lake due to their ability to out-compete trout for food resources in weed beds (Johannes and Larkin 1961). Golden shiners exhibit a pelagic behavior in the night but remain in littoral weed beds during the day. Such diurnal behavior may provide them with more efficient exploitation of littoral and pelagic food items. Stomach analyses of golden shiners in 2005 indicate consumption of zooplankton and macroinvertebrates. Currently, high densities of large zooplankton indicate good prey availability for salmonids, despite the presence of shiners. However, changes in algae community composition that will accompany increased phosphorus loading and algae productivity will decrease zooplankon populations.

Juvenile bass, golden shiner, and salmonid diets overlap in Twin Lakes. Because the mean size of largemouth bass sampled from Twin Lakes ranged from 180-186 mm, it is worthy to note that the majority of the bass are consuming similar prey items as shiners and salmonids. Large bass are also feeding upon brook trout, illustrating a direct competitive relationship. However, because bass and shiners prefer warmer water and can tolerate lower DO than salmonids thermal isolation may minimize competition between the species during certain times of the year. Such resource partitioning is evident between warm water littoral largemouth bass, littoral/pelagic warm water shiners, and pelagic cold water salmonids (Horne and Goldman 1994).

### *Management Considerations*

Control programs can be created in order to minimize or prevent negative trophic relationships from occurring in the lakes. There are essentially three methods of control; biological, mechanical and chemical (Wydoski and Wiley 1999).

Biological control of nuisance species is generally done by the introduction of top predators such as tiger musky and largemouth bass (Chris Donnelly, personal communication, Meronek et. al. 1996, Wydoski and Wiley 1999). Sterile hybrids predators, such as tiger musky, that cannot reproduce are often used in many states,m including Washingtoon, to increase piscivory. Long-term cropping and structuring of nuisance prey populations by stocking such predators has had only limited success. Of 29 control projects with predatory fish reported across the US, only 24% were considered successful (Meronek et. al. 1996). Of these, the most successful were those that introduced piscivorous salmonids to control rough fish (Meronek et. al. 1996). Stocking larger and more aggressive trout species such as triploid brook trout or tiger trout can increase predation on problem fish while providing an increased trout fishery. This method of control offers an inexpensive alternative but may take years to achieve the ultimate goal, results may not be significant, and introductions can have detrimental impacts on other species (Chris Donnelly, personal communication, Li and Moyle 1999, Wydoski and Wiley 1999). Introducing another species runs the risks of damaging trophic balances and perpetuating resourse competition in unintended ways.

Mechanical control such as electrofishing and gill netting can be used to physically remove fish (Wydoski and Wiley 1999). Nets and electrofishing alone offer the least effective measure of control and can be very labor and cost intensive for little results. If results are obtained they are usually short-term (Wydoski and Wiley 1999). A review conducted by Meronek et. al. (1996) found that out of 68 control projects that used either nets, electofishing, traps, drawdowns, or a combination of all the methods, only 43% were successful. Despite high costs, this method was reported to be more successful than biological methods at controlling unwanted fish species.

Combining mechanical and biological control may be effective. For example, reducing macrophyte densities would allow for increased predation on nuisance prey fish. Increased predation provides long-term control and reduces negative trophic implications to the fishery (Bettoli et. al. 1992, Olson et. al. 1998, Savino and Stein 1982, Savino and Stein 1989, Trebitz et. al. 1997). However, this method can also be expensive and may not produce sufficient results. Creating slot-limits on predator species such as bass at the same time as utilizing other methods may help maximize this impact on prey species. Slot-limits would protect the mid-size bass that make up the bulk of the predators in a population (Noble and Jones 1999, Novinger 1990).

Mechanical removal through angling has been successful at reducing invasive salmonids populations in small streams (Larson et. al. 1986). Utilizing anglers for control in this situation was more successful than electrofishing (Larson et. al. 1986). However, in lentic systems, liberalized bag limits have had little success at altering northern pike size structure (Goeman et. al. 1993). Angler control mechanisms depend entirely on angler participation to achieve results (Goeman et. al. 1993). Mechanical and biological control would not eliminate the nuisance species but, combined with other methods, may reduce negative trophic interactions sufficiently to maintain viable salmonid populations (Li and Moyle 1999).

Chemical control, such as the use of rotenone and antimycin, can be efficient at eradicating a species from a water body. However, rotenone and antimycin are not selective and can potentially kill all fish species present as well as other non-target organisms such as zooplankton (Wydoski and Wiley 1999). For example, complete recovery of zooplankton populations may take 1 week to 3 years (Anderson 1970, Neves 1975). Chemical control is the most widely used due to the cost-efficient method of application and long lasting results (Wydoski and Wiley 1999). Complete fish removal is nearly impossible but most management agencies report satisfying results of up to 10 years if done properly (Wydoski and Wiley 1999). This method can offer the most cost-efficient means of effective nuisance fish control (Chris Donnelly, personal communication). However, agencies that utilized rotenone or antimycin reported only 45-48% success out of 68 projects utilizing the chemicals for selective control (Meronek et. al. 1996). Agencies that utilized these chemicals for complete eradication of rough fish species reported 63-73% success rates out of 43 control projects reported (Meronek et. al. 1996). Combined chemical and mechanical control methods of unwanted fish species have shown 66% success rates of agencies that reported across the United States (Meronek et. al. 1996).

The choice of a control project is based on the water body, fish species present, societal concerns, economics, and time constraints. Many considerations need to be made before a project is implemented.

# CHAPTER 6

# CONCLUSIONS

Largemouth bass are piscivorous and primarily consumed golden shiners and crawfish throughout the summer in both lakes. Piscivory was the greatest during July and August due to availability of smaller shiners at that time. Crawfish are important in bass diets in June and September. Cannibalism on other bass and consumption of brook trout occurred in September. Small bass consumed the largest variety of prey organisms. Most of their prey consisted of macroinvertebrates, while golden shiners only contributed to a small portion of their diet. Extra small bass fed on zooplankton, midges, scuds and damselflies.

Largemouth bass have a strong top-down controlling effect on shiners in Twin Lakes. Just over 50% of large bass stomachs contained shiners. Less than 20% of medium bass stomachs contained shiners. No significance of bass predation on shiners between North and South Twin Lakes was seen despite differing macrophyte coverage. However, piscivory of largemouth bass does appear to be linked to macrophyte density and golden shiners behavior. Golden shiners utilize macrophte beds during the day and at night move into pelagic waters to avoid bass predation. Reducing macrophyte densities and protecting large bass( >300 mm) that feed the greatest on shiners may enhance bass piscivory. This would provide increased control on shiner populations. A combination of methods would likely maximize control of shiners and habitat availability for salmonids.

The presence of largemouth bass and golden shiners has had an effect on the trophic relationships in Twin Lakes. Resources utilized primarily by salmonids are now partitioned between four species constitute indirect competition. Bass predation on salmonids provides a direct competitive relationship. Despite the dietary overlap, resources are currently are effectively, partitioned due to varying habitat requirements between species. Only during portions of the year do all four species interact in the same space due to thermal requirements, thus minimizing competition.

Relative condition and growth of salmonids indicates that conditions may not be optimum in Twin Lakes, but currently the fish are satisfactory. Although some diet overlap exists between salmonids, largemouth bass, and golden shiners, habitat availability may be the greatest limiting factor in Twin Lakes. Oxygen depletion in the hypolimnion due to decomposition limits salmonid habitat availability from the bottom up, just at the time when the colder, deeper waters are most needed as a refuge. Increased temperatures also decrease habitat availability from the surface down. The combination of the two defining variables reduces habitat by 81% during summer stratification. Congregation of the entire trout population in a small area occurs. This can lead to stress, reduced growth, disease, and mortality.

The results of this study indicate that controlling golden shiner populations may be important tools to improve conditions for salmonids. Mechanical, biological, and chemical are three categories of control for species such as golden shiners. Although beyond the scope of this work, data indicate that water quality impacts related to increased phosphorus loading and increased algae productivity may pose the greatest long-term threat to the Twin Lakes fishery. It is strongly recommended that measures to control both internal phosphorus cycling and external loading be considered in order to prevent future loss of water quality and fisheries.

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