Smallmouth Bass Mortality During Parental Care: Implications for Year-class Strength

by

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(ABSTRACT)

I tested hypotheses that daily mortality rates (DMR) of smallmouth bass offspring were influenced by life stage, density and growth, parental male attributes, fungus infection (egg stage), and predation during parental care in the North Anna River, Virginia.

In 1994, stream discharge was relatively low and stable during spawning, and nest success was high (64%). Mortality (attrition) averaged 9.5% per day (range 5.2-13.9%) and 94.1% total (range 80.9-99.5%) for broods that survived to dispersal. Mean DMR for the interval from swim-up to larval metamorphosis (14.0%) was higher ($p=0.04$) than earlier (egg to swim-up, 6.7%) and later (metamorphosis to juvenile, 9.1%) periods.

Persistent factors during parental care (e.g. nest habitat, male attributes) did not strongly influence survival. Brood size and DMR also were unrelated ($r<0.34$, $p>0.07$) during each developmental period, suggesting density-dependent regulation was not prominent at the brood scale. Clutch size and nest success were important determinants of juvenile production for mating males. Larger males received more eggs ($r=0.40$, $p<0.01$) and, since variation in DMR was minimal among broods, maintained larger broods until dispersal ($r=0.55$, $p=0.01$).

Fungus (*Saprolegnia parasitica*) infection was a major source of egg mortality. In field and laboratory studies,

severity of infection was enhanced on clutches with higher dead egg abundance ("colonization points") and egg densities. Fungus growth rate also was strongly influenced by temperature and level of bacterial contamination. Predation was a primary cause of nest failure (70% of nest loss in 1994) and brood attrition. Diurnal nest predators were generally successful only in the absence of parental males, but American eels (*Anguilla rostrata*) were common nocturnal predators of larval and juvenile (14-20 mm SL) offspring and contributed to brood losses prior to swim-up.

Brood mortality information (1994) and annual data (1992-94) on nest success, swim-up larvae production, and August juvenile abundance suggest post-larval survival is an important determinant of annual cohort abundance.

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CHAPTER 1

EARLY SURVIVAL AND MORTALITY FACTORS FOR AGE-0 SMALLMOUTH BASS

INTRODUCTION

Large variation in fish recruitment may be precipitated by small changes in the timing and magnitude of mortality during early development (Ware 1975; Houde 1987). Recognition of initial population "bottlenecks" and underlying mechanisms is critical in order to explain and predict fluctuations in year-class strength. A complete understanding of these processes requires identification of the timing and primary sources of mortality during early life stages, as well as the relative importance of density-dependent and density-independent mechanisms acting on mortality.

It is widely accepted that density-dependent processes are important in regulating fish population stability (Crecco et al. 1986). Compensatory mortality is often predicted by models of age-0 growth and survival (DeAngelis et al. 1991; DeAngelis et al. 1993) and has occasionally been observed in field studies (Elliott 1985). However, the incidence, timing, and scale of density effects in most wild populations is controversial and probably varies with reproductive life-history.

Limited field evidence for density-dependence may be due, in part, to the nature of sampling methods; population estimates for early life stages are often made infrequently and over large spatial scales. As a result, the small scale processes of short duration, which may determine population abundance of larval fish, can be overlooked (Rothschild 1986)

or masked by climatic (density-independent) effects (Forney 1976; Crecco et al. 1986). The most suitable and, possibly, the only way to understand these processes is to investigate age-0 survival at small spatio-temporal scales.

Centrarchids, such as the smallmouth bass (*Micropterus dolomieu*), offer a unique opportunity to investigate mortality during early life stages. Smallmouth bass are solitary nesters that provide parental care to broods of offspring for up to seven weeks. In an evolutionary context, the *Micropterus* life-history pattern is intermediate between the "equilibrium" strategy (adaptive in resource-limited or K-selective environments; often includes parental care, low clutch size, and high juvenile survivorship) and the "periodic" strategy (expected in variable or seasonal environments; includes delayed maturation, large clutch size, and low juvenile survivorship) described by Winemiller and Rose (1992). This reproductive strategy and the conspicuousness of nesting males (hereafter "males" or "parental males") and offspring allow direct field enumeration of offspring, as well as observation of behavior, inter- and intraspecific interactions at the nest, and other processes that influence mortality during early development.

Despite extensive research on the species, major gaps still exist in the understanding of smallmouth bass recruitment and population dynamics. Losses for a cohort are greatest during the first summer of life (Pflieger 1966; Clady 1975; Vogele 1981), but the exact causes, timing, and extent of mortality during this period remain unclear. Variable reproductive success to larval swim-up (Pflieger 1975; Goff 1985; Reynolds and O'Bara 1991) and vulnerability of brood offspring to a host of mortality factors (Eipper 1975) indicate that parental care may be an important time for age-0 survival.

Smallmouth bass brood losses are attributed to whole-nest failures and attrition in successful nests. Both types of mortality are caused by interacting abiotic and biotic factors that vary in importance annually and with ontogeny of young. Abiotic factors (e.g., flow, temperature, and pH extremes), which often result in egg and larval brood destruction, are well documented in the literature (Cleary 1956; Eipper 1975; Lukas 1993). Biotic factors such as fungus infection of eggs, starvation, and predation are also recognized as causes of mortality, but are seldom investigated or quantified in field studies (Pflieger 1975; Vogele 1981).

Parental males may also directly or indirectly influence brood size. Larger, presumably more experienced, males often have larger clutches (Weigmann et al. 1992) and rear greater numbers of swim-up larvae (Ridgway and Friesen 1992; Lukas 1993). Although male size and aggressiveness have been positively associated with brood size and nest success in smallmouth bass (Reynolds and O'Bara 1991; Lukas 1993) and rock bass (*Ambloplites rupestris*, Noltie and Keenleyside 1986; Sabat 1994), these attributes do not necessarily deter offspring attrition. Large brood size may simply reflect mating success with larger, more fecund females (Weigmann et al. 1992) or with multiple females (Vogele 1981).

Knowledge of factors influencing mortality can provide a basis for understanding patterns in brood abundance. For example, consistent differences in parental male defense, microhabitat, or growth among broods could affect mortality rates throughout parental care and explain why certain nests produce many more juveniles at dispersal. An alternative hypothesis is that variation in (egg) clutch size is reduced at later life stages if individual broods are subject to some source of compensatory mortality. However, any realistic depiction of brood mortality includes a combination of factors

that may act in a compensatory, depensatory, or density-independent manner. Addressing these possibilities requires a mechanistic understanding of early survival.

The primary goal of this study was to examine life-stage specific survival and principal factors that influenced age-0 mortality in the North Anna River, Virginia. Estimation of nest success and brood size at successive life stages during parental care allowed me to track offspring survival and detect variation in mortality rates among individual broods and developmental periods. To account for differences in mortality, I investigated the effect of offspring density and growth, parental male characteristics, and suspected mortality factors at each life stage. This information, combined with data on annual reproductive success, sources of nest failure, and relative juvenile abundance in late summer shed light on specific processes, developmental periods, and sources of mortality that were important in defining year-class strength.

Specific hypotheses tested in this study include:

1) Nest failure and brood mortality rates are constant during early life stages.

2) Relative attrition rates among broods are consistent through time.

3) Mortality is density-dependent at the brood scale.

4) Larger, more aggressive parental males rear more young through enhanced brood survival.

5) Fungus infection and predation are important sources of mortality for egg and post-larval stages, respectively.

METHODS

STUDY SITE

The North Anna River was selected as the site for this study for several reasons: 1) the stream contains a sizable population of reproducing smallmouth bass (35.4/ha; Groshens 1993), 2) good water clarity permits data collection by direct underwater observation, and 3) previous research projects on this river provide information on spawning, reproductive success, and mortality factors for the smallmouth bass population (Groshens 1993; Lukas 1993; Sabo 1993).

The North Anna River spans approximately 130 km in the York River basin of east-central Virginia (Figure 1). The river was impounded in 1972 to create Lake Anna, a 3,885-hectare reservoir that provides cooling water for Virginia Power's North Anna Nuclear Power Station (Virginia Power 1986). Hydropower turbines were later installed in the dam which releases epilimnetic water. Minimum discharges of 1.1 m^3/sec are released through a hydro turbine. When the level of Lake Anna reaches 250 ft above msl, a second turbine is opened and 5.3 m^3/sec is released. Mean daily discharges ranged from 1.1 m^3/sec to 432 m^3/sec for 1972-1986 (Virginia Power 1986). The mean annual flow near Partlow, Virginia from 1979-1994 was 8.5 m^3/sec and percent exceedence flows were 16.5 m^3/sec (10%), 2.0 m^3/sec (50%), and 1.3 m^3/sec (90%). Because Lake Anna has minimal flood storage capacity, high flows are typical in the spring and often contribute to mortality for early life stages of age-0 smallmouth bass (Lukas 1993; pers. obs.). Elevated discharges are often extended over time, in part to reduce peak discharges and flooding (Bob Graham, Virginia Power, pers. comm.). Sabo

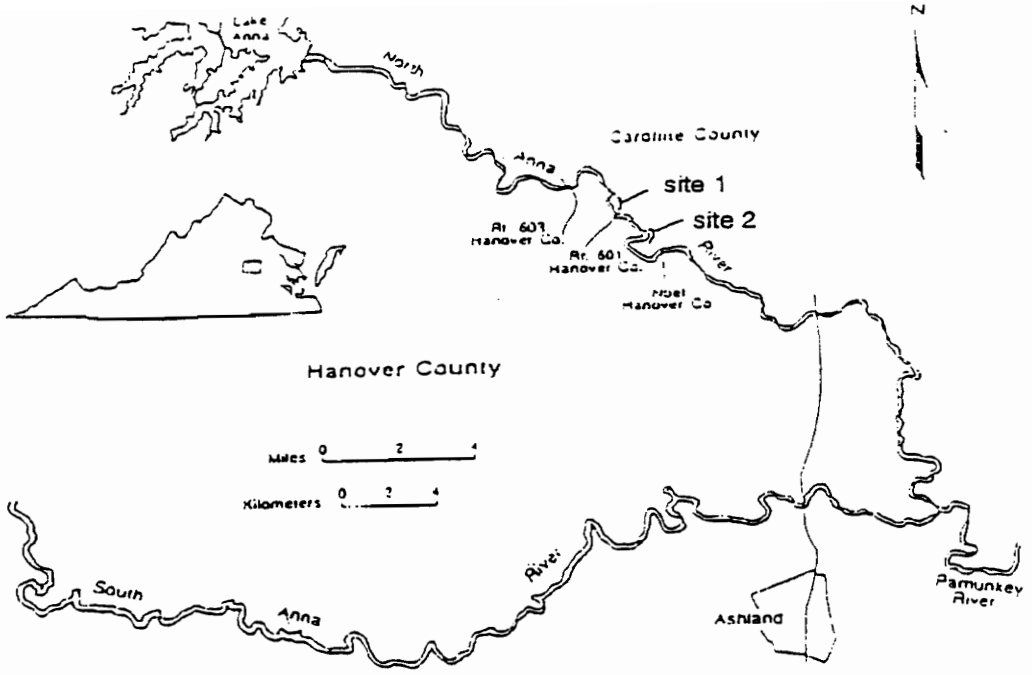

Figure 1. Location of study sites on the North Anna River, Virginia.

(1993) suggested that elevated discharges are maintained for several days longer than would naturally occur after heavy rains, which may affect the timing and success of smallmouth bass reproduction.

The North Anna River includes two physiographic regions. The upper, piedmont region has a relatively low mean gradient (0.4 m/km) and extensive, sandy run habitat. The lower region, which flows through the fall zone, has a higher mean gradient (1.6 m/km). This section includes more rocky substrata (Graham and King 1988) and offers better smallmouth bass habitat (Lukas 1993, Sabo 1993). The transition between these regions lies near the Route 601 bridge in Hanover County (Figure 1). The reach from this point downstream

approximately 9 km to U. S. Route 1 includes the best habitat and highest densities of smallmouth bass in the river (Graham and King 1988; Lukas 1993).

Within this section, the North Anna River averages 34.6 m wide (Groshens 1993). Pools are often longer than 100 m and generally less than 2.5 m deep at minimum flows (Lukas 1993). Most habitat units contain adequate rock and woody cover with a sand and bedrock/boulder substratum. Excellent water clarity (2-3 m visibility at stable flows) allows considerable growth of attached algae and aquatic macrophytes. Riparian vegetation also is abundant, consisting of a variety of plants and deciduous trees which shade a large portion of the river. In addition to smallmouth bass, 46 species of fish have been collected in the North Anna River (Virginia Power 1993). The most common species are swallowtail shiner (*Notropis procne*), satinfin shiner (*Cyprinella analostana*), redbreast sunfish (*Lepomis auritus*), rosyface shiner (*Notropis rubellus*), margined madtom (*Noturus insignis*), shield darter (*Percina peltata*), and American eel (*Anguilla rostrata*), which collectively comprise more than 80% of fish samples (Groshens 1993; Robert Graham, Virginia Power, pers. comm.).

I conducted field studies at two sites that were 22-26 km downstream of the Lake Anna dam (Figure 1); a 0.6-km (2.4 ha) reach immediately above the Route 601 bridge (site 1) and a 0.5-km (2.0 ha) section located approximately 4.0 km downstream of the bridge (site 2). Physical characteristics such as substratum, cover, depth, and flow velocities are similar for the two sites (Groshens 1993).

FIELD METHODS

Field studies were focused on determination of mortality rates for age-0 smallmouth bass during early life stages and identification of factors that influence survival. First, I

report techniques for locating nests and tracking their survival. Abundance estimate methods are described for individual broods at selected developmental stages during parental care and for juveniles at site 2 in August. I then summarize procedures for measuring factors suspected to be associated with brood mortality: offspring growth, parental male attributes and duration of care, fungus infection, and predation. Finally, procedures used to summarize and analyze these data are presented.

Data on smallmouth bass nest densities and locations, reproductive success (nests producing swim-up larvae), the number of swim-up larvae produced at each nest, and parental male attributes were collected at site 1 for three years (1992-1994, data for 1992 from Lukas 1993). Additional data were collected at sites 1 and 2 in 1994 to estimate life-stage specific mortality. The following procedures are described for 1994, but protocols used for all three years were identical except where noted otherwise. Because of a less intensive nest search effort in 1992 (less frequent and often did not include snorkeling), the total number of nests likely was underestimated and nest success overestimated (since nests may have failed prior to discovery) for this year.

A technician and I began searching for smallmouth bass spawning sites (nests) at both study sites when water temperature reached 15°C (April 12 in 1994), based on Graham and Orth's (1986) model which predicts initiation of spawning at this temperature. Nests were located by snorkeling both sites daily. We searched all habitats within each reach, but concentrated effort in pools and slow water areas. I placed a numbered stone next to each active (containing young) nest to mark its location and checked brood progress daily until juvenile dispersal. Since I was primarily interested in

offspring abundance and survival, streambed depressions (suspected nests) that did not contain young were not included in nest counts. Therefore, mating success for males could not be determined.

The cause of nest failure was determined for all unsuccessful nests. Nests that failed during high flows were assumed to perish due to "flooding". All other nest failures were attributed, directly or indirectly, to predation. I interviewed anglers observed in the study reaches to assess which, if any, parental males were angled. Angler reports were then confirmed by checking for the presence of parental males with each brood and inspecting them for hook scars.

I obtained daily temperature readings taken at the Route 603 bridge in Hanover County (1992-1994) from Virginia Power Company. Flow records for the same period were provided by the United States Geological Survey (gauge number 01670400 near Partlow, Virginia).

Age-0 Smallmouth Bass Abundance

The number of smallmouth bass offspring in each brood was estimated at four different developmental stages during parental care (Table 1). These counts are the basis for describing the timing, magnitude, and mechanisms of brood mortality.

Eggs in each nest were counted within two days of spawning using an area-density estimate (count 1). I used SCUBA to lie on the river bottom adjacent to the nest. The entire clutch was then covered with a grid (2x2 cm cells) superimposed on a piece of clear plexiglass. I traced the perimeter of the egg mass and counted the number of eggs in six of the cells. Cells were chosen along a line spanning the diameter of the egg mass so that counts were made on both edges, at approximately 0.25 diameter from each edge,

Table 1. Timing and methods for age-0 smallmouth abundance estimates in 1994.

Count No.	Stage	Days After Spawn	Method[b]
1	Egg	0-2	Area-density Estimate on Nest
2	Swim-up	9-13	Area-density Estimate on Nest
3	Metamorphosis	19-23	Capture with Net or Video Count
4	Juvenile	29-36	Capture with Net
5[a]	Late Summer	119-149	Petersen Mark - Relocate

[a] Count 5 was not brood-specific.
[b] See text for descriptions.

and near the center (2 counts). The outline of the clutch was then traced on a piece of clear acrylic. I estimated the total number of eggs in the nest by calculating the total area of the clutch with a digital planimeter and multiplying it by the average egg density from the cells (Raffetto et al. 1990).

The accuracy of the area-density technique was evaluated by applying it to a model (dry) nest created with substratum typical of natural smallmouth bass nests. I weakly adhered a known number of egg-sized (2 mm diameter) beads to the substratum and applied the field protocol. The estimated number of "eggs" ($mean_{estimate}$=1272) was significantly correlated ($r=0.97$, $p=0.001$, $n=6$) with the actual number ($mean_{actual}$=1252), and the average absolute error was 10.4%.

I recounted broods that survived to the larval stage (count 2). Young were sampled just prior to the free-swimming

phase (swim-up) as they attained a dark pigmentation and absorbed their yolk sac (9-13 days after spawn). I carefully removed any large rocks or debris from the nest that may have concealed larvae and applied the same area-density estimation technique used in count 1. Occasionally, a few larvae were scattered outside the plexiglass grid. These individuals were counted separately and added to the number estimated using the grid to calculate the total number in the brood. After counting the larvae, rocks and debris were returned to their original position in the nest.

Broods that persisted 7-11 days after swim-up (19-23 days after spawn) were counted again (count 3). Offspring at this stage (larval metamorphosis, 10-12 mm SL) were loosely dispersed in the nest vicinity and difficult to capture, especially during the day. Therefore, larvae were counted at night when they became lethargic and settled on the streambed in a condensed school. The location of each brood was marked with an anchored float slightly before dusk. The number in each brood was counted using one of two methods. Good underwater visibility allowed me to count 12 broods using an 8-mm underwater camcorder equipped with underwater lens and infrared lighting. I counted individuals directly by carefully placing a wire grid (8 cm^2 cells) on the streambed below the brood. I slowly filmed close-up footage of each cell in the grid and later reviewed the tapes. The total number in each brood was calculated as the sum of individuals in all grid cells.

The second method used in count 3 was brood netting (14 broods). In this technique, a ~4 m^2 piece of 1/32-inch mesh netting was spread on the streambed where the brood had previously been observed settling for the night. After dark, the net was carefully lifted and immediately placed in a ~35-

liter tank with river water. After removing the net, I collected the young 5-10 at a time with a dip net, counted individuals, and transferred them to another holding tank. After the captured individuals were counted, I used a spotlight and SCUBA to survey the area where the brood was netted. Individuals that avoided capture were added to the number netted to calculate the size of the brood. Once the count was complete, I transferred the captured offspring to a large fine-meshed dip net and returned them to their original position.

I did not directly compare abundance estimates from the two methods used in count 3 (I could not apply both techniques to the same brood on the same night). However, successive estimates (4-5 days apart for the same brood) using both methods were never conflicting; rates of attrition were consistent with mortality rates of broods at this life stage.

I recounted (count 4) 24 broods that remained in the nest vicinity (juveniles that did not perish or disperse) an additional 8-14 days using the same netting technique described for count 3. However, broods that had less than 100 individuals were counted visually at night using SCUBA and an underwater light. I counted all individuals and surveyed the surrounding vicinity three times, recording the mean of the three counts as the total number. For broods that had 100-200 offspring, visual counts were routinely made prior to netting to verify that estimates from the two methods were consistent.

Only count 2 (larval swim-up) was made in 1992 and 1993. In these years, a different protocol was used (Lukas 1993). Larvae were pumped into a sieve bucket with a hand-operated bladder pump. The brood was then transferred to a shallow pan with a silicon grid (to prevent young from swimming around in the pan and aid in counting). All individuals in the pan were counted, as well as any larvae remaining on the nest. The

total number of larvae in the brood was the sum of these two numbers. After counting, larvae were poured into a plastic bottle and returned to the nest by inverting the bottle just above the streambed. During all of the counts described above, parental males remained in the nest vicinity and resumed guarding the brood when counts were completed.

A final abundance estimate for age-0 smallmouth bass was made in late summer at site 2 (count 5) in 1994. From July 22-24, individual bass (31) were captured using a large camouflaged seine and 1x1 m cages with black plastic mesh. Fish were chased into the net or cage by a snorkeler, marked with a caudal fin clip, and immediately released. On August 9, the entire reach at site 2 was slowly searched in a downstream direction by four snorkelers. Snorkelers remained in a line perpendicular to the bank and in close proximity to prevent counting any fish more than once. All age-0 smallmouth bass observed were checked visually for a caudal fin clip. We also searched for bass approximately 100 m upstream and 100 m downstream of site 2 to confirm that marked individuals did not emigrate from the study reach. I estimated the total number of age-0 smallmouth bass in the reach (N) using a Petersen estimate with Chapman's modification (Ricker 1975):

$$N = \frac{(M+1)(C+1)}{(R+1)} \qquad (1)$$

where M is the number of individuals marked, C is the total number observed in the snorkel survey (August 9), and R is the number of individuals with fin clips seen in the snorkel

survey. Confidence limits (95%) for this estimate were based on the Poisson distribution (Ricker 1975).

Annual Age-0 Relative Abundance in August

From 1990 to 1994, the relative abundance of age-0 smallmouth bass at site 1 was estimated in August (08/07 - 08/14). Once each year, the reach was carefully searched visually in a downstream direction by 2-5 snorkelers. Snorkelers remained in a line perpendicular to the bank and in as close proximity as possible to prevent counting any fish more than once. The total number of age-0 bass observed by all snorkelers was used as an index of abundance.

Offspring Growth During Parental Care

I measured standard lengths (SL) to the nearest 1 mm over time for 31 broods. Every two to eight days from larval swim-up through brood dispersal, measurements were recorded for ten individuals per brood. These measurements were used to assess variability in growth and calculate brood-specific growth trajectories. Total length also was measured for 20 individuals (7-19 mm SL) to obtain the relationship between SL and TL ($TL=1.2(SL)+0.2$, $R^2=0.99$).

Size of Parental Males

In 1994, parental male total length (TL) was estimated by either a) holding a clear plexiglass ruler next to the fish (passive individuals) or b) laying the ruler on the streambed at the nest rim and waiting for the male to return to his position over the nest. I verified length estimates by angling and measuring six of the parental males (all estimated TL were within 15 mm of actual TL). In 1992 and 1993, most parental males were angled from their nest during the egg

stage, measured directly, and immediately released.

Parental Male Brood Defense

I measured the aggressiveness of parental males toward potential nest predators (Ridgway 1988) the day following discovery of each nest in 1994. Defense indices were developed to detect differences in the level of brood defense among parental males. Undisturbed males were cautiously approached underwater using SCUBA (between 1000 and 1600 hr), and males were presented with a fish model after a 2-3 min quiet period. Models of 165-175 mm bluegill had been constructed with methods described by Helfman (1983), mounted on wire extensions, and attached to a 1.8 m clear plexiglass rod. A model was presented approximately 0.65 m from the nest rim, held for one min, then moved steadily to the nest rim, where it was held for another min. At each position I recorded the number of attacks (bites) and time the male remained within a body length of the model. For males that attacked the model, I also recorded this attack distance. At five nest sites, this procedure was repeated the following day to verify precision. Relative scores for each of the indices were consistent among nests.

For data analysis, I used the total number of bites (sum at both model holding positions) and total time the male was within 1 body length of the model (out of 2 min). The distance where the male first attacked the model (attack distance) was simplified as: 1 = first attack at 0.65 m from nest rim, 2 = first attack at rim, and 3 = no attack on model.

Duration of Parental Care

Knowledge of spawning date and daily visits to each nest until brood dispersal allowed me to calculate the total number of days each parental male guarded his offspring. The male

was usually seen immediately within 2 m of the brood until the young began to metamorphose (10-12 mm SL). From larval metamorphosis through brood dispersal, the male appeared near the brood less frequently. During this period, I used SCUBA to lie on the streambed near the brood until he appeared. The parental male nearly always appeared within 10 min as long as the brood had not dispersed.

Male Nest Departure Frequencies

I used an 8-mm camcorder with underwater lens to observe parental male behavior and potential nest predators near several nests. The camera was set up approximately 1 m from nests on a small tripod. Nests (n=25) with young at the egg (n=16) and larval (n=9) stages were observed within 1 hr of sunset (observations sometimes continued after sunset) for 20 min, following a 5-min quiet period after the camera was in position. I used male departure frequency as an index of predation intensity (Hinch and Collins 1991; Lukas 1993). Departures are defined as parental male bursts away from the perimeter of the nest, apparently in pursuit of predators (pers. obs.). I also noted any potential nest predators observed.

Parental male departure frequencies were quantified to describe temporal differences in male defense. I analyzed the data by comparing male departure frequencies between egg and larval (pre-swim-up) stages, as well as between diurnal periods for each life stage. In the latter, my observations during crepuscular periods were compared with Lukas' male departure frequencies recorded during mid-day in 1992 (0900 to 1800 hr using the same protocol, Lukas 1993).

Fungus Infection on Eggs

Fungus infection and several factors related to fungal

growth were quantified for each egg clutch. Upon discovery of an active nest, I carefully examined all of the eggs for aquatic fungi and counted the number of dead (opaque) eggs. The white, fluffy mycelia were easily recognized once fungus colonized the eggs. If fungus was present, I laid a piece of plexiglass over the eggs, outlined the total area of the clutch and areas infected with fungus, traced the outlines to an acrylic sheet, and later calculated the total area of the egg mass and portions infected with fungus using a digital planimeter. This procedure was repeated for each nest every two days until the eggs hatched.

Fungus samples were collected during various stages of the spawning season (04/16, 04/20, 04/29, 05/18). I removed infected eggs (5-10) from seven clutches and placed them in small plastic bags with river water. The samples were then transported on ice to the Virginia Tech Mycology Laboratory, where the fungi were identified to species.

Predation

American eels were the most common piscine predator seen in the vicinity of smallmouth bass broods. Although small (<120 mm) eels were observed in nests diurnally during embryonic stages, most eels were observed serendipitously near young at night while I was counting larvae and juveniles (counts 3 and 4). The number of eels observed was recorded each time eels were seen within approximately 2 m of a brood.

Time-lapse Photography of Broods

I suspected that nocturnal predators were an important source of mortality for larval and juvenile smallmouth bass. An 8-mm camcorder with underwater lens and intervalometer was used to observe individual broods at night for long periods of time. I mounted two infrared lights on the top of the

underwater lens and set the unit up on a small tripod, approximately 0.5 m from a brood. The camcorder was then set to record 8 frames every 10 sec. Broods (N=11) were filmed for 4 to 8 hr (between 2000 and 0600 hr) on 11 different nights. I later viewed each of the video tapes twice at reduced speed and recorded the number and species of predators.

Eel Diet Analysis

Eels were sampled at selected locations and time of day to determine the extent of predation on age-0 smallmouth bass. Twenty eels were collected from within 1 m of broods (inactive larvae or juveniles on the streambed between 2100 and 0100 hr) between May 18 and June 5. I captured the eels with a hand-held dip net and immediately placed them in 10% formalin. In the laboratory, eel specimens were measured to the nearest mm and the upper digestive tract (above the large intestine) was removed. I separated age-0 smallmouth bass from other food items and measured their length, width, and maximum body depth (to calculate total volume). Diet items were classified as fish, insects, crayfish, or "other" material. Volume of each item was determined by measuring average length, width, and depth under a dissecting microscope and calculating the volume of a cylinder with those dimensions.

Eels also were collected from random locations near smallmouth bass spawning habitat (but >10 m from any brood) at both study sites. I used a backpack electrofishing unit to collect specimens during three different time periods (0600-0730 hr = "morning", 1500-1800 hr = "day", and 2200-0100 hr = "night" between May 17 and June 7) while most smallmouth bass young were at the juvenile stage. Captured eels were processed in the same manner as above, but total volume of food items was measured using volume displacement (Hyslop

1980).

A third collection of eels was completed on larger spatial (just below the Lake Anna dam to Route 1 Hanover bridge) and temporal (between May 31 and September 7) scales by Virginia Power as part of their annual sampling surveys on the North Anna River. Specimens were collected between 0900 and 1600 hr using backpack electrofishing units or electric seines and immediately placed in 10% formalin. I dissected these eels as described above and measured diet items using volume displacement.

DATA ANALYSIS

The first four age-0 smallmouth bass abundance estimates allowed brood-specific survival rates to be calculated for the three intervening intervals. I estimated daily mortality rate (DMR) using the following equation from Ricker (1975):

$$DMR = 1 - S^{1/T} \qquad (2)$$

where S is the proportion of individuals surviving from the preceding count and T is the interval (days) between counts. These mortality rates represent losses due to attrition for successful nests and do not include whole-nest failures.

DMR also was estimated for the period from the juvenile brood counts (4) to count 5 using equation 2. Because juvenile estimates (count 4) were made over a large interval of time (due to variation in spawning date and development rate among broods), a range for DMR was calculated using the intervals from the earliest (05/13) and latest (06/05) juvenile counts to count 5 (08/09) as end points.

A Friedman test with multiple comparisons was used to test for differences in DMR among early life stages. I tested for autocorrelation of DMR among developmental periods to investigate the relative consistency of mortality rates for individual broods. Pearson's (parametric) and Spearman's (nonparametric) correlation procedures were used for this and other correlation analyses involving brood size, DMR, and male attributes. Since results of parametric and nonparametric analyses were similar, only Pearson's correlation coefficients are reported. Unless noted otherwise, $\alpha=0.05$ was used as the level of significance for all tests.

I tested for temporal differences in male defense by comparing male departure frequencies between egg and larval (pre-swim-up) stages, as well as between diurnal periods for each life stage. All comparisons were made using one-tailed two sample t-tests. Tests were one-sided because I predicted that brood defense (departures) would increase as young developed (Ridgway 1988) and be greater during crepuscular periods.

Field observations provided data on the frequency of nests infected with fungus, fungus growth rates, clutch area, and the total proportion of egg masses (area) covered by fungus. I used correlation procedures to examine the relationship between mean daily water temperature, spawning date, number of dead eggs, and egg density with fungus growth rates and the proportion of eggs infected with fungus. Nonlinear regression (SAS Institute 1988) was used to delineate the effects of fungus growth on DMR during the embryonic period (excluding complete nest failures attributed to other causes). I used a one-way repeated measures analysis of variance (ANOVA) design to test for differences in clutch size at days 1, 3, and 5 after spawn and a multiple comparisons test to identify days at which clutch areas were

significantly different.

I used multiple regression analyses to select the "best" models for prediction of DMR for periods 1, 2, 3, and 1-3 (total). Developmental periods were defined as follows: period 1 was the interval from spawning (count 1) to swim-up (count 2), period 2 was from swim-up to larval metamorphosis (count 3), and period 3 was from metamorphosis to the juvenile count (4). Independent (predictor) variables were chosen for each model using backward stepwise regression (F-to-enter=4.0, F-to-remove=3.9). Initial predictor variables included parental male TL, total bites (male aggression index), initial brood size, proportion of clutch with fungus (period 1 and total DMR models), brood growth rate (not used for period 1 DMR model), and eel presence (whether an eel was observed in vicinity of nest, not used in period 1 DMR model).

RESULTS

ANNUAL VARIATION IN SPAWNING TIME AND REPRODUCTIVE SUCCESS

Timing of Spawning

The timing of smallmouth bass spawning varied among years in 1992-1994 (Figures 2-4). In all years, spawning was initiated in April under stable or receding flow conditions when water temperatures reached 15°C. Subsequent nesting activity was dependent on flow and temperature conditions. In 1992 and 1993, multiple high flow periods (floods) destroyed previously constructed nests. Pulses of renesting occurred as flows receded and stabilized after floods. Low, stable flows in 1994 resulted in an early, abbreviated spawning season (spawning began 04/12, total spawning period 33 days) relative to the previous two years (1992; spawning initiated 04/15 and lasted 54 days, 1993; spawning initiated 04/26 and lasted 36 days). All spawning occurred when water temperatures were between 15°C and 23°C.

Reproductive Success

Smallmouth bass nest densities, reproductive success, and factors influencing offspring survival varied annually (Table 2). The total number of nests spawned was highest in 1992 and 1993 due, in part, to frequent renesting after flooding. Nest success rate (to swim-up) was higher in 1994 (70%) than the previous two years (46% in 1992, 38% in 1993).

There were more nest failures attributed to biotic (predation related) causes than abiotic (flooding) causes before swim-up in 1993 and 1994. The source of all nest failures could not be determined in 1992 because of less frequent visits to nests.

The mean number of swim-up larvae produced per nest

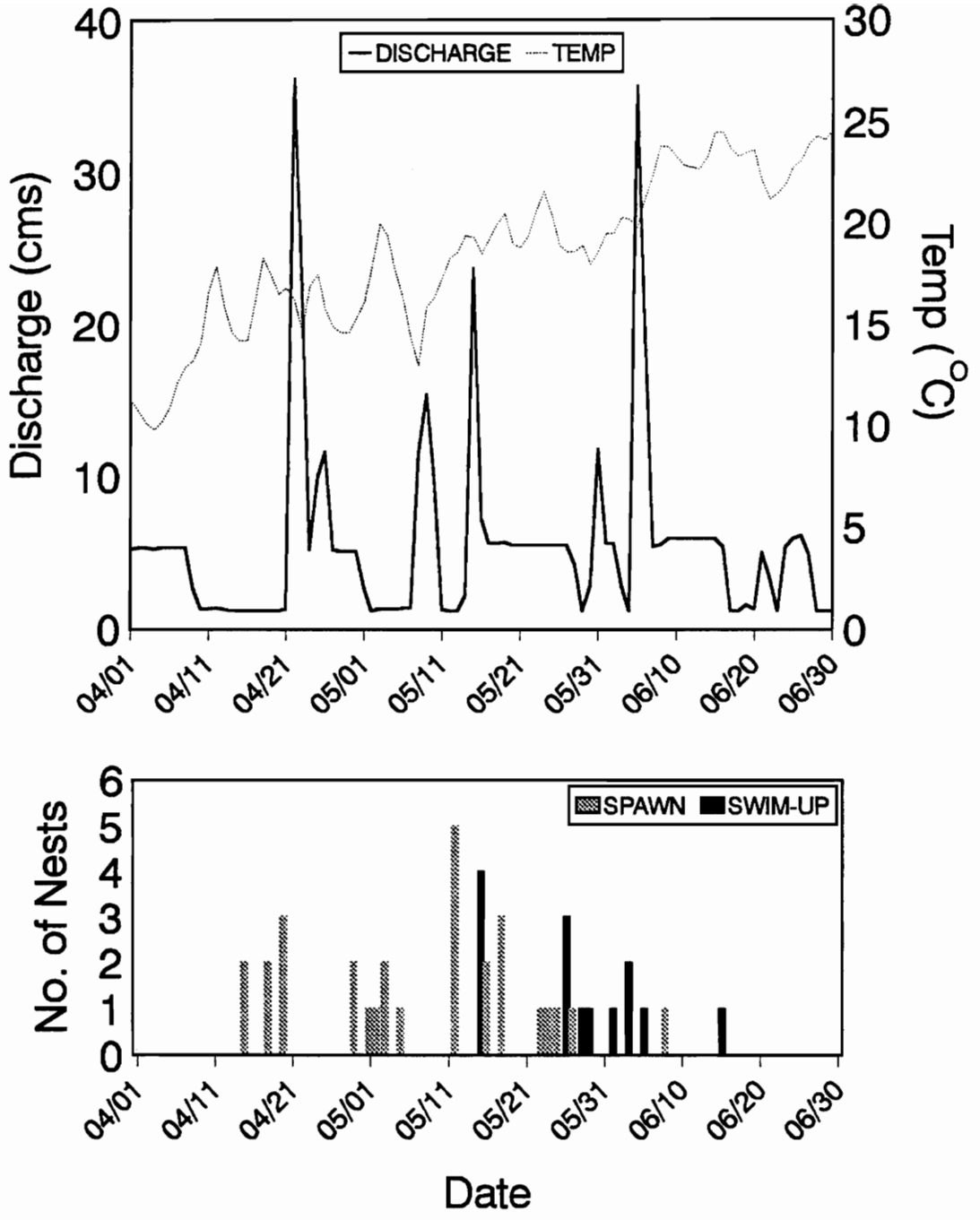

Figure 2. Timing of spawning and nest success to swim-up at site 1 relative to temperature and flow conditions in 1992. The total number of broods that were spawned or reached swim-up is shown (lower diagram) for each date.

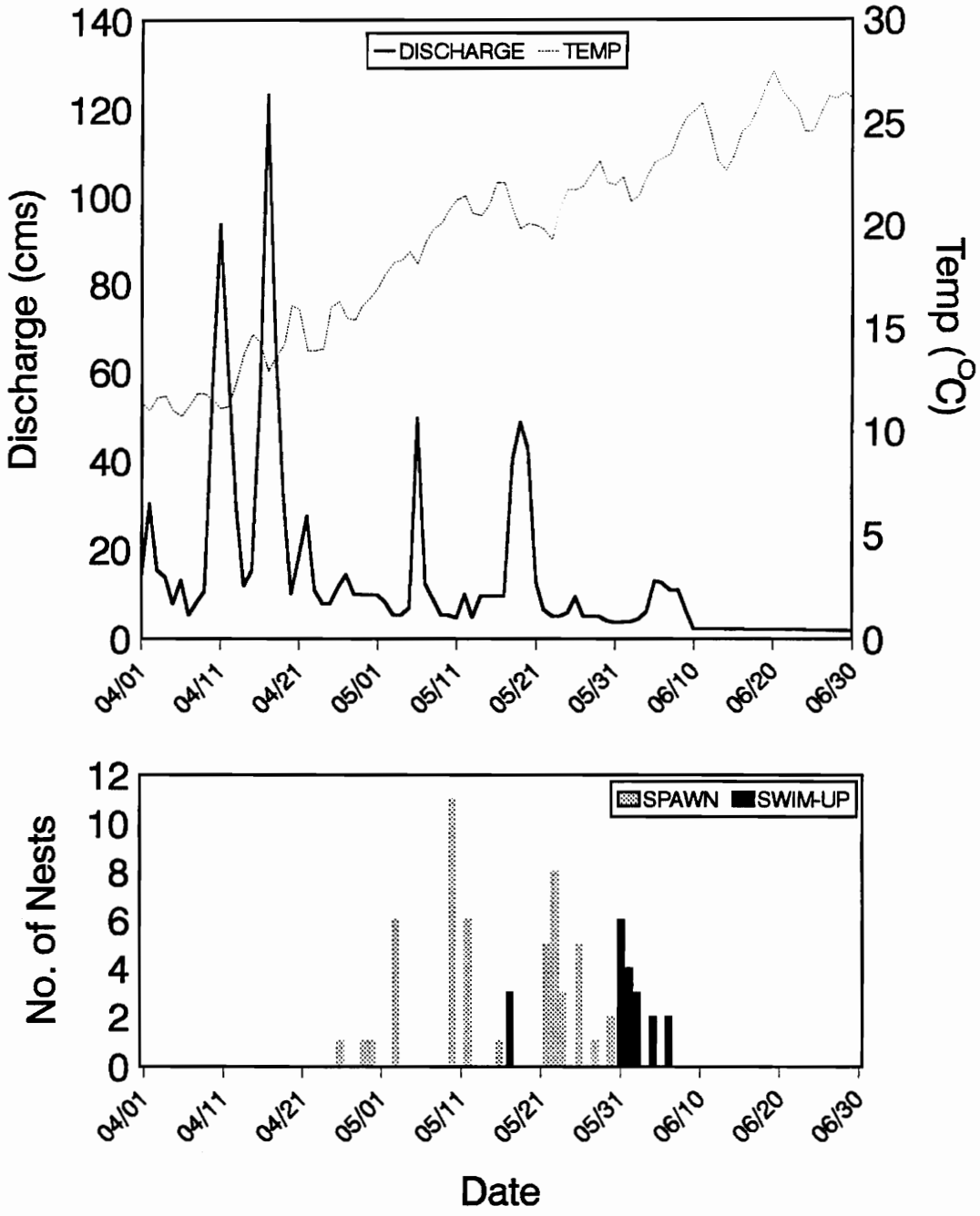

Figure 3. Timing of spawning and nest success to swim-up at site 1 relative to temperature and flow conditions in 1993. The total number of broods that were spawned or reached swim-up is shown (lower diagram) for each date.

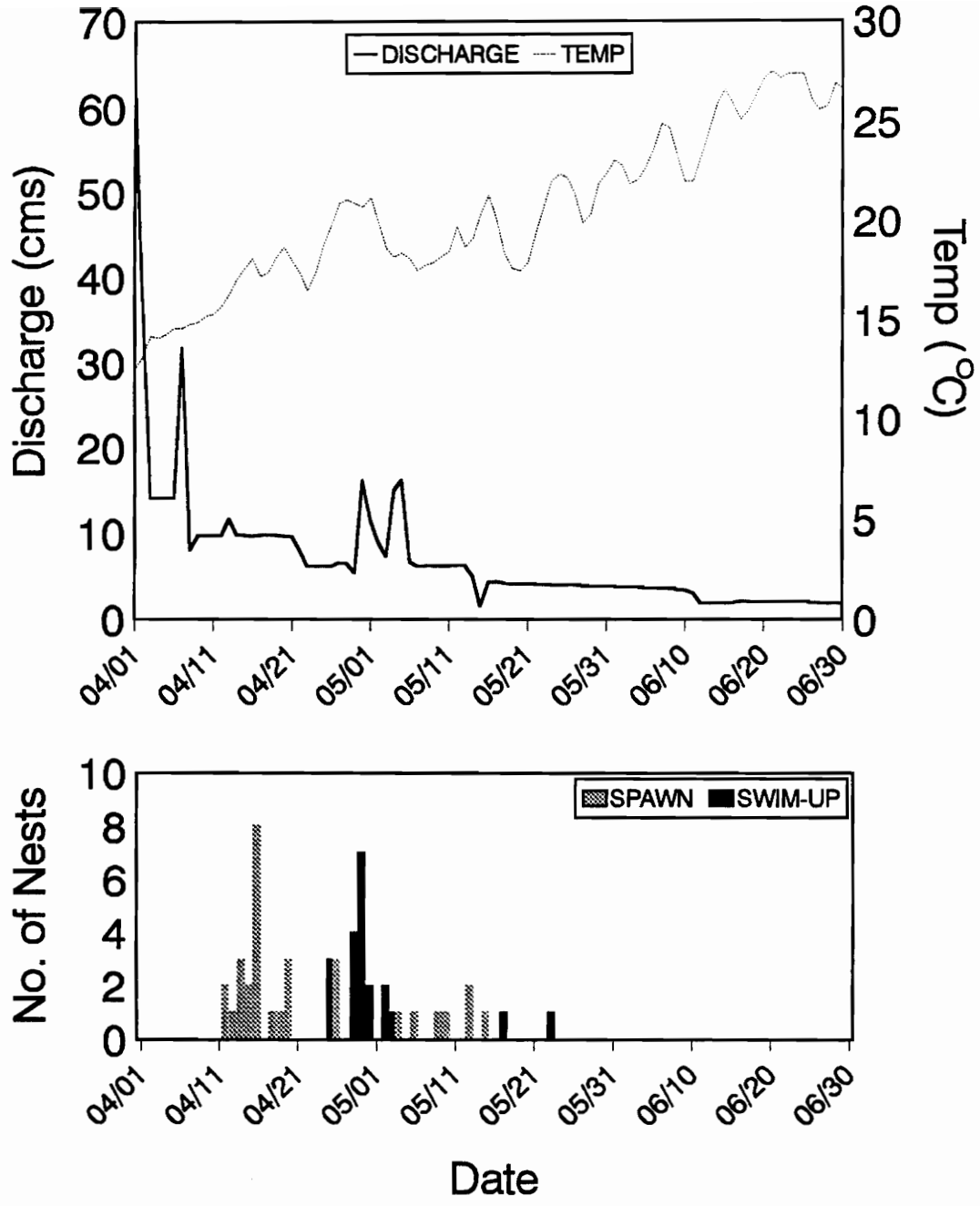

Figure 4. Timing of spawning and nest success to swim-up at site 1 relative to temperature and flow conditions in 1994. The total number of broods that were spawned or reached swim-up is shown (lower diagram) for each date.

Table 2. Comparison of smallmouth bass annual reproductive success at site 1, North Anna River, Virginia.

	1992	1993	1994
Total Nests	35	60	33
Nests Successful to Swim-up	16 (46%)	23 (38%)	23 (70%)
Swim-up larvae per Nest[a]	627 ±465	996 ±884	2449 ±1166
% Biotic Nest Failures[b]	-	54	70
% Abiotic Nest Failures[c]	-	46	30

[a] Mean ± 1 SD.
[b] Whole nest failures due to predation, male abandonment, or angling of parental male prior to swim-up.
[c] Whole nest failures caused by flooding prior to swim-up.

increased each year from 1992 to 1994 (Table 2). The use of different count methods (1992-93 versus 1994) may have contributed to the discrepancy among years, but visual estimates of broods confirmed large annual differences in larval abundance.

Parental Male Attributes

The size distribution of parental male smallmouth bass also varied annually from 1992-1994 (Figure 5). Spawning males ranged from 188 to 475 mm TL, indicating that all age classes of mature males participated in spawning (based on Groshens 1993). However, no males greater than 370 mm TL spawned in 1993 and no males less than 230 mm TL spawned in 1994. Lukas (1993) reported that approximately 25% of parental males constructed more than one nest in 1992, compared with 3% in 1993 and 0% in 1994 (excluding simultaneous multiple broods).

Although flow and temperature conditions were primary determinants of when spawning could occur, male size also influenced spawning time. Larger parental males tended to

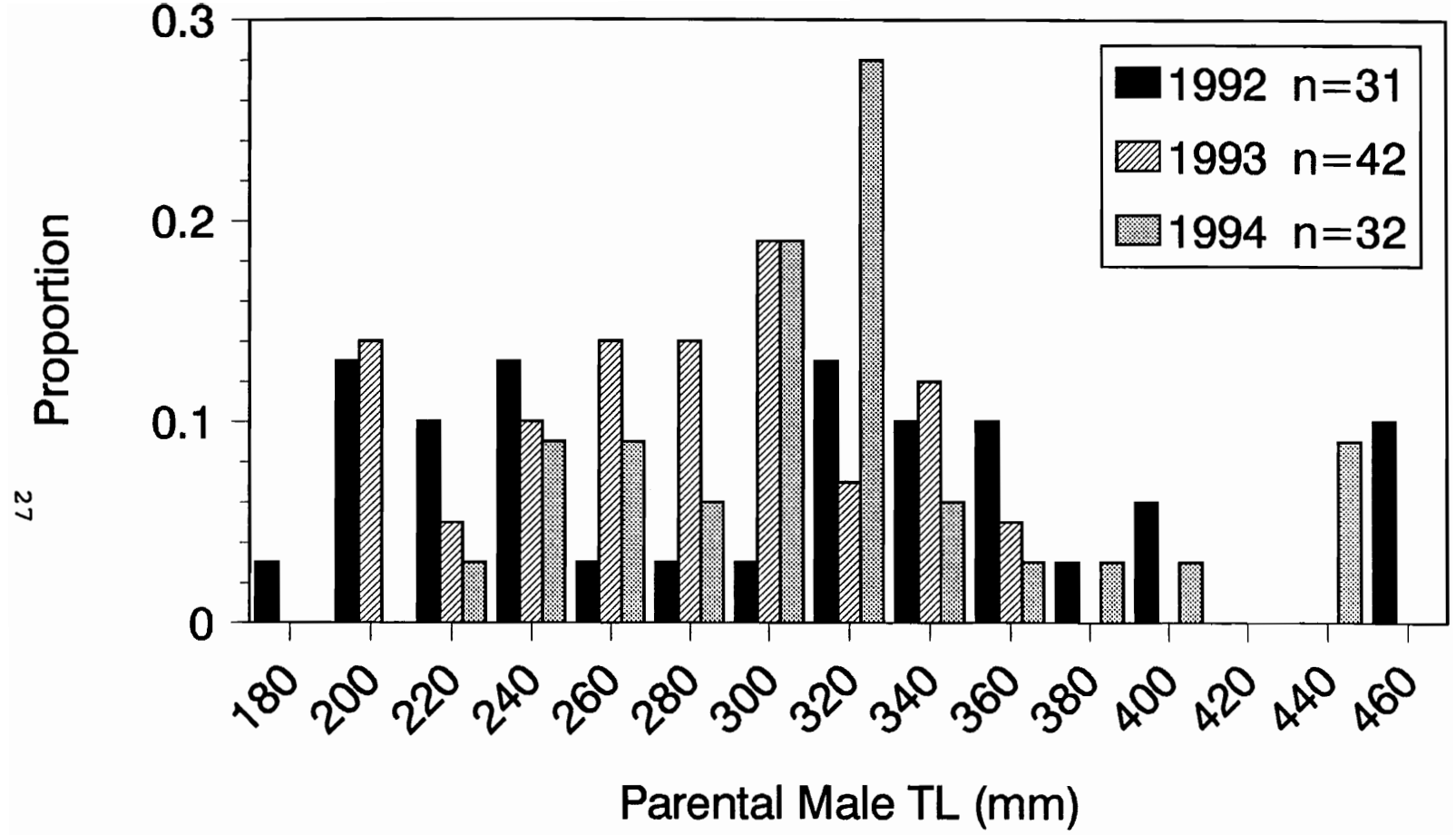

Figure 5. Parental male length distributions for 1992-1994 at site 1.

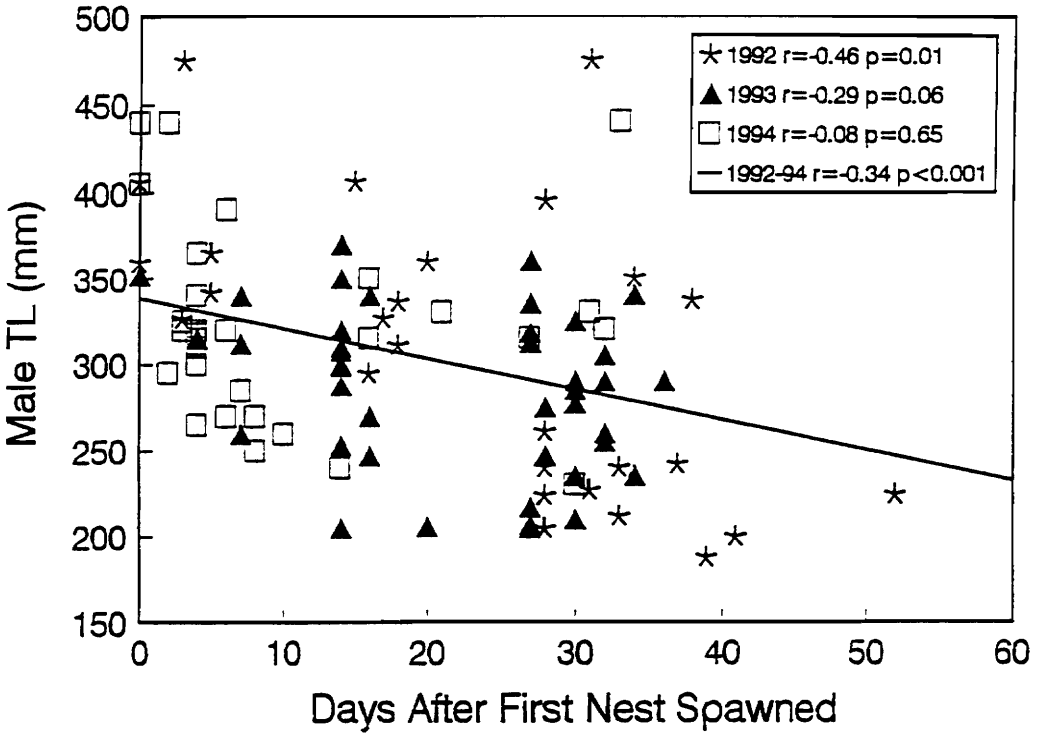

Figure 6. Relation of size and the timing of spawning for parental male smallmouth bass at site 1, 1992-1994. Each symbol represents one nest. The regression equation for all years combined is MALE TL=338.6-1.77(DAYS AFTER FIRST NEST SPAWNED), R^2=0.12.

spawn before smaller males (Figure 6). The inverse relationship of male TL and spawning date was significant for 1992 (r=-0.46, p=0.01, n=28), 1993 (r=-0.38, p=0.01, n=41), and all years combined (r=-0.35, p<0.001, n=99), but not for 1994 alone (r=-0.08,p=0.38, n=30).

Larger males also tended to rear more swim-up larvae (Figure 7). However, the relationship was only significant for 1994 (r=0.69, p<0.001, n=20) and all years combined (r=0.46, p<0.001, n=57).

ANNUAL ABUNDANCE OF AGE-0 SMALLMOUTH BASS

Abundance estimates at swim-up (April - May) and in

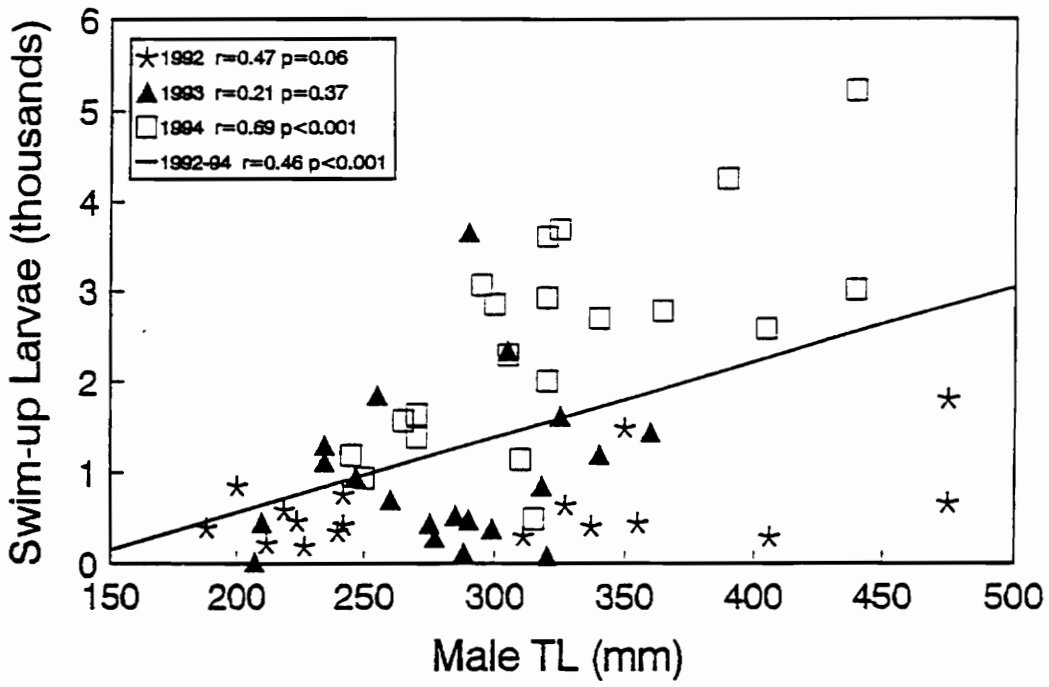

Figure 7. Relation between number of swim-ups (Y) reared by parental male smallmouth bass and male size (X) at site 1, 1992-1994. Regression equations are: Y=-40+2.2(X) for 1992, Y=-269+4.5(X) for 1993, Y=-2200+14.4(X) for 1994, and Y=-1077+8.2(X) for all years combined.

August indicate that age-0 mortality is high during the first summer. Relative abundance of juveniles in August at site 1 was 41, 79, and 40 for 1992-1994, respectively. From May to August, estimated mortality was greater than 95% for all three years. Although the total number of swim-up larvae annually varied as much as 50,000 individuals (range ~10,000 - 60,000, estimated total from all successful nests), the relative abundance of juveniles in August varied by a maximum of 39.

1994 REPRODUCTIVE SUCCESS AND AGE-0 SURVIVAL

A total of 56 nests were constructed in 1994 at sites 1 and 2. Parental males (50 total, 230-470 mm TL) spawned

between 04/12 and 05/14 (Figure 8) in slow water zones (behind obstructions and in back-current areas) at depths of 0.4 m to 2.0 m. Although most males spawned once in a single nest, one male spawned in two nests (about 0.5 m apart) simultaneously and four males renested and spawned while their initial brood was at the free-swimming (juvenile) stage. Nest densities, success rates, and causes of nest failure were similar at both sites (Table 3). All biotic nest failures (14 nests) were attributed to predation due to poor parental male defense, male abandonment, or male removal (angling). Six nests with eggs were destroyed by high flows (abiotic failures) between 04/28 and 05/06 (Figure 8). Most active broods had reached the larval swim-up stage during this period and were not destroyed by the increased discharge. Of the 36 broods that reached larval swim-up, 34 survived to metamorphosis (count 3). Broods that disappeared after count 3 (19-23 days after spawn) were assumed to have dispersed.

Table 3. Smallmouth bass reproductive success in the North Anna River in 1994.

	Site 1	Site 2	Both Sites
Total Nests	33	23	56
Nest Density (no./ha)	13.8	11.6	12.8
Nest Density (no./ river km)	48.5	46.2	47.5
Nests Successful to Swim-up	23	13	36
% Nest Success to Swim-up	70	56.5	64
% Biotic Nest Failures[a]	70	70	70
% Abiotic Nest Failures[b]	30	30	30

[a] Whole nest failures due to predation, male abandonment, or angling of parental male.
[b] Whole nest failures caused by flooding.

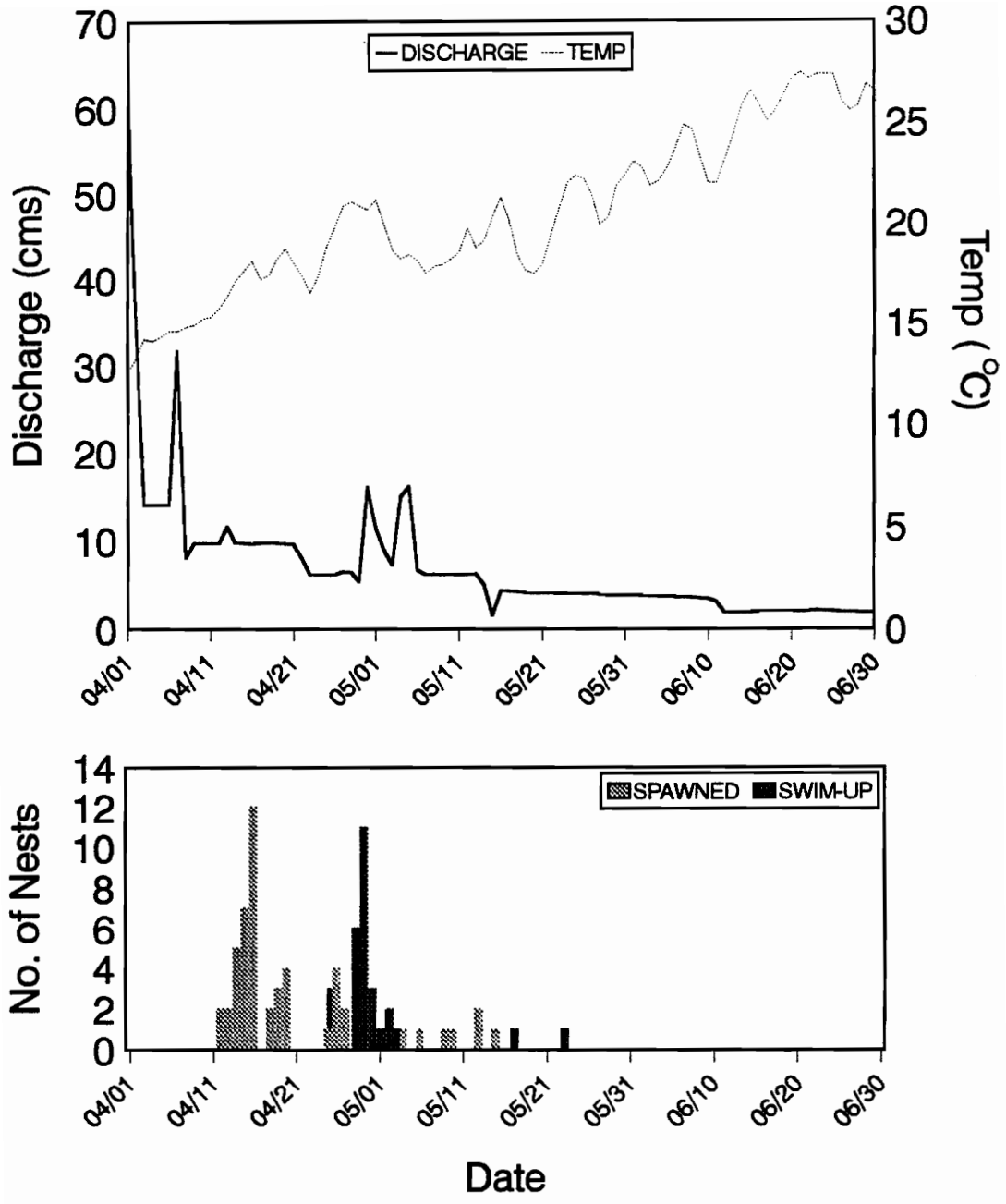

Figure 8. Timing of spawning and nest success to swim-up at sites 1 and 2 relative to temperature and flow conditions in 1994. The total number of broods that were spawned or reached swim-up is shown (lower diagram) for each date.

Brood Size, Survival Rates, and Density Effects

The size of smallmouth broods decreased with each of the four counts during parental care (Table 4). Overall, more than 94% of spawned offspring in successful nests died during

Table 4. Age-0 smallmouth bass abundance during early developmental stages in 1994.

Developmental Stage	Days After Spawn	Broods Counted	Mean No. Per Brood	SD
Egg	0-2	47	4527	2960
Swim-up	9-13	30	2392	1296
Metamorphosis	19-23	26	552	312
Juvenile	29-36	24	267	245

parental care (Figure 9). Including whole-nest failures and attrition, greater than 99% of young died during the parental care period.

Daily mortality rates (DMR) of young averaged 9.4% during parental care, but varied among life stages from 6.7% (period 1) to 14.0% (period 2, Table 5). DMR was significantly different (Friedman test, $0.01<p<0.025$) and higher (Friedman multiple comparisons test, $p<0.05$) during period 2 (swim-up count to larval metamorphosis count) relative to periods 1 (egg to swim-up) and 3 (metamorphosis to juvenile).

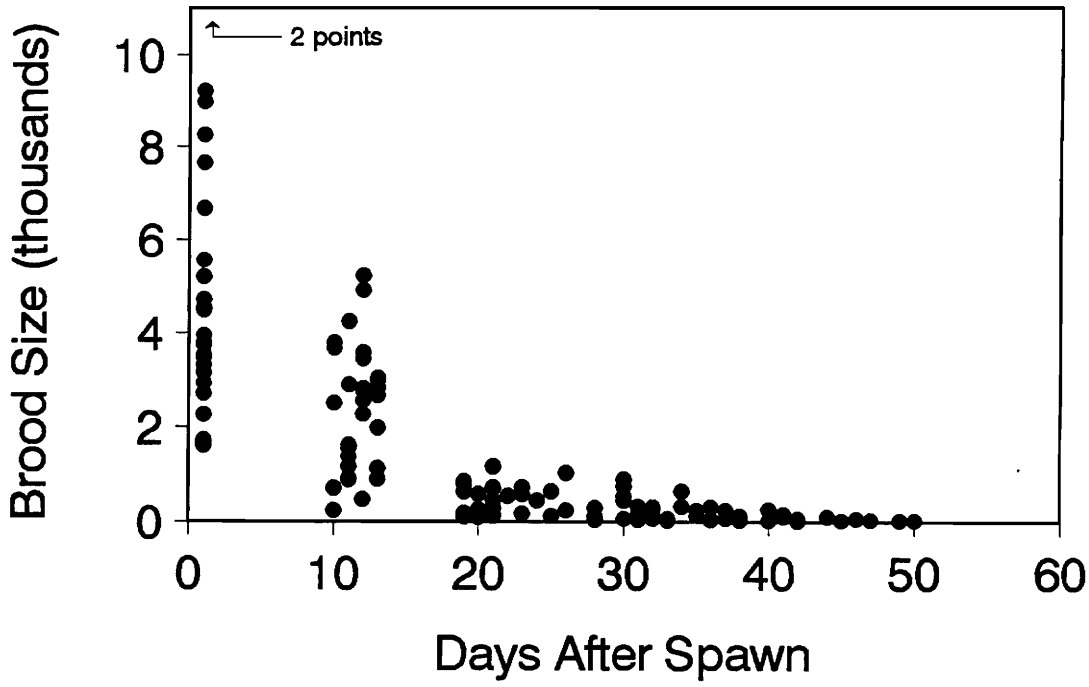

Figure 9. Number of smallmouth bass offspring per brood over time in 1994. Each point represents the number of individuals in one brood.

Table 5. Daily mortality rates for early life stages of age-0 smallmouth bass in 1994.

Period	Days[a]	DMR	SD	n
(1) Egg to Swim-up	9-13	0.067	0.051	29
(2) Swim-up to Meta[b]	7-11	0.140	0.045	26
(3) Meta to Juvenile	8-14	0.091	0.063	22
(1-3) Egg to Juvenile	29-36	0.094	0.023	22

[a] The number of days (range) in each developmental period.
[b] Meta = Larval Metamorphosis.

I anticipated that persistent characteristics of habitat and parental males (size and aggressiveness) would lead to

consistently higher or lower mortality rates for certain broods throughout parental care. Contrary to expectations, the relative magnitude of DMR was inconsistent for successive developmental periods (Figure 10). Autocorrelation of DMR for individual broods was not significant for either comparison (period 1 vs. 2, $r=-0.40$, $p=0.06$, $n=23$; period 2 vs. 3, $r=0.18$, $p=0.44$, $n=21$).

The initial number of offspring in each developmental period was unrelated to DMR for that period, indicating density-independent mortality for individual broods during parental care (Table 6 and Figure 11).

Table 6. Correlation of developmental stage-specific mortality rates and initial numbers at each stage for smallmouth bass broods in 1994.

Comparison	n	Pearson's r	p
# Eggs vs. DMR Period 1	29	0.33	0.08
# Swim-ups vs. DMR Period 2	26	0.31	0.12
# Meta vs. DMR Period 3	22	0.24	0.29
# Eggs vs. DMR Periods 1-3	21	0.18	0.44

Although DMR was density-independent and inconsistent through time for individual broods, mortality rates were not variable enough to alter relative brood size during parental care. The number of offspring per brood was positively correlated among all four brood counts (Table 7), suggesting nests that received the most eggs produced the most juveniles at dispersal (Figure 12).

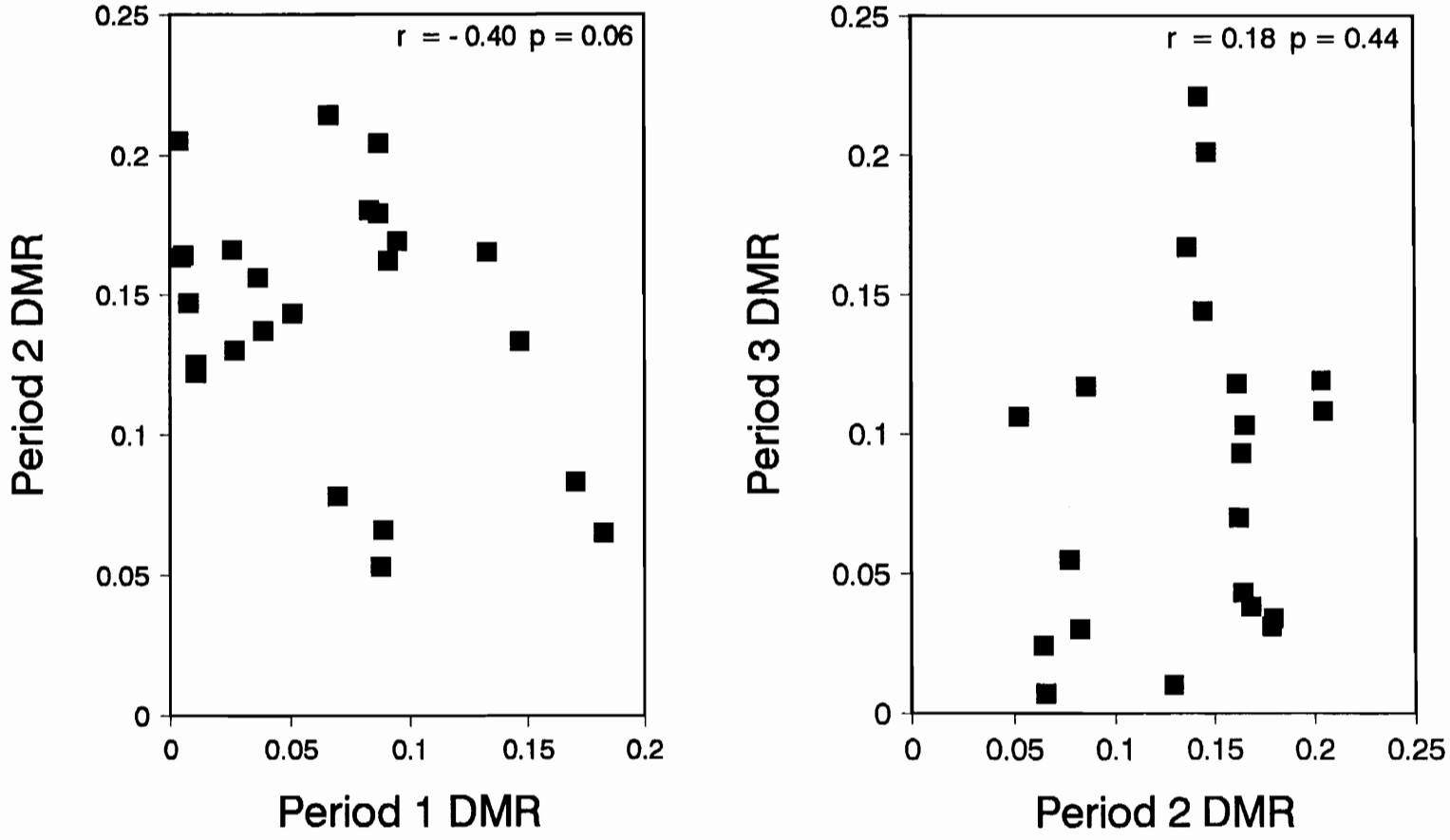

Figure 10. Scatterplots of DMR for successive developmental stages for individual broods.

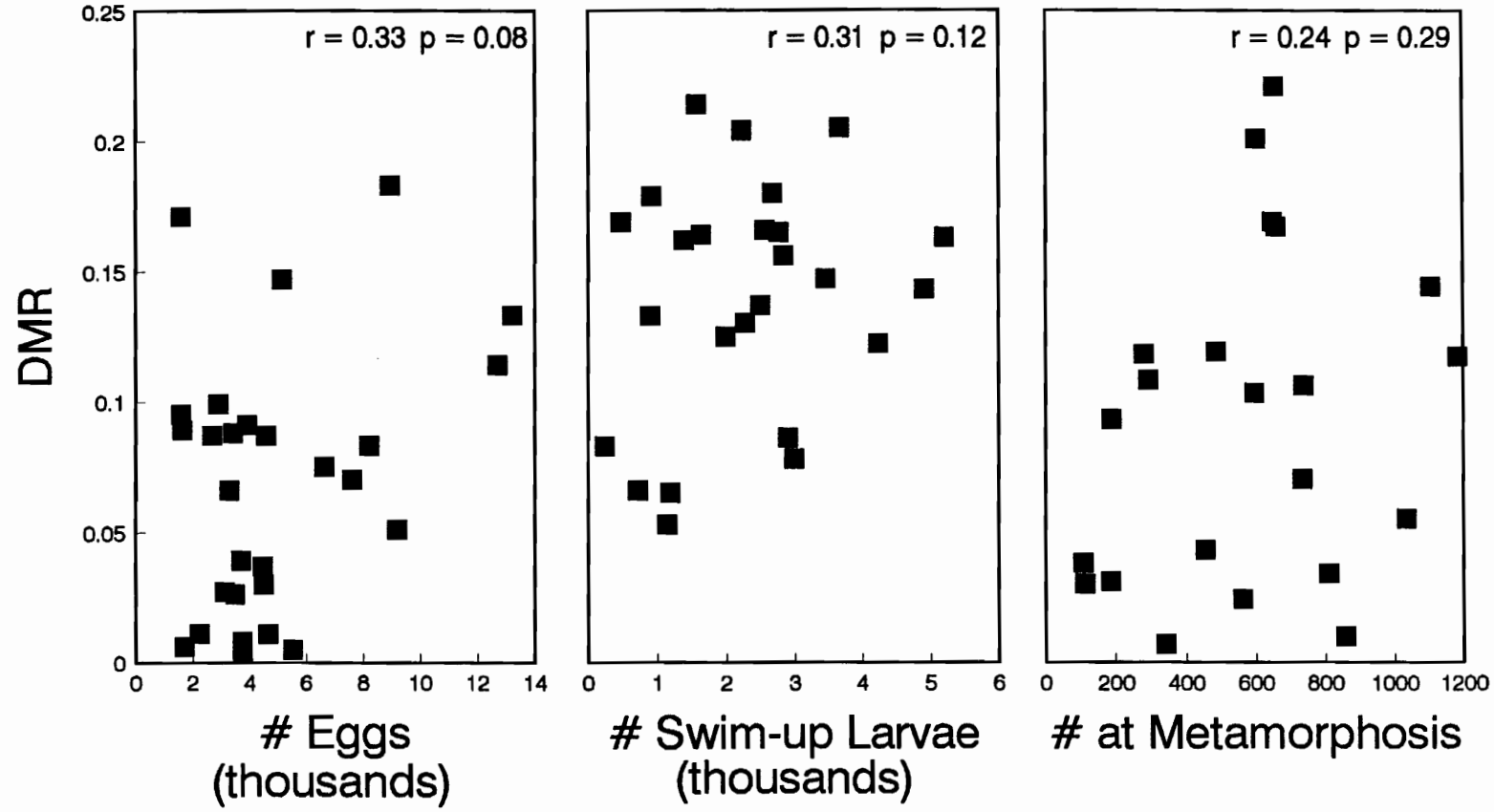

Figure 11. Relationship of brood size and DMR for three developmental periods during parental care. Mortality rates for each plot were calculated for the period immediately following the brood count specified on x-axis.

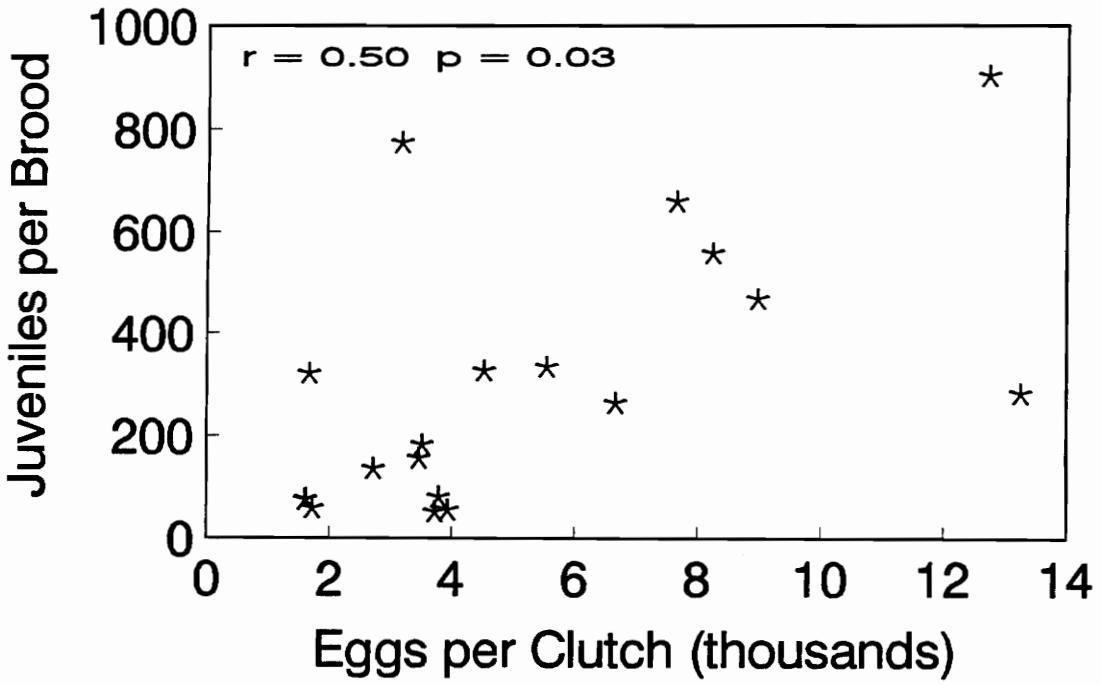

Figure 12. Correlation of brood size at egg (count 1) and juvenile (count 4) stages.

Table 7. Correlations of brood size at each count during parental care in 1994.

Counts Compared	Nests	Pearson's r	p
Egg (1) and Swim-up (2)	27	0.49	0.01
Egg (1) and Meta (3)	22	0.38	0.08
Egg (1) and Juvenile (4)	18	0.50	0.03
Swim-up (2) and Meta (3)	22	0.69	< 0.001
Swim-up (2) and Juvenile (4)	18	0.60	0.01
Meta (3) and Juvenile (4)	18	0.70	< 0.001

Decreasing Clutch Size

As part of procedures for quantifying fungus infection, the total area of substratum covered by each clutch was measured on days 1, 3, and occasionally 5 (if eggs hadn't hatched) after spawn. These measurements revealed that the size (area) of clutches decreased over time ($p=0.002$, repeated measures ANOVA, n=13 nests). Clutch areas were significantly larger ($p<0.05$, student-Newman-Keuls multiple comparison method) on day 1 (mean 603 ± 225 sd) than on day 5 (mean 465 ± 175 SD) and on day 3 (mean 552 ± 225 sd) relative to day 5. These data indicate that, in addition to egg mortality from fungus, nest predators (likely eels) were consuming eggs. Unlike fungus, which occurs in patches throughout the clutch, eels were often seen around the edges of nests and probably removed most eggs from the clutch perimeter.

August Abundance

The number of age-0 smallmouth bass at site 2 was estimated on August 9, 1994. Of the 52 fish observed in our snorkel survey, 7 were marked (31 fin-clipped previously on July 22-24). The population estimate using Petersen's method with Chapman's modification was 211, 95% confidence limits: (110,446). DMR for the period from juvenile brood counts to the late summer estimate was 0.03 - 0.04 at site 2.

Offspring Growth During Parental Care

Mean growth rates for young between swim-up and brood dispersal were 0.49 mm/day (± 0.07 SD, range 0.34-0.60, Appendices 1-3). Age-0 lengths (TL) were approximately 9-10 mm at swim-up (count 2), 12-15 mm at larval metamorphosis (count 3), and 18-24 mm at the juvenile count (4). Some individuals reached 30 mm before dispersing from the nest

site.

No strong correlations were found between growth trajectories and stage-specific DMR or brood size at counts 3 and 4 (Table 8), suggesting growth rates were independent of offspring density and mortality rates. However, evidence for density effects was greatest at the swim-up stage ($r=-0.44$, $p=0.05$), when abundance of free-swimming young is highest and larvae are beginning to feed exogenously (Figure 13). The negative correlation indicates slower growth in broods with more swim-up larvae.

Table 8. Correlations of growth trajectories (slopes) with brood-specific DMR and abundance for individual smallmouth bass broods after larval swim-up.

Suspected Growth Correlate	Number of Nests	Pearson's r	p
# of Swim-up Larvae	21	- 0.44	0.05
# at Metamorphosis	19	0.09	0.72
# of Juveniles	18	0.06	0.82
DMR Period 2	19	- 0.41	0.08
DMR Period 3	18	- 0.30	0.24

PARENTAL MALE ATTRIBUTES AND SIZE CORRELATES

Fifty male smallmouth bass (230-470 mm TL) spawned at sites 1 and 2 in 1994. The male size and spawning time distributions for both sites combined were similar to findings for site 1 only (see Figures 4 and 5).

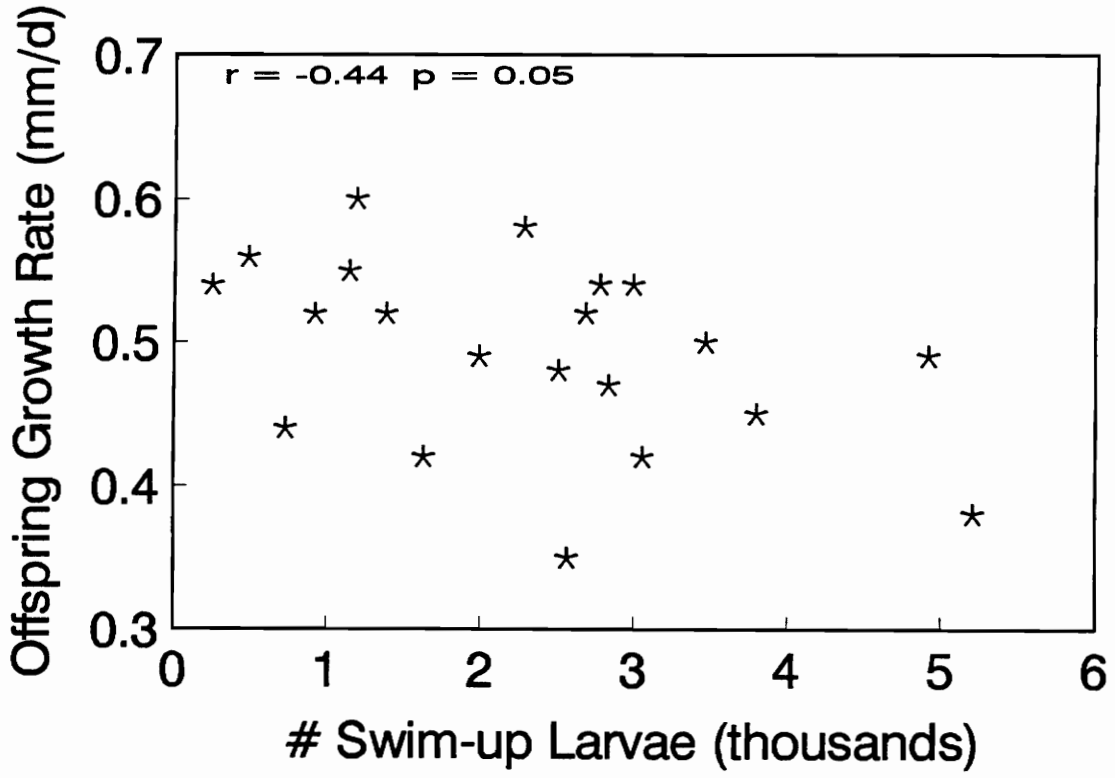

Figure 13. Relationship of offspring growth rates (swim-up to dispersal) and the number of swim-up larvae per brood.

Parental Male Size

Although there was no difference in the size of parental males for successful (n=29) and unsuccessful (n=16) nests (t-test, p=0.52), the number of offspring per brood was positively related to parental male TL for all 4 counts during parental care (Figures 14-17). Male TL was not strongly associated with DMR during parental care (Table 9), although male size was positively related to mortality during period 3 (p=0.05). These data suggest that larger males receive more eggs at the time of spawning and continue to have larger larval and juvenile broods than smaller males, but are not necessarily better brood defenders.

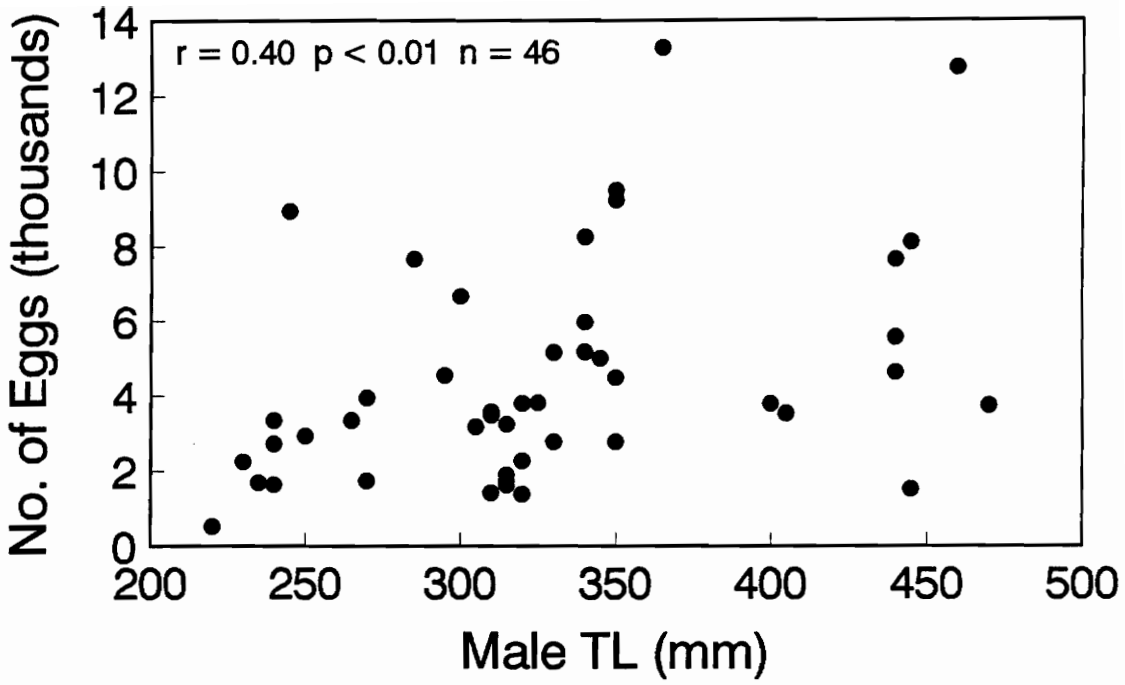

Figure 14. Clutch size for parental male smallmouth bass.

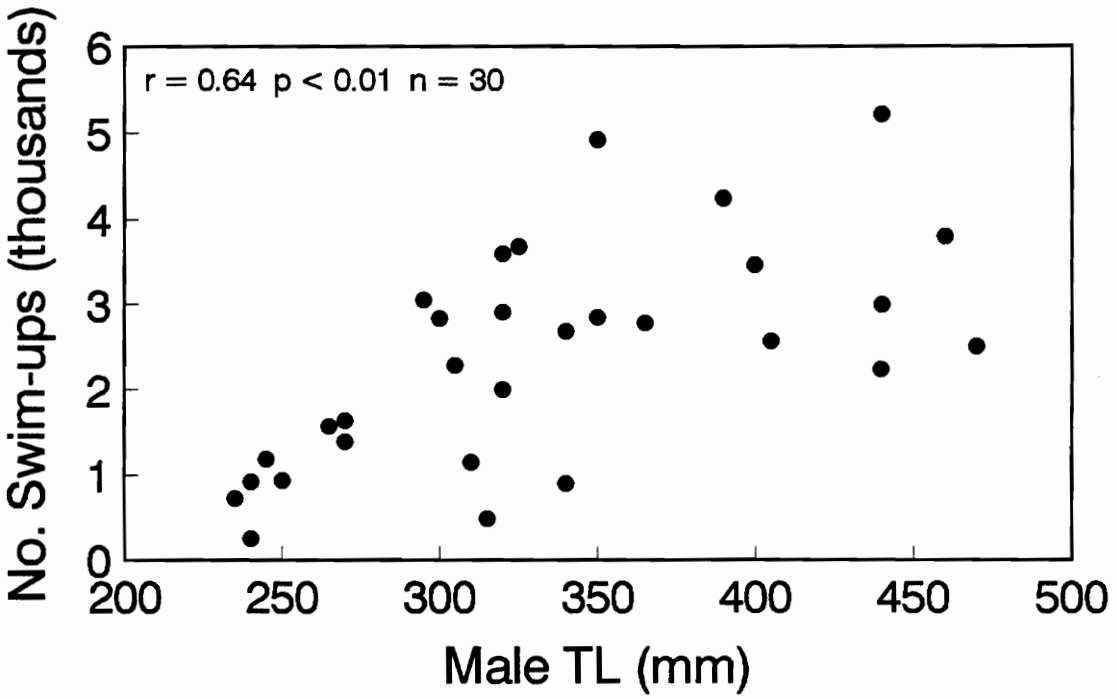

Figure 15. Number of swim-ups reared by parental male smallmouth bass.

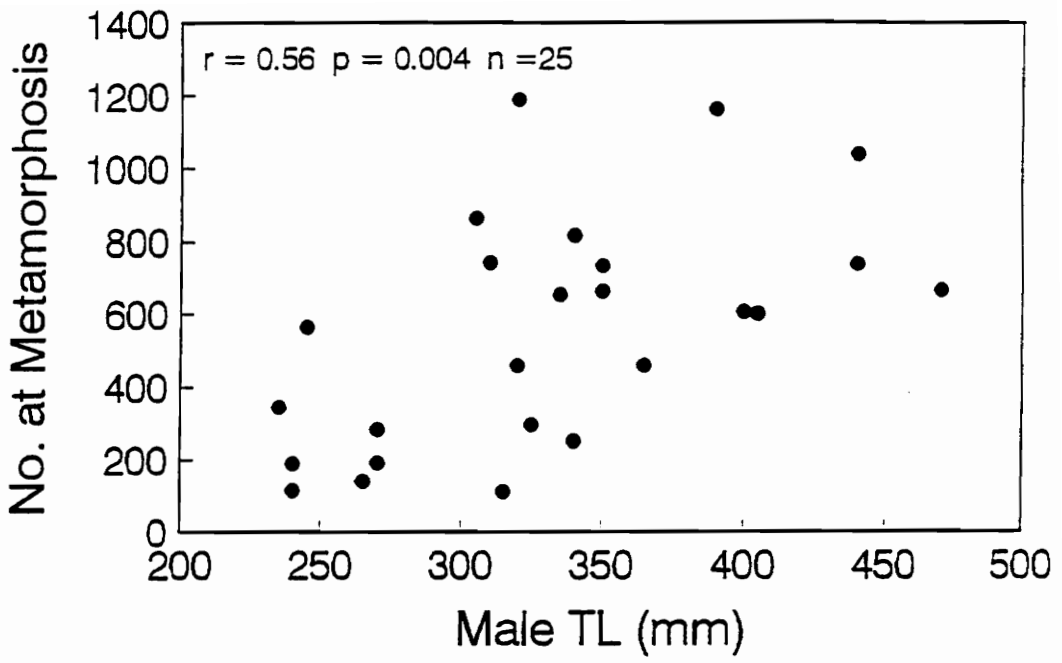

Figure 16. Number of metamorphosed larvae reared by parental male smallmouth bass.

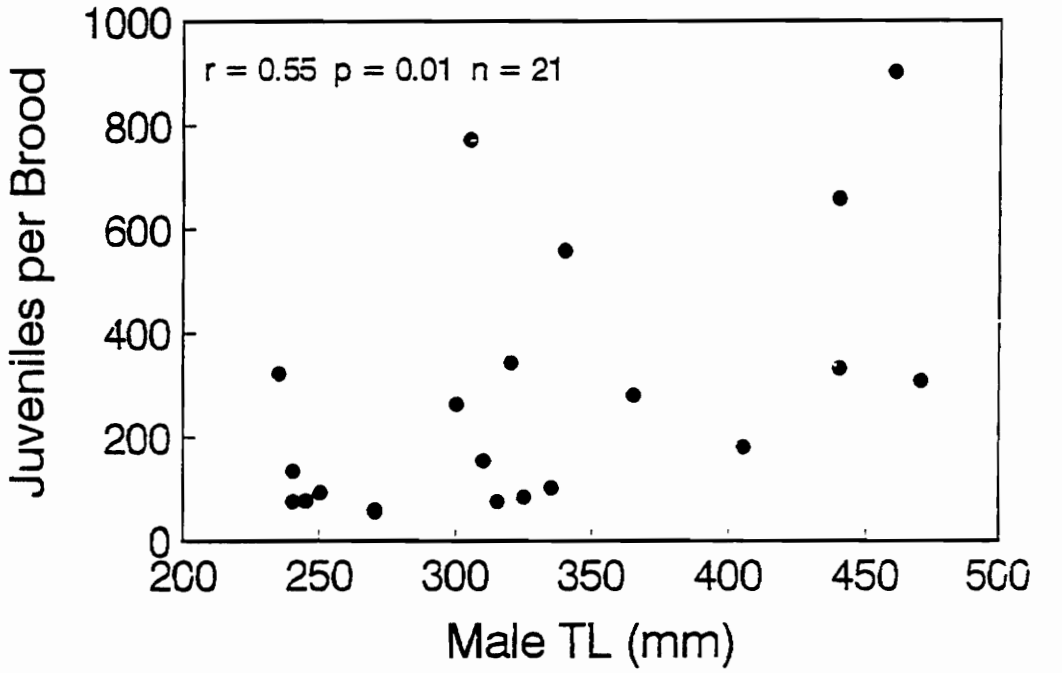

Figure 17. Number of juvenile offspring reared by parental male smallmouth bass.

Table 9. Correlation of parental male size with daily mortality rate (DMR) during different developmental periods during parental care in 1994.

Comparison	No. of Nests	Pearson's r	p
Male TL vs. Period 1 DMR	30	- 0.30	0.10
Male TL vs. Period 2 DMR	26	0.19	0.35
Male TL vs. Period 3 DMR	25	0.39	0.05
Male TL vs. Periods 1-3 DMR	22	0.15	0.97

Parental Male Brood Defense

Indices of male aggression toward nest predators (male defense indices) were developed to determine if male behavior alone or in combination with male size affected brood survival during period 1 (egg to swim-up). Conversely, I also investigated whether offspring abundance affected the level of defense (aggressive behavior).

The three male defense indices were highly correlated (total bites vs. total time near model, r=0.65, p=0.0001; total bites vs. attack distance, r=-0.055, p=0.0004; attack distance vs. total time near model, r=-0.51, p=0.001). Therefore, results of correlation analyses comparing male defense with male TL, DMR, and brood size are reported only for total bites (Table 10).

Since more aggressive brood defense should lead to better protection from diurnal predators and larger males are assumed to be more experienced parents, the number of bites on the model predator was expected to be negatively associated with DMR (increased survival) and positively correlated with the number of offspring and male size. However, index scores were not related to male size, DMR during periods 1-3, or the number of offspring in the brood.

Table 10. Correlation of total bites of a predator model by parental male smallmouth bass (male defense index) with parental male size, period 1 mortality rates, and egg abundance.

Comparison	n	Pearson's r	p
Total Bites vs. Male TL	37	0.10	0.54
Total Bites vs. DMR (Periods 1-3)	22	0.32	0.15
Total Bites vs. # Eggs	37	0.30	0.07
Total Bites vs. # Swim-up Larvae	23	0.04	0.86
Total Bites vs. # at Meta.	20	-0.04	0.86
Total Bites vs. # Juveniles	17	0.09	0.72

Duration of Parental Care

Parental care was provided by males from spawning to brood dispersal (juveniles ~18-30 mm TL). The mean duration of care was 39.6 days (SD 6.4, range 29-54 d). The span of parental care was not associated with male TL ($r=-0.127$, $p=0.55$, $n=25$), DMR for periods 1-3 ($r=-0.347$, $p=0.13$, $n=20$), clutch size ($r=-0.08$, $p=0.73$, $n=23$), or the number of juveniles at count 4 ($r=0.07$, $p=0.75$, $n=22$). In all cases, brood dispersal seemed to coincide with termination of parental care, but I could not conclusively determine if the male or offspring initiated dispersal.

Male Nest Departure Frequencies

Male nest departure frequency was considered an index of predation intensity. For nests investigated in 1992 (Lukas 1993) and 1994, the frequency of male departures was significantly higher when offspring had reached swim-up (relative to the egg stage) and increased during crepuscular periods (relative to day periods, Table 11).

Table 11. Comparison* of parental male nest departure frequencies at different life stages and diurnal time periods.

	Egg		Swim-up	
Time	Depart./Hr	n	Depart./Hr	n
Day (1992)	20.7	15	54.2	7
Evening (1994)	38.6	16	72.3	9

* All comparisons among time periods and life stages were significant at $\alpha=0.05$.

Nest predators were occasionally observed (momentarily) in the vicinity of a nest before the parental male chased them away. However, only one predation event was seen in 1992 (one swim-up larva; Lukas 1993) and no predation was observed during day or crepuscular filming in 1994. In 1994, potential predators observed near nests included largemouth bass, American eel, redbreast sunfish, bluegill, various cyprinids, and other juvenile and adult smallmouth bass.

FUNGUS INFECTION

All smallmouth bass egg masses sampled (n=34) in 1994 were infected with fungus. Fungus samples from seven nests were identified as the water mold *Saprolegnia parasitica*. Infections appeared to begin on dead or unfertilized (opaque) eggs within 1-2 days after spawning and grew outward, encompassing adjacent live eggs. By the time eggs hatched, a thick mat of mycelia sometimes covered the clutch. Casual investigation of several nests (post-hatch) revealed clusters of dead eggs bound by the mycelia. However, even nests with the most severe fungus infection (100% egg coverage) were able to produce larvae. Neither fungus colonization nor differences in the severity of infection among nests caused obvious changes in male behavior.

Infection Rates and Effects on Brood Mortality

The final proportion of egg masses (area) covered with fungus ranged from 3-100%, but 28 of 34 nests sampled had less than 40% (Figure 18). The proportion of clutches covered with fungus was related to mean water temperature, egg density, and the abundance of dead eggs. Eggs that developed at temperatures (mean) greater than 18°C (n=5) had less fungus infection (Figure 19). This suggests that a threshold for fungus colonization or growth may occur at this temperature. The level of fungus infection also tended to increase with egg density (Figure 20). Although the proportion of the clutch covered with fungus became more variable as egg density increased, severe infections (>50% of clutch covered) only occurred at high (>7/cm^2) densities.

S. parasitica first colonized dead eggs (present at day 2 after spawn) or organic matter in the nest. The proportion of clutch area covered by fungus was positively related to dead egg abundance (Figure 21). After initial colonization, a significant portion of the variation in fungus growth rate was explained by egg density (R^2=0.18, p=0.01, n=33). A threshold temperature of 18°C (similar to Figure 19) also appears to be important for fungus growth rate and may explain the variable levels of infection reported above.

DMR for the interval from spawn to swim-up (period 1) was positively related to the proportion of clutch area infected with fungus (Figure 22). These data were best represented by a nonlinear (power function) model. High mortality occurred in all nests where fungus covered >25% of the clutch area, but losses were much less predictable at lower levels of

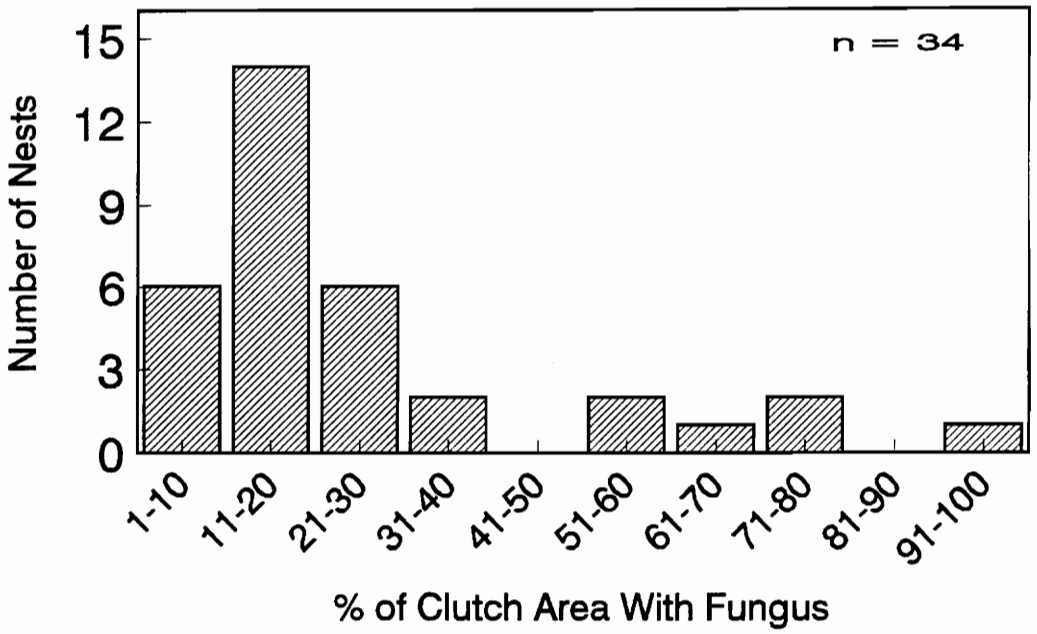

Figure 18. Number of smallmouth bass egg clutches with different levels of fungus infection.

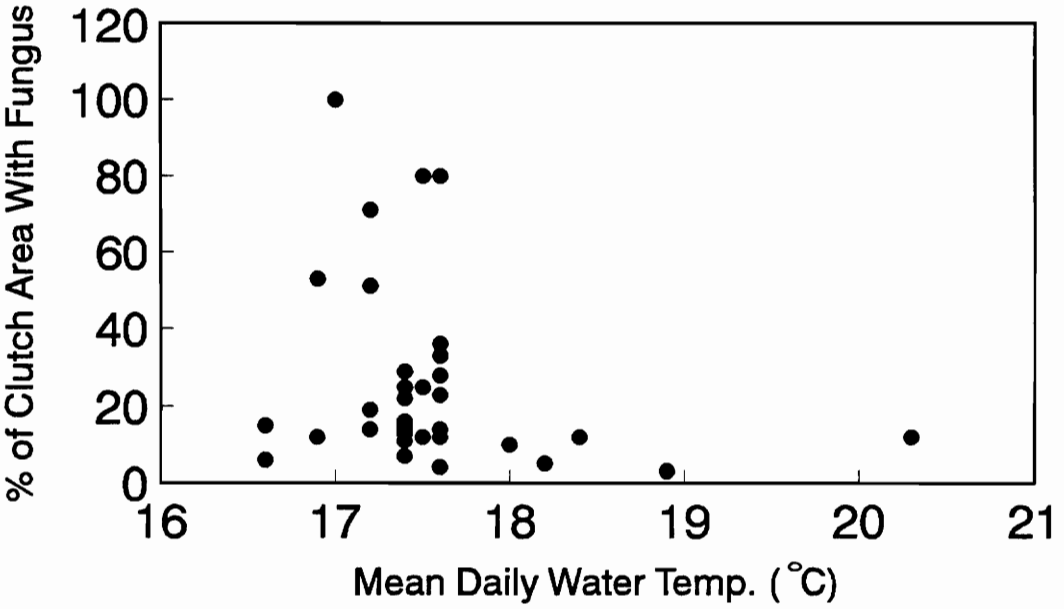

Figure 19. Proportion of smallmouth bass clutches covered by fungus at different water temperatures.

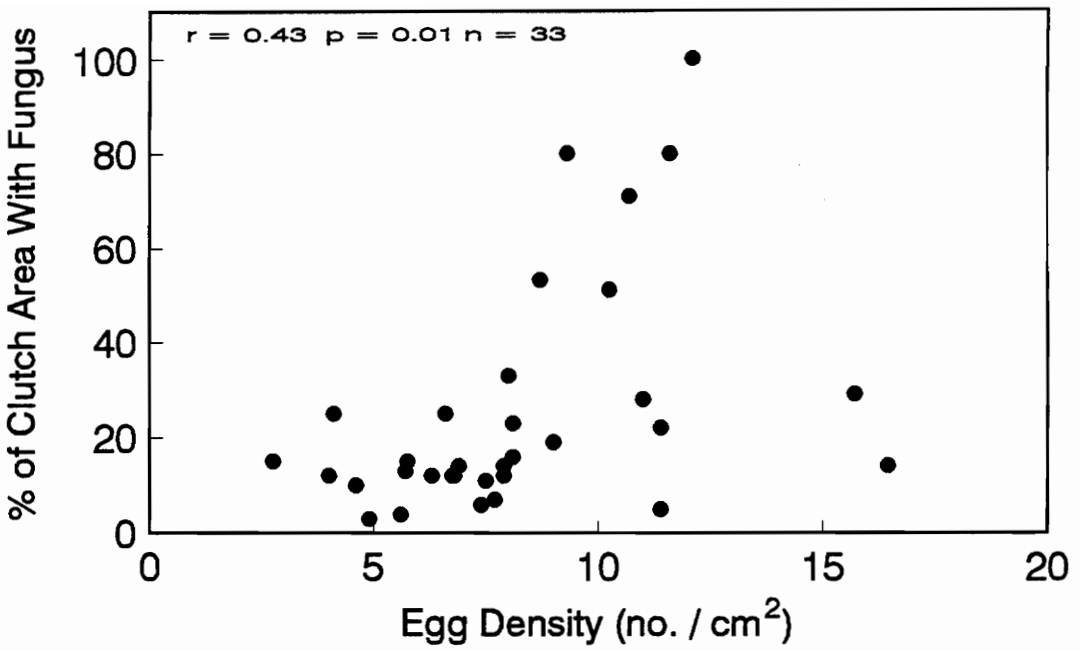

Figure 20. Relation of clutch egg density and the proportion of clutch area covered with fungus.

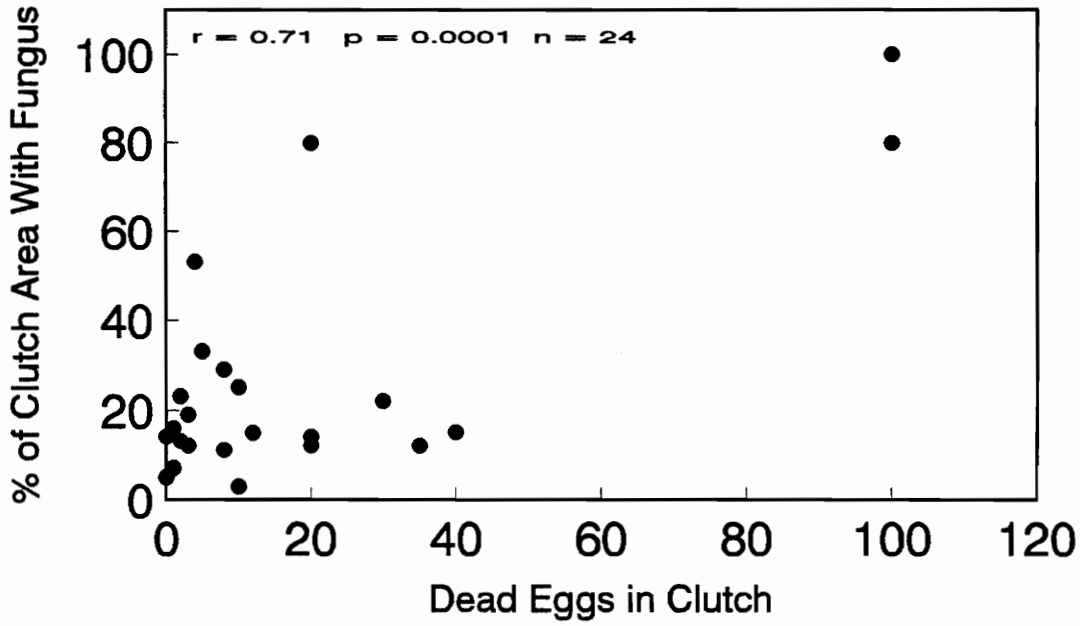

Figure 21. Relationship of percent clutch area covered by fungus and the number of dead eggs for smallmouth bass egg clutches.

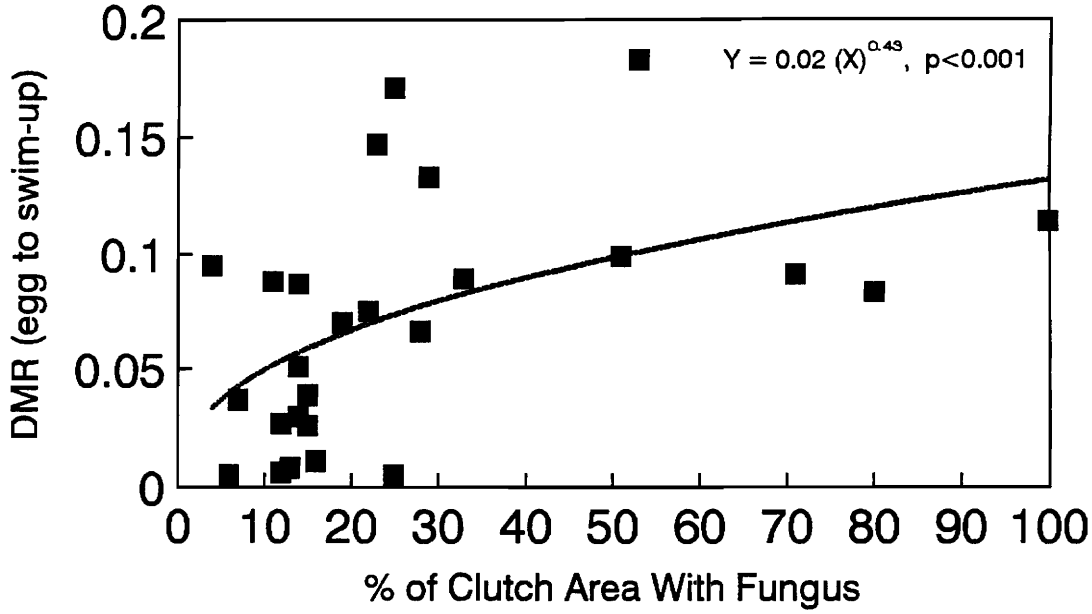

Figure 22. Scatterplot of period 1 DMR (Y) as a function of the proportion of clutch area infected with fungus(X). Curve was fitted using least squares nonlinear regression.

infection. Variable effects of infection may been related to additional mortality sources and differential susceptibility of live eggs to infection.

BROOD PREDATION

No brood predation was observed during diurnal periods in 1994 (except for occasional small eels in nests prior to swim-up). Pelagic nest predators (centrarchids and cyprinids) were rarely seen in the vicinity of the brood (when the parental male was present) during daily snorkel surveys and other diurnal data collection activities. Nest predators were seen more frequently during crepuscular nest filming (see male departure frequency section), but were not observed eating

young. However, night observations revealed that American eels were common predators of smallmouth bass larvae and juveniles.

Eels (~125-500 mm) were frequently observed near inactive broods and, in many cases, were seen consuming larvae and juveniles at night. On 47 occasions, 63 eels were observed within 1 m of an inactive brood (between 2200 and 0200 hrs). Eels were consuming or pursuing smallmouth bass young (14-20 mm SL) on 12 different occasions. In addition, small eels (<100 mm) were seen in nests containing eggs or embryos at least three times. Overall, eels were observed in the vicinity of 23 of the 36 broods that survived to dispersal.

Time-lapse Photography of Broods

A total of 51.7 hr of night video footage was recorded for 9 different smallmouth bass broods (young 10-20 mm SL). Four eels and one redbreast sunfish were the only predators observed. However, the combination of a confined visual field and intermittent recording surely limited the effectiveness of the camera. In one instance, an eel was observed feeding in the midst of a brood by a diver for several minutes, but could not be seen on the concurrently recorded video tapes.

Eel Stomach Contents

The stomach contents of 20 eels (125-287 mm) collected directly from nest sites included 64 smallmouth bass (14-20 mm SL, Figure 23). Only one eel in electrofishing samples (159 eels, 95-470 mm) from my study sites and throughout the river contained smallmouth bass (4 juveniles, 17-20 mm SL). A summary of eel stomach contents from all samples is shown in Appendices 4 and 5.

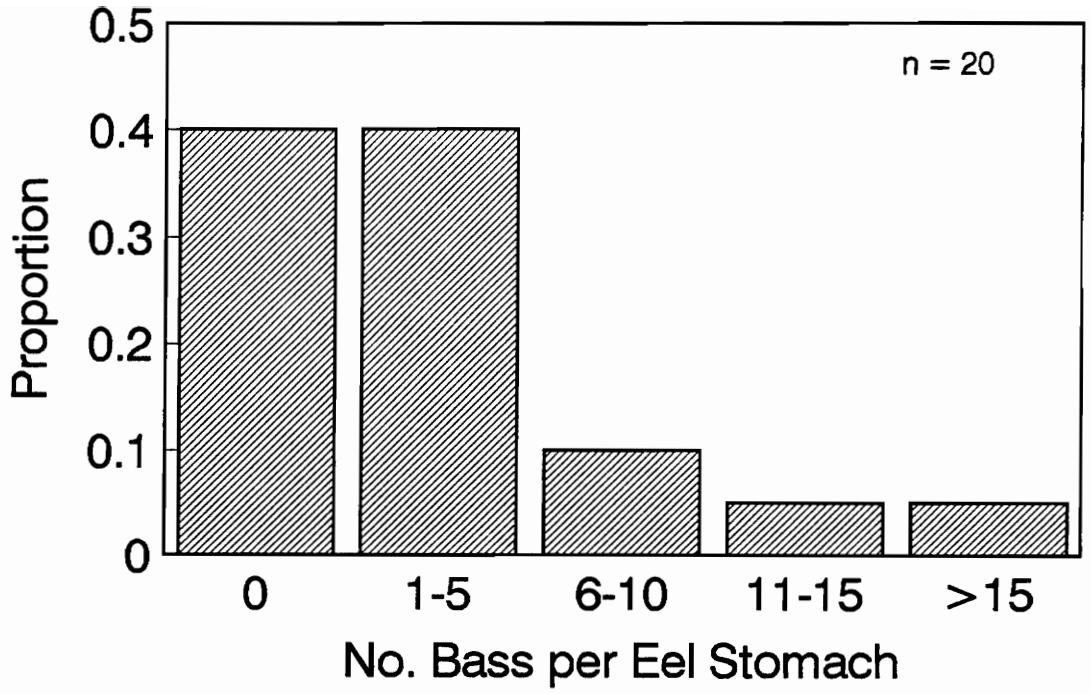

Figure 23. Occurrence of age-0 smallmouth bass in stomachs of eels captured in the vicinity of active nests.

MULTIPLE REGRESSION MODELS FOR DMR

Regression models were developed to account for variation in DMR among broods during different ontogenetic periods (Table 12). DMR for period 1 was dependent on parental male TL, parental male aggression index, and egg abundance. Any variables selected as predictors of DMR for other periods did not explain a significant proportion of the variation in these variables ($p<0.05$).

Table 12. Multiple regression models for developmental stage-specific DMR. Predictor variables were chosen using backward stepwise regression.

Dependent Variable	Predictor Variables Chosen	p (Model)	R^2
DMR1[a] Egg to Swim-up	Male TL, Male Aggression Index (Male Bites), No. Eggs	0.001	0.57
DMR2 Swim-up to Meta.	None	-	-
DMR3[b] Meta. to Juvenile	Male TL, Eel Presence/Absence	0.09	0.29
DMR Total Egg to Juvenile	None	-	-

[a] Regression equation is DMR1 = 0.151 - 0.0004 (Male TL) + 0.002 (Male Bites on Predator Model) + 0.00001 (No. Eggs), n=23.
[b] Regression equation is DMR3 = -0.027 + 0.0004 (Male TL) - 0.04 (Eel Presence/Absence), n=17.

DISCUSSION

Results of this field study indicate that smallmouth bass mortality is extremely high and variable among years during early life stages. Reproductive success and brood survival were influenced by many biotic and abiotic factors that varied in importance among years and ontogenetic stages. In 1994, mate acquisition, clutch size, and nest success were critical determinants of juvenile production for parental males. However, no apparent relationship of brood success or swim-up larvae production with late summer abundance emphasizes the significance of factors governing summer juvenile survival. These findings imply a need for further investigation of age-0 movements and mortality during summer and at larger spatial scales in order to better understand factors and processes influencing smallmouth bass year-class strength determination

and recruitment dynamics.

NESTING AND ANNUAL REPRODUCTIVE SUCCESS

Annual variability in nest abundance and reproductive success is common in wild populations of smallmouth bass (Pflieger 1975; Vogele 1981; Raffetto et al. 1990). Plasticity in spawning behavior (e.g. renesting) permits reproduction and persistence under extremely variable environmental conditions. Annual differences in nest density on the North Anna River were primarily due to changes in the frequency and timing of spring spates. High flows destroyed previously constructed nests, but floods were often followed by a pulse of renesting as flows receded. Although each cycle of flooding and renesting augmented total nest numbers, frequent high discharges often destroyed nests before they reached swim-up (nest success). Thus, recurrent floods (as in 1992) tended to increase nest abundance and decrease overall nest success rates.

Temperature and flow conditions under which spawning was initiated and transpired agreed with accounts from other streams (Pflieger 1966; Winemiller and Taylor 1982; Reynolds and O'Bara 1991; Lukas 1993), but annual differences in male size distribution may have affected the timing of spawning. The inverse relationship of male size and spawning date (1992-94) agrees with findings where accumulated degree days (>10°C) was negatively correlated with male size in an Ontario Lake (Ridgway et al. 1991) and in the North Anna River (1992 and 1993 only, D. J. Orth, unpublished data). This information is further evidence that the timing and duration of spawning is predictable (see Graham and Orth 1986) and may prove useful in modelling efforts, hydro-assessments, and instream flow recommendations.

Reproductive success (to swim-up) and swim-up larvae production per nest in this study were within reported ranges

and conform to the high variability observed among other populations (Table 13). Yearly differences in nest success and brood size is one reason why nest density alone is a poor indicator of juvenile production and eventual year-class strength (Cleary 1956). Since nest densities often do not reflect the number of nests and offspring that survive to swim-up (e.g. 1993 vs. 1994 in Table 1; Ridgway and Friesen 1992), predicting cohort abundance at much later stages is not justified.

Table 13. Comparison of mean number of swim-up larvae per nest and annual nest success for smallmouth bass from various studies. For the North Anna River, 1992 data are from Lukas (1993), 1993 data are for site 1 only, and 1994 includes sites 1 and 2.

Investigator or Location	Swim-up Larvae per Nest	Nest Success to Swim-up
Latta 1963	2054	54%
Pflieger 1966	2363	80%
Neves 1975	3943	67-77%
Vogele 1981	2855	30-84%
Goff 1985 - Windy Conditions	720	33%
Goff 1985 - Calm Conditions	1778	88%
NORTH ANNA RIVER 1992	608	42%
NORTH ANNA RIVER 1993	996	38%
NORTH ANNA RIVER 1994	2392	64%

Previously, reproductive success has been associated mainly with abiotic factors including flooding and flow attributes, water temperature, distance of nests from shore or cover, and date of spawn in streams (Reynolds and O'Bara 1991; Lukas 1993), and the number of hours of strong winds on lakes

(Goff 1985). In this study, "flooding" mortality was common, but biotic (predation) sources of nest failure also were important. Even when flooding interrupted spawning several times (1993), more than 50% of nest failures were attributed to biotic sources. Similarly, Pflieger (1975) emphasized that while abiotic disturbances are a major challenge during the nesting period, biotic factors may control age-0 smallmouth bass abundance in Missouri streams.

Nest success and total larval production were highest in 1994, a year with relatively stable, low flows. Similar physical conditions also have led to high reproductive success in Missouri (Pflieger 1966) and Tennessee (Reynolds and O'Bara 1991) streams. In the North Anna River (1994), limited flood-induced nest destruction resulted in high nest survival early in the season and, consequently, little renesting. Stable physical conditions and success of early nests probably also contributed to high larval (swim-up) production because larger males tended to spawn earlier and rear more swim-up larvae than smaller males.

AGE-0 SURVIVAL DURING PARENTAL CARE

Mortality during early life stages was due to a combination of nest failures and attrition in successful nests. In 1994, nest success was high and most nest failures occurred prior to swim-up; 94% of nests that reached swim-up survived to juvenile dispersal. Friesen (1990) also found that whole-nest (density-independent) mortality was most prevalent prior to larval swim-up in Lake Opeongo, Ontario.

Despite high reproductive success (1994), overall survival (<1%) and survival in successful nests (<6%) were extremely low during parental care. In reproductive strategies that require substantial parental investment (e.g., parental care), high larval and juvenile survivorship are anticipated (Winemiller and Rose 1992). The drastic decrease

in brood size was unexpected based on the smallmouth bass life-history pattern and some previous studies of smallmouth bass reproduction (e.g., Pflieger 1966; Friesen 1990), but brood size at each life stage generally corresponded with other reports (Figure 24).

Brood DMR generally corresponded with previous studies, except during period 2 (Table 14). Friesen (1990) reported high rates of nest failure just after swim-up, but did not observe high brood attrition. Toetz (1966) suggested that low

Table 14. Comparison of mortality rates in successful nests at selected developmental periods during parental care in literature reports and North Anna River data in 1994.

Period	DMR	% Mortality	Source
Egg to Swim-up*	7.6-16.6%		Latta 1963
(1)	0.4-0.8%	6	Pflieger 1966
	6.6-17.5%	67-74	Clady 1975
	6.0-13.2%	63	Vogele 1981
	6.7%	46.3	NORTH ANNA RIVER
Swim-up to Meta.	0.06%	0.05	Friesen 1990
(2)	14.0%	73.4	NORTH ANNA RIVER
Meta. to Juvenile	10.6%	59.2	Friesen 1990
(3)	9.1%	48.7	NORTH ANNA RIVER

* Range of possible DMR for egg to swim-up in literature reports estimated based on variable development time: 7-16 days (Lukas 1993).

foraging success during this phase, as young begin free-swimming and shift from endogenous to exogenous feeding, may contribute to high mortality in bluegill sunfish (*Lepomis macrochirus*). Hjort's (1914) "critical period" concept also predicts that larval mortality (related to starvation) will be

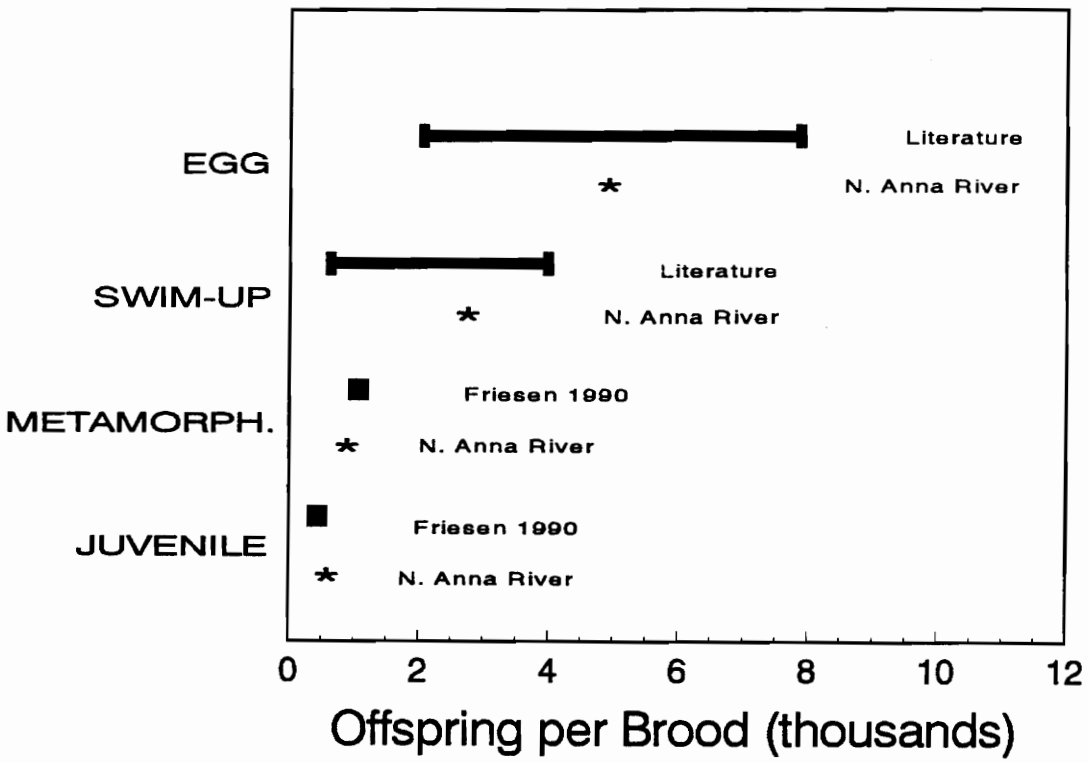

Figure 24. Comparison of brood abundance at selected life stages during parental care for literature reports and North Anna River data in 1994. Literature egg and swim-up values represent the range of mean abundances from Latta 1963, Pflieger 1966, Clady 1975, Neves 1975, Vogele 1981, and Goff 1985.

greatest at this stage. Although inadequate forage at first-feeding may have affected mortality through increased predation risk due to limited growth (Toetz 1966; Easton and Orth 1992) or reduced swimming ability (Laurence 1972), there was little direct evidence of larval starvation in the North Anna River based on stomach fullness data (Ed Pert, Virginia Tech, unpublished data) and growth rates after swim-up (Sabo and Orth 1995; Appendix 1).

Most larval and juvenile brood attrition was attributed to eel predation (see Predation section). Pflieger (1975) and Friesen (1990) also noted that offspring are most vulnerable to predators just after swim-up. Young are poor swimmers

during this period, especially prior to metamorphosis when fins are undifferentiated (Figure 25). Although swimming speed and maneuverability were most limited for larvae (TL<15 mm, Easton and Orth 1992) and probably contributed to predation losses, nocturnal behavior likely had a greater effect; larvae and juveniles were very susceptible to eel predation as they lay inactive and aggregated on the streambed at night. Vulnerability to eels and other predators appeared to be much lower during daylight as young fed in the water column under more vigilant parental male protection.

PATTERNS IN BROOD MORTALITY

Life-stage specific mortality rates for individual broods were not closely associated with brood size, male attributes, offspring growth, or most other variables that were suspected to influence survival. Relative attrition rates among broods were also inconsistent through time, despite (presumed) persistent differences in nesting habitat quality, male defense ability, and inherent (genetic) variability among broods. These findings suggest that mate acquisition, clutch size, and nest success were more important determinants of juvenile production for individual males.

Density-dependence is often a key assumption or prediction in models of larval fish ecology. Simulations of growth and survival using an individual-based model for age-0 smallmouth bass predicted strong density-dependent mortality during early development and density-independence later in the growing season (DeAngelis et al. 1993). Limited empirical data also suggest that density effects are important during certain life stages. In Lake Opeongo, Ontario, smallmouth bass brood mortality was density-independent prior to larval metamorphosis, while density-dependent and density-independent processes were evident for juveniles (Friesen 1990). Several mechanisms that may explain compensatory effects have

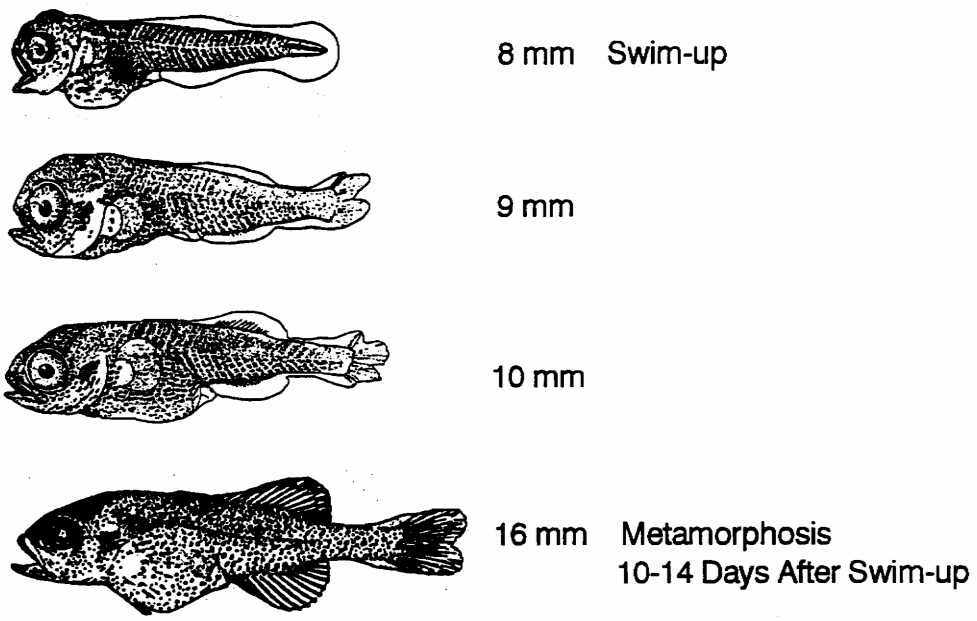

Figure 25. Changes in smallmouth bass morphology from larval swim-up to metamorphosis (8-16 mm TL). Diagrams copied from Lippson and Moran 1974.

been proposed: fungus infection (Brown 1956; Hoffman 1969), starvation or competition for food which may limit growth and increase susceptibility to predation (Easton and Orth 1992; DeAngelis et al. 1993), predator aggregation, and cannibalism (Brownell 1985, Eipper 1975).

The lack of density-dependent survival in this study indicates that offspring were not self-regulating at the brood scale. Density-independent brood attrition was particularly unexpected since physical disturbances, which may obscure density effects, were minimal (1994). Density-dependence may have been precluded by high egg or larval mortality caused by fungus infection and eel predation. However, the number of swim-up larvae per nest in 1994 was high relative to previous

years and several other field studies (Table 13).

Brood attrition rates were also unrelated to parental male size and aggression during most early life stages. Larger, more experienced males were expected to limit predation and enhance brood survival (Reynolds and O'Bara 1991; Lukas 1993), but the period 1 multiple regression model was the only strong evidence that male attributes affected brood survival rates. In this model, DMR was a function of male size, male aggression, and egg abundance. Male effects seem conflicting since DMR decreased with increasing male size and increased with increasing level of aggression. However, males that were overzealous in pursuing intruders may have left offspring vulnerable to other predators at the nest.

The inability of parental males to reduce fungal growth (egg stage) or offer adequate protection from eel predation may have limited the influence of measured male attributes. Male defenses were very effective against diurnal brood predators with which they likely evolved (e.g., minnows and sunfishes). Defenses were apparently not as adaptive for protecting offspring from efficient nocturnal predators (eels) that are uncommon in the native range of smallmouth bass.

Although male attributes did not profoundly affect offspring survival rates, they were important indirect determinants of juvenile production. The positive correlation between male TL and clutch size supports the hypothesis that larger males are more successful in mating with larger, more fecund females (Weigmann et al. 1992; Lukas 1993). Because survival was not density- or size-dependent (through time) during parental care, larger parental males were able to maintain larger broods until dispersal (agrees with Friesen 1990). These results corroborate findings of inconsistent, density-independent (but not highly variable) brood mortality rates and indicate that mating success and clutch size were primary determinants of abundance at dispersal.

DURATION AND TERMINATION OF PARENTAL CARE

The duration of parental care (mean 39.6 d, range 29-54 d) was longer than previously reported. The mean annual duration of care was 20.3-34.0 d over 4 years (range 19-45 days) in an Ontario Lake (Ridgway and Friesen 1992), but only lasts up to four weeks in most accounts (Emig 1966; Vogele 1981). Slow offspring development in successful early nests probably contributed in prolonging the care period in 1994.

Although development rates of young were surely important, care duration differences could be related to variation in male energy reserves (Ridgway and Friesen 1992; Ridgway and Shuter 1994); parents with greater fat stores may be able to defend broods longer. In addition, Ridgway and Friesen (1992) suggested that ontogenetic changes in offspring behavior may influence the timing of care termination. For example, developing offspring occupied (diurnally) an increasing area near the nest site over time and may have, at some point, become too scattered to warrant further protection.

Cessation of parental care seemed to closely coincide with brood dispersal. I could not determine if care termination was initiated by the male or brood emigration because both are likely involved. However, two lines of evidence highlight the influence of the parent. First, there were extreme physical and behavioral differences among offspring that were 29 days (~15-16 mm SL) and 54 days old (30-34 mm SL) at dispersal. Younger individuals were much less motile and did not stray as far from the nest during diurnal feeding. Second, two broods (18-21 mm SL) dispersed (or were all consumed) within one day of removal (angling) of the parental male. Friesen (1990) also noted that parental males were absent from broods 24-48 hr prior to dispersal.

AGE-0 SUMMER SURVIVAL

The interval from brood dispersal (May or June) until August is when most age-0 growth occurs and may be a critical time for determination of year-class abundance. There are no previously reported survival estimates for this period, but mortality from swim-up to early September was extremely high in an Ozark stream (~99.9%, Pflieger 1966) and in Lake Katherine, Michigan (85.3-97.0%, Clady 1975). Estimated mortality for this period was also high (>99%) at site 2 in 1994. However, estimated DMR after brood dispersal was much lower than during parental care. Juveniles are probably less susceptible to predation after dispersal because of their larger size, increased motility, and solitary nature.

SOURCES OF BROOD MORTALITY

Specific causes of brood attrition varied in importance with life stage in 1994 (Figure 26). Fungus infection was prevalent during the egg stage, but predation caused most whole-nest failures, contributed to egg losses, and was the most important mortality factor identified after swim-up.

FUNGUS INFECTION

In previous reports, fungus was often mentioned or suspected as a source of smallmouth bass egg mortality, but was seldom investigated (Cleary 1956; Turner and MacCrimmon 1970; Vogele 1981; Lukas 1993). In this study, *Saprolegnia parasitica* infections on clutches were widespread and caused significant egg loss.

Variability in the level of fungal infestation was attributed to factors affecting colonization and growth rates: number of dead eggs, temperature, and egg density. Field and laboratory (Chapter 2) observations confirmed hypotheses that: 1) *S. parasitica* colonizes dead eggs or dead organic material, 2) fungus grows outward from the point of colonization,

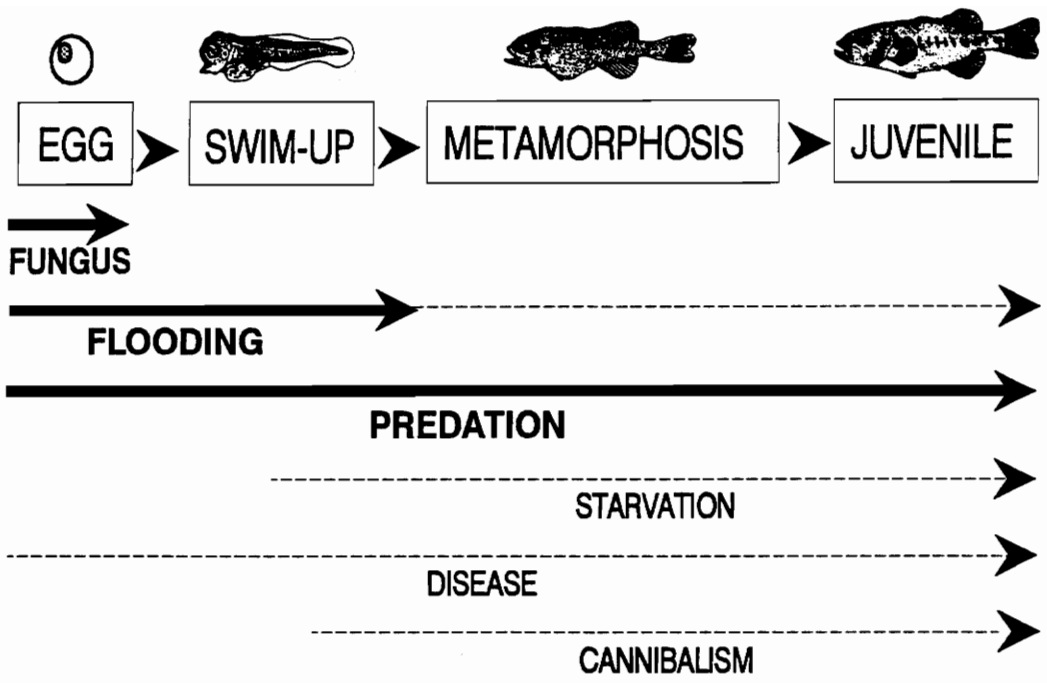

Figure 26. Conceptual model for age-0 smallmouth bass mortality during parental care. Primary sources of mortality (solid lines) and factors that are less important (dashed lines) are indicated for each life stage.

encompassing and killing adjacent live eggs, and 3) growth rates are enhanced by increased egg density (Kanouse 1932; Brown 1956; Hoffman 1969).

Infections were most severe early in the spawning season. Although egg density did not vary appreciably among early and late spawned clutches in 1994, temperature generally increased over time. However, fungus growth is not expected to be highest at cool, early season temperatures (~15-18°C). *S. parasitica* growth rates are positively correlated with temperature up to 30°C under sterile conditions (Olah and Farkas 1978, Chapter 2). This apparent contradiction and low temperature threshold for fungus in the field (~18°C) may be

related to co-occurring bacteria, which outcompete fungi for nutrients at warmer (i.e. >18°C) temperatures (Olah and Farkas 1978). Specific mechanisms in the egg-fungus interaction, including effects of egg density, temperature, and competitive bacteria, are discussed further in Chapter 2.

Since fungus growth rates and the proportion of eggs infected increased with egg density, mortality rates were expected to be negatively density-dependent during period 1. Evidence of this relationship ($p=0.08$) was stronger for period 1 than any later life stages. Significant density-dependence may have been masked by other mortality factors or affected by differential survival rates after the eggs hatched (the latter half of period 1). Several lines of evidence (described below) suggest that small eels (<100 mm) consumed eggs. If egg predation by eels was density-independent or depensatory, any density effects would likely have been overshadowed. When young hatched and became yolk-sac larvae (wrigglers), they generally migrated into the substrata and may have become even more accessible to eels (which frequently burrow into the streambed and may escape detection by the parental male).

Variable mortality rates in nests with little fungus infection (<25% of clutch area) could reflect unquantified effects of eel predation and losses after eggs hatched (prior to swim-up). Mortality was also variable, but consistently higher, for clutches with more fungus (>25% of area). Live eggs may have been more susceptible to hyphal penetration in these nests as abundant mycelia overwhelmed membrane defenses.

PREDATION

Predation was an important source of mortality for all life stages during parental care. Whole-nest failures (biotic) prior to swim-up were likely caused by diurnal predators, but high rates of attrition were mainly attributed to nocturnal eel predation. The magnitude of predation losses

at certain developmental stages was affected by aspects of parental defense, as well as diel and ontogenetic changes in offspring behavior.

Centrarchids, cyprinids, and other fish that feed diurnally are typically implicated as primary brood predators (Vogele 1981; Reynolds and O'Bara 1991), despite the fact that predation is not normally observed under natural conditions unless the parent is disturbed or removed (Neves 1975; Pflieger 1975). When I removed one nesting male, three redbreast sunfish began consuming eggs within several minutes. Pflieger (1975) reported similar results for unprotected nests in a Missouri stream. However, I rarely saw potential predators in the nest vicinity during daily snorkel visits. Video of nest sites and male departure frequencies verified that diurnal predation of eggs and swim-up larvae by pelagic fishes was rare. Elevated male vigilance (increased departure frequency) during crepuscular periods at both life stages may reflect an increase in offspring vulnerability at night (Friesen 1990) and nocturnal predator activity.

American eels were common predators of larval and juvenile bass, and contributed to brood losses prior to swim-up. Diet analyses revealed that eels fed primarily on benthic invertebrates and fish, which is consistent with results of other eel feeding studies (Ogden 1970; Lookabaugh and Angermeier 1992; Denoncourt and Stauffer 1993). Eels may also be important fish egg predators. Although eel collection (for diet analysis) occurred when most broods were past the swim-up stage, four eels (104-275 mm) collected near chub (Nocomis spp.) and fallfish (*Semotilus corporalis*) spawning mounds contained cyprinid eggs. Eels also preyed on greater redhorse (*Moxostoma valenciennesi*) eggs in the upper St. Lawrence River (Jenkins and Jenkins 1980).

Eel densities are high in the North Anna River. In the York River drainage (containing the N. Anna River at the

piedmont-coastal plain junction), eels constituted 12.6% (by number) of all fish collected (Paul Angermeier and Roy Smogor, Virginia Tech, unpublished data). Although eel abundance decreased with distance from the ocean as described by Smogor et al. (1995), eel densities in the piedmont region of the York River basin were higher than in any other Virginia drainage.

Eels may feed at any time, but seem most active at night (Sorenson et al. 1986, pers. observ.). Eels were frequently observed (nocturnally) consuming or pursuing larval and juvenile offspring. Vulnerability to eel predation apparently increased after dark as parental male defense declined and free-swimming young congregated on the streambed. Nocturnal behavior of parental males was similar to descriptions of nonbreeding bass (Emery 1973; Hinch and Collins 1991); most males lay idle on the bottom near their brood. In contrast, Hinch and Collins (1991) observed high parental activity (rotating over nest) at night prior to swim-up. However, "activity" does not necessarily indicate protection because parental males' ability to detect predators seemed to decrease at night (based on direct and video observations). Male protection seemed especially difficult because eels often burrowed into the streambed and ambushed young from beneath rocks.

Offspring camouflage seemed to be the best (though limited) nocturnal predator defense. After dark, individuals were tightly aggregated, motionless, and showed a distinct change (paling) in pigmentation that produced a cryptic appearance. When larvae were captured at night (brood counts 3 and 4) and exposed to light, they regained their usual (diurnal) black pigmentation within several minutes.

The importance of eel predation during larval and juvenile stages was confirmed by diet analyses. Although stomach contents consisted primarily of invertebrates over all

sample periods and locations, age-0 bass predominated in eels collected directly from active nest sites at night. Rapid evacuation rates extend the implications of these data; larval bass (11-22 mm) fed to captive eels (157-275 mm) at 23°C were digested within 3-4 hrs (Appendix 6). Therefore, the impact of eels on age-0 bass would likely be underestimated unless eels were collected frequently at night from smallmouth bass nesting habitat.

Using available data, it can be demonstrated that eel predation accounts for a large proportion of brood losses at certain life stages (Table 15). For example, during period 3 (metamorphosis to juvenile stage), an average of 50 individuals per brood were lost to all mortality sources each day (based on mean initial numbers per brood for this period). A single eel feeding on one brood each night could account for 16-21 deaths or about 32-42% of these losses. This is likely a conservative estimate of total eel predation since multiple eels were often seen feeding on a brood and individual eels contained up to 19 bass.

Table 15. Computation and comparison of mean age-0 brood mortality and estimated losses attributable to eel predation during period 3 in the North Anna River.

OBSERVED LOSSES:			
552 (mean #/brood, count 3)	X	0.09 (period 3 DMR)	= 50 bass/brood/d
ESTIMATED CONSUMPTION BY EELS[a]:			
12 hrs X 0.25 hr^{-1} X 5.3 bass/stomach	=	16 bass eaten/eel/d	
12 hrs X 0.33 hr^{-1} X 5.3 bass/stomach	=	21 bass eaten/eel/d	

[a] Daily consumption (C) calculated using the Bajkov method: C = 12 X E X S, where 12 = estimated number of hr per day when bass were most vulnerable to eels, E = instantaneous evacuation rate (3-4 hrs) estimated from data in Appendix 6, and S = mean number of bass per stomach for eels collected directly from nest sites (primarily during period 3).

Additional evidence suggests that eels were an important source of mortality prior to swim-up. Similar DMR and clutch area decline rates, eel presence in nests, and apparent lack of other nest predators implicate eels as egg predators. Eels (<100 mm) were seen consuming young bass (eggs or embryos) in nests on three occasions. The period 1 DMR for these three broods was much higher than predicted by the fungus regression model shown in Figure 22. Daily rates of clutch area decline (mean 6.3%, days 1-5, n=13) were similar to period 1 DMR (mean 6.7%). Assuming decreases in area represented commensurate reduction in egg abundance, decline rates may be an alternative measure of egg mortality. Fungus infection probably acted with predation in causing clutch area reduction, since egg penetration by hyphae (vegetative filaments) leads to removal of nutrients and may cause eggs to shrivel or burst (pers. observ.).

EFFECTS OF ANGLING PARENTAL MALES

Whole-nest failures attributed to predation are often caused by parental male abandonment (Webster 1948; Vogele 1981). Male removal by angling could have the same effect, but angler visits to my study reach were not concurrent with most nest failures. Angling became more frequent later in the season, when most broods had reached swim-up. Although several parental males were caught and released, there were not appreciably greater mortality rates for young (periods 1-3) from nests having angled (hook scarred) males (n=10, mean DMR = 10.0%) relative to unangled males (n=10, mean DMR = 9.5%). Similarly, Neves (1975) suggested that angling of males may have affected nest mortality, but most males were able to return before significant predation occurred. Lukas (1993) also proposed that brood mortality is limited as long as males are released quickly and unharmed.

AGE-0 MORTALITY AND DETERMINATION OF YEAR-CLASS STRENGTH

In studying age-0 mortality and recruitment dynamics, two central questions are: When is year-class strength set? and What determines it? In this study, I attempted to identify patterns in the timing, magnitude, and causes of mortality during early development that influenced year-class formation.

Previous investigators have linked juvenile smallmouth bass densities with habitat quality and offspring abundance at various life stages. Hoff (1991) found a strong correlation between quality of nesting habitat (cover) and number of fall fingerlings. Other authors speculated that year-class strength was determined: before larval metamorphosis (for fish in general, Toetz 1966), by rates of mortality prior to swim-up (Eipper 1975), or after swim-up (Pflieger 1966). Data collected in this study suggest that relative fall abundances were determined after swim-up, and possibly after brood dispersal.

Variation in yearly age-0 abundance may initially stem from different numbers of successful nests. Because of abiotic (e.g., discharge) and biotic (e.g., spawning adult abundance or size distribution, predator density) instability in natural environments, variable reproductive success is the norm. However, reproductive success was not a good indicator of subsequent summer densities; relative abundance of juveniles in August was not related to the number of successful nests or the total number of swim-up larvae produced at site 1 (Figure 27). For instance, August juvenile abundances in 1992 and 1994 were similar despite a six-fold difference in the number of swim-up larvae produced. Age-0 fall abundance was also independent of nest densities in Iowa streams (Cleary 1956) and unrelated to fry production in Missouri (Pflieger 1975).

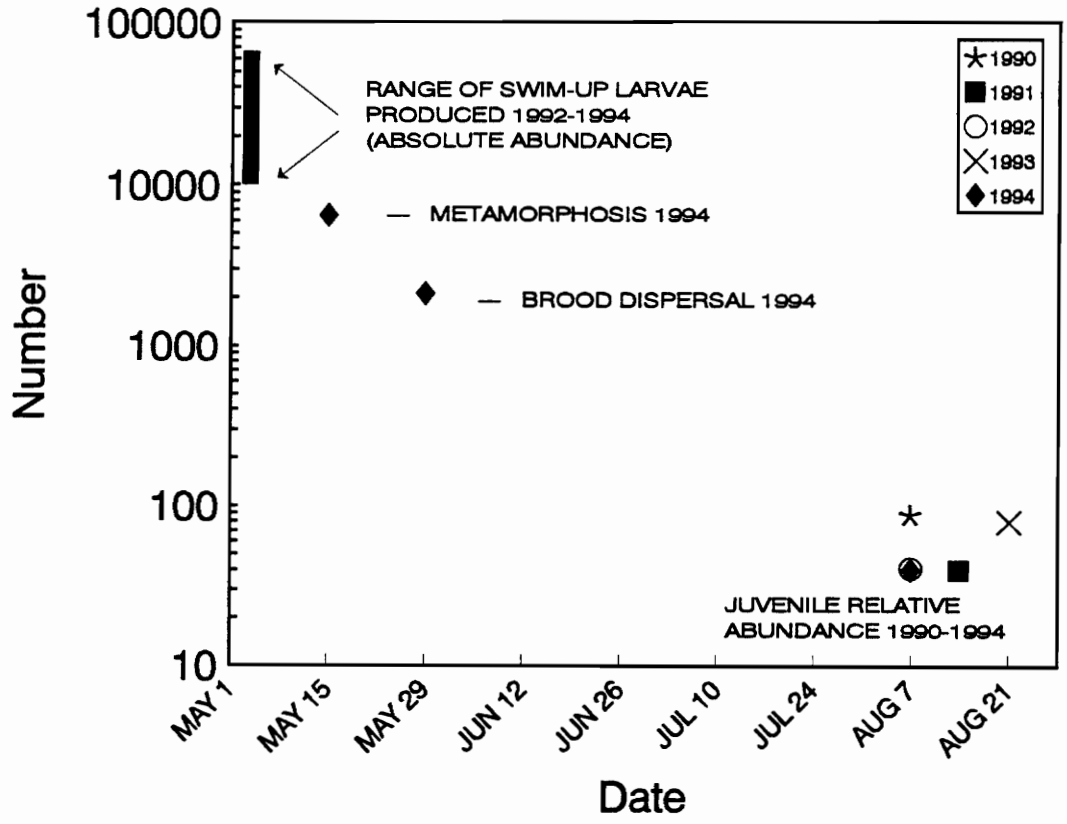

Figure 27. Annual absolute (swim-up to brood dispersal) and relative (August juvenile) offspring abundance at site 1. Relative abundances for 1990-1991 are from Sabo 1993.

Pflieger (1975) proposed that other factors, such as fluctuations in age-0 carrying capacity, are more critical determinants of year-class strength than numbers of fry. This does not imply that variation in reproductive success does not influence recruitment. Low nest success may limit the number of potential recruits. High success may be one basis for high juvenile production and indicate conditions are favorable for continued survival.

In the North Anna River (1994), juvenile abundance at dispersal depended primarily on the number of young produced at earlier (i.e, egg or swim-up) life stages. This information and findings discussed previously: 1) no

correlation of the number of successful nests or total swim-up larvae production with August juvenile abundance in 1992-94, 2) high variability in total swim-up larvae production, but much lower variability in relative August abundance 1990-1994 (1990-1991 data from Sabo 1993), and 3) few whole-nest failures after swim-up, suggest that post-larval mortality was an important determinant of cohort abundance and emphasize the importance of processes affecting summer juvenile survival.

Offspring emigration was assumed minimal and not addressed in this study, but may have contributed to decreased abundance at certain life stages. Young did not appear to disperse from broods prematurely and survive (based on daily snorkel surveys) - any young that strayed from parental protection too early were likely eaten. However, unequal juvenile emigration and immigration at study sites could have affected summer abundance and mortality estimates. The extent of movements among river reaches could not be determined and exemplifies the danger of extrapolating results of this study. Data collected on small spatio-temporal scales may not reflect processes occurring throughout the river or annual variability in the timing and magnitude of mortality. It is also unclear whether new findings (e.g., density-independent brood attrition, widespread fungus infection, and eel predation) are unique to the North Anna River or simply have not been recognized in other systems. Similar investigations over longer periods of time and in other locations should provide further insight regarding the applicability of this information.

IMPLICATIONS FOR MANAGEMENT AND FUTURE RESEARCH

Information gained from this study is useful for identifying specific periods of age-0 vulnerability and small-

scale processes that may contribute to differences in cohort abundance. Because of the socio-economic importance of smallmouth bass, this information has practical applications in resource management. Knowledge of processes underlying age-0 mortality can aid fisheries managers in interpreting year-class fluctuations and in assessing impacts of fish harvest. For example, results of this study indicate that mate acquisition, clutch size, and nest success are important determinants of final brood production. Since male size is positively related to the number of offspring per brood and, possibly, mating and nest success (Winemiller and Taylor 1982; Reynolds and O'Bara 1991; Weigmann et al. 1992), overharvest of large adults could reduce total juvenile production and have significant impacts in situations where population size is limited by low recruitment.

Current water use practices necessitate clarification of the relationship between habitat modification (including flow and channel alterations) and reproductive success. The Federal Energy Regulatory Commission (FERC) mandates that utilities applying for hydropower licenses provide protection for fishery resources. This study provides information on effects of streamflow and other factors influencing recruitment at specific life stages and could contribute to recommendations for appropriate flow regimes. Procedures used to develop these recommendations, such as the Instream Flow Incremental Methodology (IFIM), may be improved with greater understanding and consideration of biological mechanisms. For instance, many whole-nest failures were attributed to floods and predation caused by parental male abandonment. Nest success may be improved by preventing rapid temperature fluctuations that lead to male abandonment (Latta 1963; Brown 1956) and flow increases that cause downstream displacement of offspring (Harvey 1987; Simonson and Swenson 1990) during predictable spawning periods. Furthermore, the Electric Power

Research Institute (EPRI) key species program and Oak Ridge National Laboratory have developed an individual-based model (IBM) for stream-dwelling smallmouth bass populations (Jager et al. 1993). Data from this and other studies on the North Anna River will aid in the development and validation of several model components.

Examination of processes affecting summer juvenile survival is the next logical step in investigating smallmouth bass recruitment. If compensatory regulation occurs, it is probably most important after brood dispersal and at larger spatial scales. The extent of growth-mediated survival and other mechanisms during this period needs to be clarified. Interpretation of findings in this and other field studies would also be improved with information on movements of age-0 bass. Fluctuations in abundance may be exaggerated or diminished by unequal emigration and immigration at any spatio-temporal scale examined.

CONCLUSIONS

1) Age-0 survival during parental care was much lower than expected for the smallmouth bass life-history pattern, with highest rates of mortality occurring after swim-up.

2) No compensatory mortality was detected at the brood scale; survival rates were density-independent throughout parental care.

3) Relative mortality rates were inconsistent through time among individual broods, indicating persistent factors such as nest site habitat and male defense ability did not control brood survival.

4) In addition to environmental extremes, biotic sources of mortality were important causes of nest failure and brood attrition in successful nests. Decreases in abundance were primarily attributed to two mortality factors that have previously been downplayed: fungus infection of eggs and eel predation.

5) Although measured male characteristics were not strongly related to offspring survival, mate acquisition, clutch size, and nest success were critical determinants of juvenile numbers at dispersal for individual males.

6) No apparent relationship of brood success or larval production with August relative abundance indicates that year-class strength was determined after larval stages and emphasizes the significance of processes influencing summer juvenile survival.

CHAPTER 2

EFFECTS OF TEMPERATURE, FISH EGG DENSITY, AND BACTERIA ON *SAPROLEGNIA PARASITICA* GROWTH

INTRODUCTION

Mortality in natural and hatchery fish populations is often highest during the egg stage. Aquatic fungus infestation is one of the most common and widespread causes of these losses (Kanouse 1932; Hoffman 1969) and has led to extensive efforts to develop treatments in fish ponds and hatcheries. However, few quantitative studies have investigated the ecology and host-parasite interaction of fungus and fish eggs in natural environments (but see Scott and O'Bier 1962; Snieszko and Axelrod 1980).

Aquatic fungi are a ubiquitous component of most freshwater environments (Hoffman 1969; Snieszko and Axelrod 1980). Water molds (order Saprolegniales) are the most common fungal pathogens of fish and fish eggs. At least six genera have been identified, but *Saprolegnia spp.* made up more than 60% of isolates on fish and fish eggs from across the United States (Scott and O'Bier 1962). *S. parasitica* is the species most frequently associated with fish eggs (Kanouse 1932).

S. parasitica is a saprophyte of dead fish eggs and apparently a facultative parasite of live eggs (Snieszko and Axelrod 1980), but the severity of effects on live eggs remains unclear. For example, smallmouth bass clutches that were more than 70% covered with mycelia (masses of vegetative filaments) produced an average of 2625 swim-up larvae in the

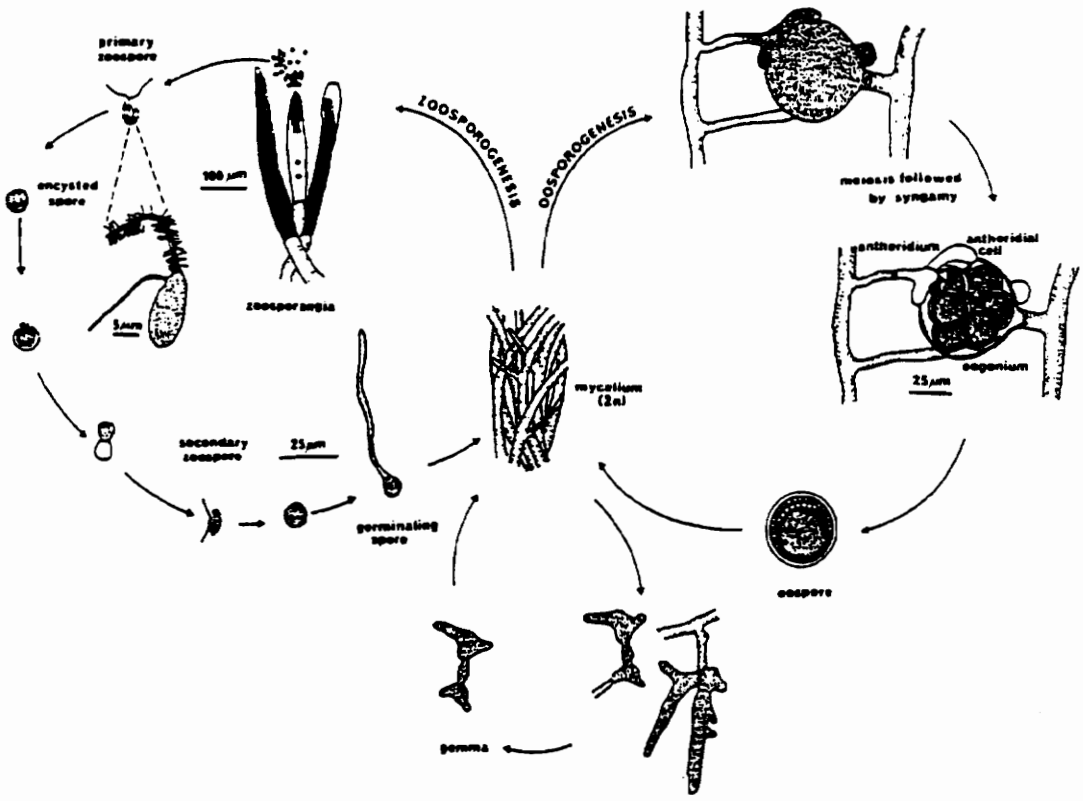

Figure 28. Saprolegnia life cycle (from Snieszko and Axelrod 1980).

North Anna River (Chapter 1). *S. parasitica* has an extremely wide range of adaptability with respect to vegetative and asexual (zoosporangial) development, but exhibits very limited sexual (oogonial) reproduction under natural and culture conditions (Kanouse 1932; see Figure 28). When given a concentrated supply of nutrients (nitrogenous organic material) such as fish eggs, asexual growth is rapid and reproduction may be exclusively asexual.

Initial fungal colonization of eggs occurs via flagellated zoospores (upper left portion of Figure 28) in most cases (Kanouse 1932; Snieszko and Axelrod 1980). Although seasonal trends may vary among years, zoospores are

often most abundant in spring and fall, reaching densities greater than 1000/L (Willoughby 1962; Olah and Farkas 1978). *Saprolegnia* infections begin on dead eggs or other organic material (Kanouse 1932; Davis 1953; Hoffman 1969). The fungus is apparently unable to colonize live eggs, but does spread outward from the initial infection point via vegetative hyphal (nonseptate filaments) growth, encompassing adjacent live eggs (Davis 1953; Hoffman 1969).

The colonization and growth rates of *S. parasitica* on fish eggs have been associated with several environmental variables. Growth rates apparently increase with temperature (<30°C) under sterile conditions (Olah and Farkas 1978). However, field observations indicate that growth on eggs is greatest at lower temperatures (15-18 °C, Chapter 1), and outbreaks of saprolegniosis in the wild are most common in spring and fall (Hoffman 1969; Olah and Farkas 1978). Olah and Farkas (1978) proposed that fungal infection in nature is maximized far below its optimal temperature for growth because bacteria outcompete the fungi for organic nutrients at warmer temperatures. Colonization and growth rates also appear to be positively associated with fish egg density (Brown 1956; Hoffman 1969), but the relationship has not been clearly established.

Field studies on the North Anna River, Virginia (Chapter 1) indicate that *Saprolegnia parasitica* is a major source egg mortality for riverine smallmouth bass (*Micropterus dolomieu*). Fungal infection on eggs was positively related to mortality from egg fertilization to larval swim-up. Laboratory experiments were designed to: 1) verify that fungus initially colonizes dead eggs, then encompasses and kills adjacent live eggs and 2) test hypotheses that *S. parasitica* growth rate is enhanced by increases in egg density and water temperature up to some threshold and is inhibited by bacterial contamination.

METHODS

CULTURE OF *SAPROLEGNIA PARASITICA*

A *S. parasitica* isolate was obtained from the American Type Culture Collection (ATCC 22284) and transferred to several types of media (see Appendix 7). Cultures were propagated on: 1) cornmeal agar, 2) Emerson agar, 3) soluble yeast-phosphate-glucose (Ypg) liquid medium (Emerson), and 4) hemp seed. Antibiotics (penicillium G and streptomycin sulfate) were added to selected media to alleviate bacterial contamination. Rapidly growing cultures that were free of contaminants were most easily produced with antibiotic liquid Ypg medium.

S. parasitica grown on all types of media was inspected for reproductive structures (zoosporangia and oogonia). Zoosporangia were abundant on all hemp seed cultures examined, but only vegetative hyphae were observed on all other media types (with occasional resting spores). Bubble (aerated) cultures of *S. parasitica* in antibiotic liquid YPG medium produced extremely rapid growth of mycelia, but did not stimulate production of reproductive structures.

LABORATORY EXPERIMENTS

Experiment 1

In experiment 1, *S. parasitica* (ATCC 22284) growth rates were measured at four different temperatures. I placed 100 ml of sterile Ypg liquid medium into 300 ml Erlenmeyer flasks. Each flask was inoculated with a 1x1 cm cornmeal agar plug cut from the perimeter of an actively growing culture of the fungus. I set incubators at four temperatures (treatments): 5, 15, 20, 25 °C and placed two covered flasks (replicates) at each temperature. Incubators were checked daily and never varied more than 1°C from the assigned temperature. After 1

week, fungus was collected on filter paper using vacuum filtration, placed in a preweighed foil dish, oven dried at 62 °C for 24 hr, and reweighed.

Three trials were conducted in this experiment. I employed the above protocol in each trial, except for the following modifications (summarized in Table 16): 1) the duration of trial 1 ("sterile media") was 7 d and trials 2 and 3 lasted 8 d, 2) treatment temperatures varied among trials, 3) in trial 2 ("antibiotic"), antibiotics (penicillium G and streptomycin sulphate, 150 mg/L each) were added to growth media to alleviate any bacterial contamination from fungus cultures, and 4) 100 ml of unsterilized water taken from the New River, Virginia was added to each flask at the time of fungus inoculation in trial 3 ("river water").

Table 16. Summary of protocols for experiment 1.

Trial	Sterile Media (1)	Antibiotic (2)	River Water (3)
Treatments (°C)	5,15,20,25	5,15,20,25,32,35	5,14,17,20,23
Duration (days)	7	8	8
Media	Ypg	antibiotic Ypg	Ypg
Unsterilized River Water Added?	no	no	yes

Differences in fungus growth among trials may have been related to level of bacterial contamination. Although bacterial abundance was not quantified, samples from each temperature in all trials were visually examined for bacteria under oil immersion using phase contrast microscopy.

Experiment 2

I investigated the colonization and growth rates of *S. parasitica* on smallmouth bass egg substrate at different temperatures and egg densities. Smallmouth bass eggs were obtained by capturing and spawning wild fish. Six adult females (330-520 mm TL) and 3 males (345-440 mm TL) were collected from the New River, Virginia using a boat electrofishing unit. Fish were immediately transported to the Virginia Tech Aquaculture Center in a 150-L, aerated holding tank with treated river water (0.02 g MS-222 anesthetic/L and 0.5% salt). Fish were held in aerated 2000-L outdoor tanks (salinity 5-10 ppt, temperature 14-17°C, dissolved oxygen 9.2-9.8 mg/L). Approximately 12 hr after capture, both sexes were anesthetized with MS-222 and injected with human chorionic gonadotropin (HCG) at 4 cc/kg body weight (Dick Luebke, Texas Department of Parks and Wildlife, pers. comm.).

We hand-stripped each female smallmouth bass as eggs became fully mature (3-26 hr after injection). The developmental progress of eggs was monitored by extracting eggs with a glass catheter and examining them under a dissecting microscope (Rottmann et al. 1991). Mature eggs were spawned into a bowl containing approximately 100 ml of sterile water while sperm was stripped from males and collected with a small pipette. We fertilized the eggs by adding the sperm and an additional 200 ml of water while agitating the mixture. The fertilized eggs were immediately transported to the laboratory.

In this experiment, two treatments were employed: 1) egg density (high = 10 ml or approximately 550 eggs, low = 6 ml or approximately 330 eggs) and 2) temperature (14°C, 17.5°C, and 21°C). Egg densities and temperatures were based on values observed in the North Anna River. Each treatment combination was replicated twice.

In preparation of each sample, I submersed eggs in an

antibiotic solution (streptomycin sulphate and penicillium "G", 150 mg/l each) for 45 sec, rinsed with sterile water, and placed them in a glass dish (500 ml) containing 300 ml of sterile water (pre-adjusted to the appropriate temperature). A hemp seed from an actively growing *S. parasitica* culture was introduced into each dish. The samples were then placed in pre-set incubators and fitted with sterile aeration units (with air filter).

Fungus colonization and growth were inspected every 12 hr after experiment initiation up to hr 60. I recorded the number of dead (opaque) eggs and area of the dish bottom infected with fungus for each sample. The area with fungus was estimated by superimposing a grid (1x1 cm cells) on the bottom of the dish and counting the number of cells that contained fungus. I replaced 250 ml of 300 ml in each dish with fresh sterile water 24 hr after experiment initiation. This was done to help reduce contaminants and simulate water replacement in rivers.

Data Analysis

Fungus weights recorded in experiment 1 were converted to fungus growth rates (g/day). I tested for differences in growth rate among temperature treatments in each trial using one-way analysis-of-variance (ANOVA) procedures. A Kruskal-Wallis (KW) test was employed in the "river water" trial because ANOVA model assumptions were violated . In experiment 2, a two-way ANOVA design was used to test for egg density and temperature effects on fungus growth. Pairwise multiple comparison procedures (Student-Newman-Keuls Method) were then employed to identify which treatment levels were significantly different.

RESULTS

EXPERIMENT 1

The effect of temperature on *Saprolegnia parasitica* growth rate varied among trials (Figure 29). Growth rate increased with each temperature treatment (15-25°C) on sterile media (temperature effect significant, p=0.004, one-way ANOVA). Fungus growth rates were higher on sterile antibiotic media and also increased with temperature (5-25°C), but decreased at 32°C and 35°C (temperature effect significant, p=0.003, one-way ANOVA). Fungus growth was lower over a similar temperature range (5-23°C) and was inversely related to temperature when unsterilized river water was added to samples, but temperature effects were not significant (p=0.15, KW test).

Bacteria were intentionally introduced in the "river water" trial, but contamination was also evident in "sterile media" by day 5. Only the trial with antibiotic media was uncontaminated and represents fungus growth under sterile culture conditions. Contaminated samples were easily identified as they became conspicuously cloudy. Bacteria were visible in microscopic examination of (initially) sterile media samples at each temperature. Bacteria were extremely abundant and often physically attached to fungal hyphae in all samples where unsterilized river water had been added.

EXPERIMENT 2

Results of experiment 2 indicate that *S. parasitica* growth rate increased with temperatures and egg density (Figure 30). Although the duration of the experiment was 60 hr, fungus growth in treatments with high egg density and temperature (17.5°C, 21°C) was physically limited by dish space after 36 hr. Therefore, temperature and density effects were

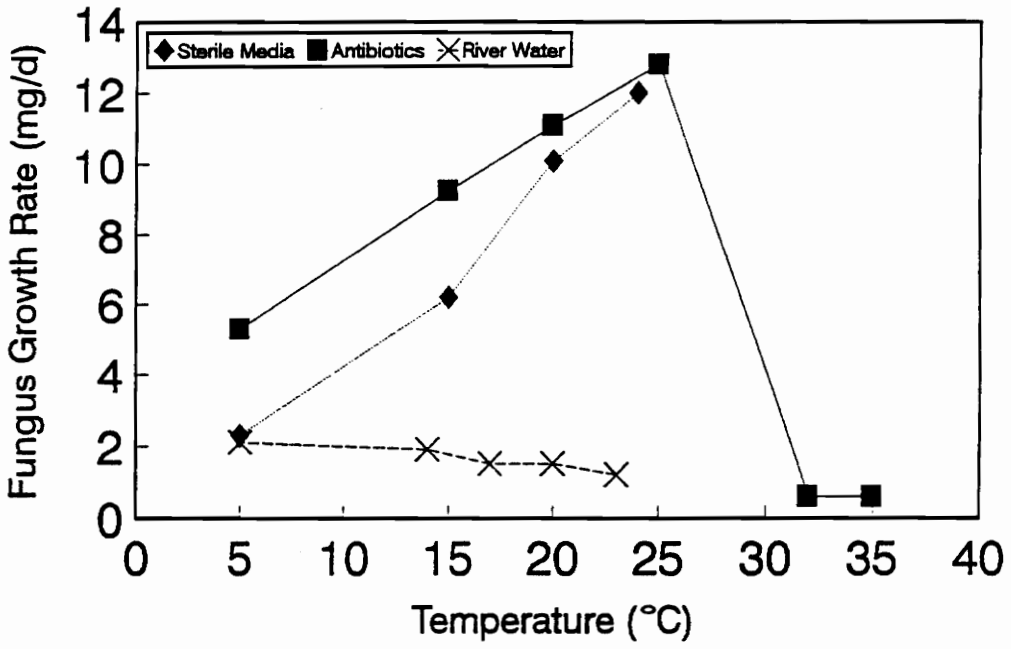

Figure 29. Fungus growth rates in experiment 1 trials. Samples that contained antibiotics were not contaminated with bacteria, but trials with sterile media and river water were mildly and extremely contaminated, respectively.

compared 36 hr after experiment initiation (Table 17). At 36 hr, there were strong temperature ($p<0.0001$) and density ($p<0.0001$) effects (two-way ANOVA), with a significant temperature-density interaction ($p=0.01$). All treatment levels were significantly different ($p<0.05$, Student-Newman-Keuls multiple comparisons method).

Table 17. Analysis of variance (ANOVA) table for effects of temperature and smallmouth bass egg density on fungus growth rate in experiment 2.

SOURCE OF VARIANCE	DF	SS	MS	F	P
Temperature	2	7170.7	3585.3	439.0	<0.0001
Egg Density	1	736.3	736.3	90.2	<0.0001
Temp x Egg Density	2	160.7	80.3	9.8	0.01
Residual	6	49.0	8.2		
Total	11	8116.7	737.9		

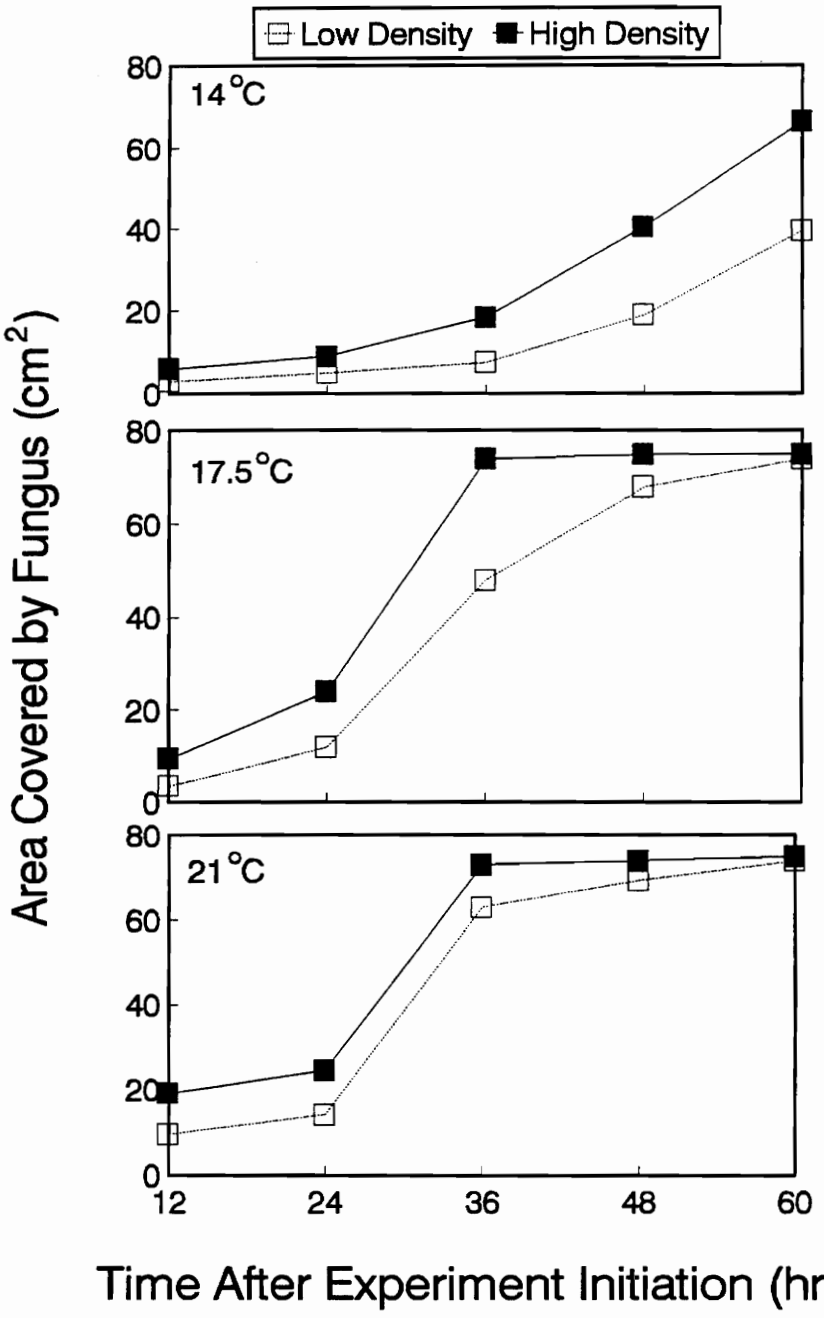

Figure 30. The area covered by fungus in experiment 2 samples over time. Growth at high and low egg densities is shown for each temperature. Each point represents the average of two replicates.

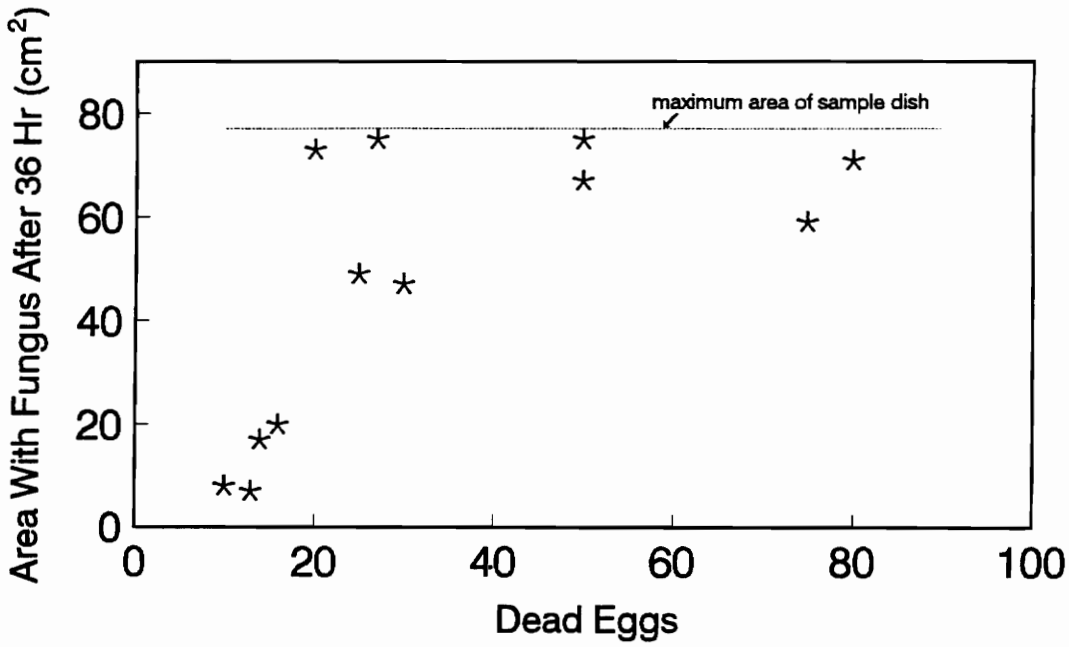

Figure 31. Relationship between the initial number of dead eggs and the area covered by fungus after 36 hours in experiment 2 samples.

S. parasitica grew outward from the hemp seed inoculum and other points of colonization in experiment samples. Most colonies appeared on dead eggs and the total area covered by fungus after 36 hr was a nonlinear function of the initial number (counted 12 hr after initiation) of dead eggs in each sample (Figure 31). The effect of dead eggs on fungus growth was positive and roughly linear at low numbers of dead eggs (<30), but fungus growth was not as sensitive to higher numbers. The small surface area of sample dishes likely limited fungus growth when dead eggs were abundant and explains why this relationship appears asymptotic. The number of dead eggs per sample did not vary with egg density, but was positively related to temperature ($r=0.89$, $p<0.0001$), indicating that the significant temperature effect on fungus growth rate may have been due, in part, to higher initial egg mortality at higher temperatures.

DISCUSSION

Field studies on the North Anna River, Virginia, indicated that smallmouth bass egg infection by *S. parasitica* is influenced by temperature, egg density, and other ambient environmental factors that affect fungus colonization and growth rates (Chapter 1). Results of laboratory experiments corroborated these and other empirical data and have important implications for fish culture.

In experiment 2, the inoculum (hemp seed) was the primary origin of hyphal growth. However, colonies also developed on peripheral dead eggs within 12 hr. Zoospores were likely the source of infection in these instances (Kanouse 1932) because outlying dead eggs were not in physical contact with inoculum, zoosporangia were abundant on hemp cultures, and oogonia were never observed in cultures. Membrane defenses (discussed below) of viable eggs apparently inhibited zoospore colonization (Kanouse 1932) and prevented *S. parasitica* from initially becoming established.

Dead eggs also quickly (<2 days after spawn) developed a puffy, white mass of hyphae in the wild. Other organic material, such as detritus and feces from the parental male, could have acted as additional sources of infection. Zoospores were likely the main propagule, but eggs may have also been infected by resting (encysted) spores or gemma in the streambed. High densities of gemma and resting spores presumably persist in the substrata at certain nest sites (though unverified) since eggs are often spawned repeatedly in the same nest depression within the same year and among years.

S. parasitica grew outward from initial points of infection and encompassed live eggs, often completely enveloping them in masses of mycelia (experiment 2 and field observations). Previous investigations indicate that fungus

causes egg mortality (Kanouse 1932; Snieszko and Axelrod 1980), but clutches that are completely covered with fungus often produce larvae in the wild (Vogele 1981, pers. observ.). When I examined several infected, but successful (to swim-up) nests, I found clumps of dead eggs entangled with mycelia. Since masses of dead or unfertilized eggs were never observed in clutches prior to infection, it is unlikely that all of these eggs were initially inviable.

Egg mortality may result from penetration or smothering by hyphae (Kanouse 1932). Shigeharu and Teshima (1991) found that the fertilization envelope of eggs from two fish species, *Plecoglossus altivelis* and *Tribolodon hakunensis*, acts as a physical barrier to fungus and contains fungicidal enzymes that prevent colonization. These defenses apparently do not stop penetration by profuse hyphae, which may infiltrate the membrane with direct pressure (Kanouse 1932) and digestive enzymes (Rand and Munden 1992). In a separate laboratory experiment, microscopic examination revealed that *Saprolegnia spp.* hyphae penetrated hybrid bass (*Morone saxatilis x M. chrysops*) eggs and may have caused mortality (W. L. Knotek, unpublished data).

Egg density and temperature are important factors influencing *S. parasitica* growth rate. Increased growth with egg density likely reflects augmented substrate (nutrients) for expanding fungus colonies. An alternative hypothesis is that denser egg masses contain more dead eggs (colonization points). However, there was no correlation between egg density and the number of dead eggs in experiment 2. Consequences of faster growth may be greater hyphal mass and egg mortality. In an experiment where smallmouth bass eggs were placed in nylon sacs to simulate clumping, fungus encompassed egg masses and caused complete mortality, while eggs scattered on nylon trays experienced lower mortality (Brown 1956).

Effects of temperature on fungus growth rate are more complex. Although growth increases with temperature under sterile conditions (5-25°C, Olah and Farkas; experiments 1 and 2), maximum growth on eggs occurred at much lower temperatures in the field and appeared to reach a threshold at 17-18°C (Figure 32). As indicated by experiment 1, inhibition of fungal growth may be attributed, in part, to competitive bacteria.

Because *saprolegnian* fungi and bacteria are common in aquatic environments and have similar requisites, they compete to assimilate accessible organic materials. At higher temperatures, faster growing bacteria tend to proliferate, but *Saprolegnia spp.* can grow well at cooler temperatures (as low as 5°C, Olah and Farkas 1978). Bacteria also inhibit fungal sporulation (Willoughby 1962). This may explain why the most severe fungal infections in nature often do not coincide with optimal temperatures for growth (e.g., spring and fall, Olah and Farkas 1978).

In the North Anna River, the heaviest infestation of smallmouth bass eggs occurred early in the spawning season (Chapter 1). Although this was the coolest portion of the season (15-18°C), bacteria severely suppressed *S. parasitica* growth at these temperatures when river water was added to sterile media in experiment 1. The replacement of water in lotic systems, which may diminish proliferating bacteria and impede rapid build-up on eggs, probably plays a major role in the fungus-bacteria interaction. Fluctuations in zoospore abundance could have also affected clutch infection rates (Willoughby 1962), but it seems unlikely that variation in propagule density was dramatic enough to account for the rapid (<2 weeks) changes in levels of infection among nests.

Temperature may have indirectly influenced *S. parasitica* growth rates in experiment 2 through effects on egg viability. Initial egg mortality was greater in warmer treatments and

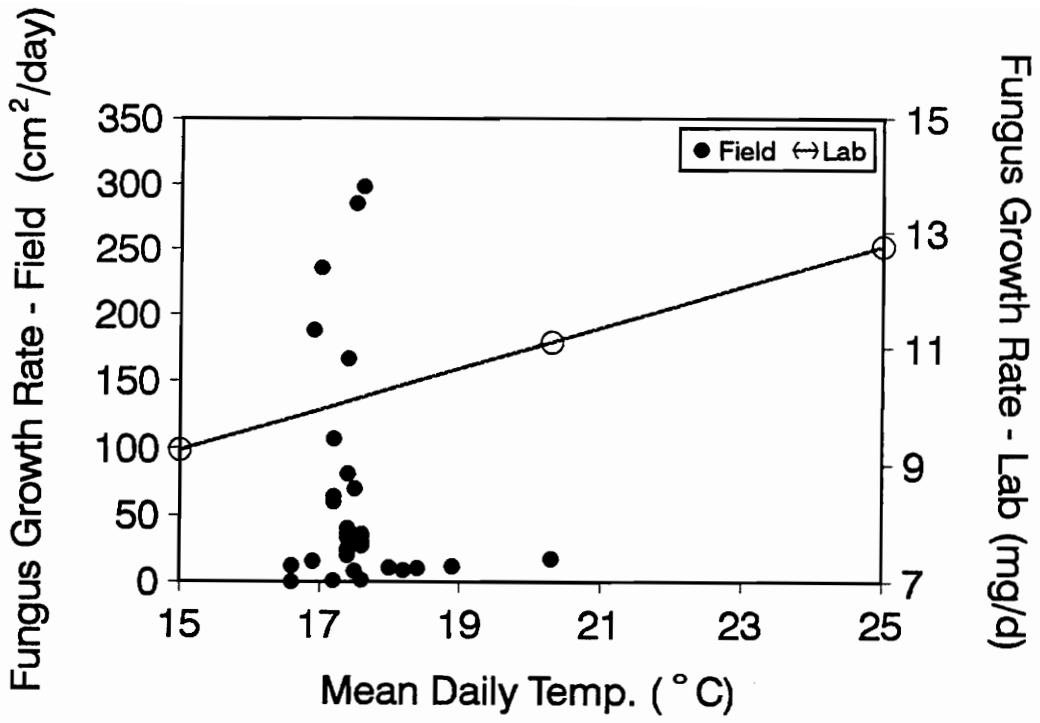

Figure 32. Comparison of fungus growth rates at different temperatures in laboratory experiment 1 (on antibiotic medium) and in the North Anna River (on smallmouth bass eggs).

probably contributed to faster colonization and growth. These effects were not apparent in field studies; the number of dead eggs per clutch was unrelated to mean water temperature.

Although this study was focused on testing hypotheses generated from field investigations, results have important implications for fish culture. Fungal infestation of eggs remains a common problem in hatcheries, despite attempts to control infections (e.g., malachite green and other fungicides). Egg survival may be enhanced by quickly removing dead eggs or other material that could be colonized by fungus. Steps should be taken to ensure high fertilization success to help reduce the number of inviable eggs. Egg density

(clumping) should also be minimized to inhibit the spread of infections. Since appropriate conditions for egg incubation vary greatly among fish species, temperature requirements, development rate, hatching success, and effects of fungus and bacteria need to be considered collectively in determining optimum water temperatures.

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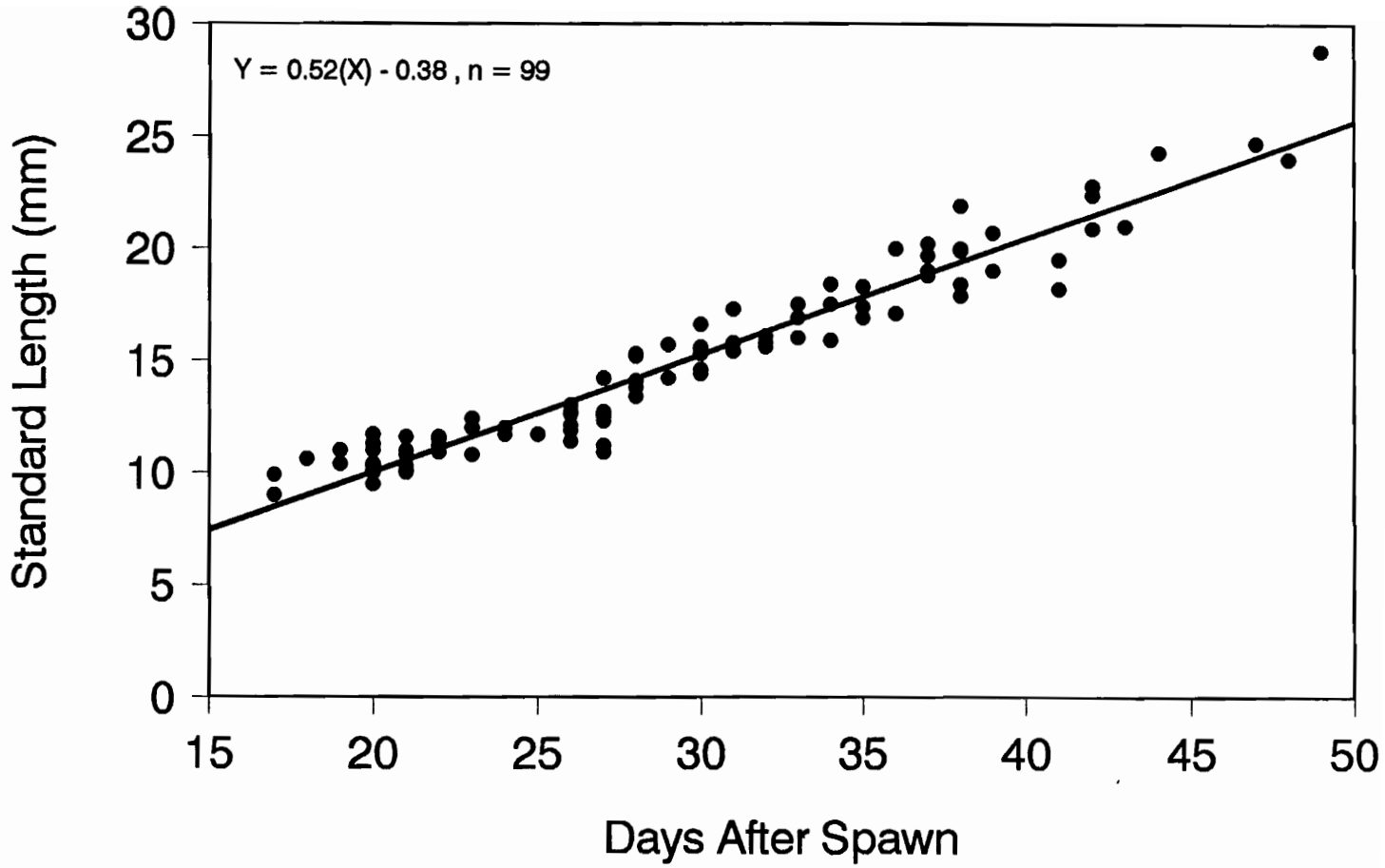
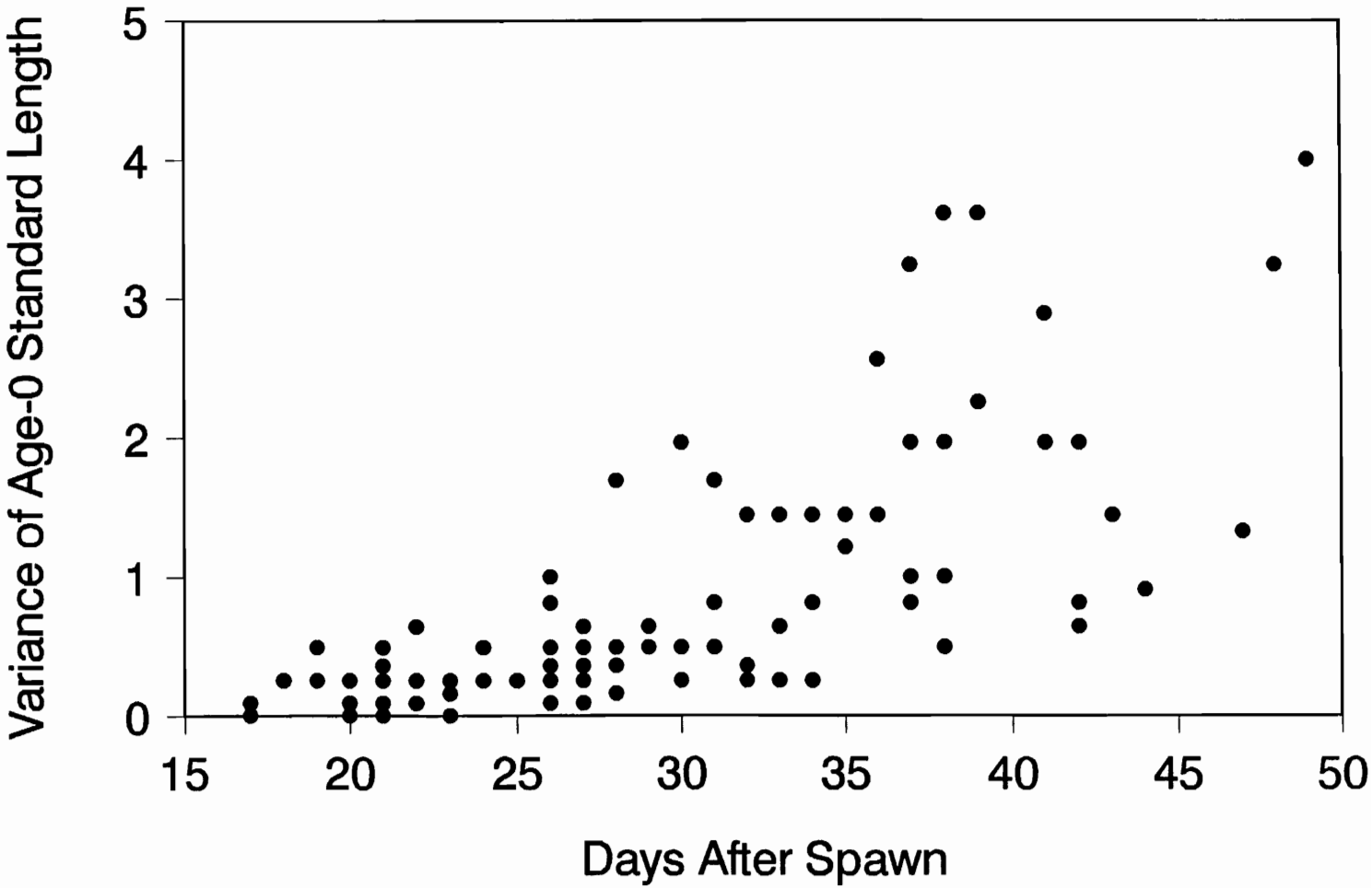
APPENDICES

Appendix 1. Slopes, intercepts, and coefficients of determination (R^2) for age-0 smallmouth bass growth (mm (SL) / d) during parental care in 1994. The regression equation for each brood, SL = a(DAYS AFTER SPAWN) + b, is based on days 17-49 after spawn. Table only includes broods that were measured on three or more occasions.

Brood No.	Slope (a)	Intercept (b)	R^2
2	0.41	2.8	0.99
12	0.35	4.0	0.99
28	0.60	- 2.4	0.99
29	0.42	1.8	0.99
32	0.52	- 0.5	0.99
36	0.52	0.6	1.0
54	0.54	- 0.7	0.99
55	0.54	- 0.5	1.0
58	0.42	2.1	0.93
70	0.47	0.04	0.91
85	0.58	- 2.0	0.99
94	0.38	2.9	0.99
95	0.56	- 1.0	0.98
97	0.49	- 0.49	0.99
100	0.55	- 2.3	0.92
16M	0.54	- 0.6	0.98
22M	0.45	1.6	0.96
23M	0.52	- 1.1	0.99
34M	0.50	- 0.4	0.96
39M	0.48	1.6	0.99
41M	0.44	1.1	0.96
46M	0.49	- 0.7	0.99
MEAN	0.49		
RANGE	0.35 - 0.60		



Appendix 2. Mean standard lengths of age-0 smallmouth bass over time during parental care. Each point represents the mean length of ten individuals from one brood.



Appendix 3. Variance of age-0 standard lengths (mm) over time during parental care. Each point represents the variance of ten individuals from a single brood.

Appendix 4. Frequency and abundance of age-0 smallmouth bass in eel stomachs.

Sampling Method, Location, and Time Period[a]	No. Eels Collected	Eel Size Range (mm)	% of Eels Containing Age-0 Bass	Mean No. Bass per Stomach When Present	% of Eels With Empty Stomach
Netted Within 1 m of Broods[b]					
Night Only	21	125-287	57	5.3	19
Electrofishing in Bass Nesting Habitat[c]					
Morning	40	95-450	0	-	42.5
Day	17	108-294	0	-	70.5
Night	49	112-299	2	3.0	35
Total	106	95-450	1	3.0	43
Electrofishing Throughout River[d]					
Day Only	52	129-470	0	0	44
TOTAL (All Samples)	179	95-470	7	5.0	41

[a]Morning samples were collected 0600-0730 hrs., day 0800-1700 hrs., and night 2200-0200 hrs.
[b]Samples collected between 5/18/94 and 6/5/94.
[c]Samples collected between 5/17/94 and 6/7/94.
[d]Samples collected on 5/31/94, 8/26/94, and 9/7/94.

Appendix 5. Stomach contents of eels from the North Anna River, Virginia. Results are shown as percent by volume and frequency of occurrence (in parentheses) for each food category.

Sampling Method, Location, and Time Period[a]	TAXON			
	Insects	Fish	Crayfish	PDUM[e] or Other
Eels Netted Within 1 m of Broods[b]				
Night Only	7% (0.62)	64% (0.66)	23% (0.05)	6% (0.19)
Electrofishing in Bass Nesting Habitat[c]				
Morning	55% (0.45)	22% (0.05)	14% (0.025)	9% (0.075)
Day	100% (0.29)	0 (0)	0 (0)	0 (0)
Night	78% (0.65)	20% (0.08)	1% (0.02)	1% (0.02)
Total	71% (0.52)	19% (0.06)	6% (0.02)	4% (0.04)
Electrofishing Throughout River[d]				
Day Only	48% (0.50)	6.5% (0.02)	44% (0.02)	1.5% (0.02)
TOTAL (All SAMPLES)	(0.53)	(0.12)	(0.02)	(0.05)

[a]Morning samples were collected 0600-0730 hrs., day 0800-1700 hrs., and night 2200-0200 hrs.
[b]Samples collected between 5/18/94 and 6/5/94.
[c]Samples collected between 5/17/94 and 6/7/94.
[d]Samples collected on 5/31/94, 8/26/94, and 9/7/94.
[e]PDUM = Partially Digested Unidentified Material

Appendix 6. Description of evacuation of age-0 smallmouth bass from eel stomachs. All eels were held at 23°C and starved for at least 24 hours prior to feeding. Stomach contents were examined immediately after eels were sacrificed.

Eel TL (mm)	TL (mm) of Bass Consumed	Time in Stomach (hrs)	Comments
157	18	~1.5	Bass partially digested, ~90% intact
185	11	2	Bass recovered in 2 pieces, ~70% intact, only head recognizable
275	18,11	3-4	Only fish material remaining was a 2x4 mm piece of tissue
260	20,22	3.5-4.5	Most remains in lower intestine, small amount of unidentifiable tissue in stomach

VITA

William Ladd Knotek was born on November 9, 1969 to parents Dale and Mary Ann Knotek. He was born and raised in Crookston, Minnesota and graduated from Crookston Central High School in 1988. He received his Bachelor of Science degree in fisheries and wildlife biology from the University of North Dakota in 1992 and began work toward a Master of Science degree in fisheries biology at Virginia Polytechnic Institute and State University in 1993. Upon graduation in December, 1995, Ladd was employed by Montana Fish, Wildlife, and Parks as a Fisheries Mitigation Biologist.