MAINTENANCE AND PRODUCTION OF OHIO RIVER MUSSELS

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PREFACE

Since their accidental introduction into the Great Lakes in the mid-1980's, zebra mussels (*Dresseina polymorpha*) have devastated mussel populations in Lake St. Clair and western Lake Erie. Moreover, the rapid spread of zebra mussels down the Mississippi River and into many of its major tributaries poses a significant threat to the most diverse mussel fauna in the world. About 40% of the mussel species endemic to the Ohio River drainage are federally listed as endangered. Many of these endangered species occur only in the Ohio River and its major tributaries – precisely those streams experiencing the greatest impact from zebra mussels.

The Scope of Work for this project included the following tasks:

1. An evaluation of the use of cortisol to produce juvenile mussels from non-host fish.
2. An evaluation of quarantining mussels from the Ohio River
3. Transfer of adult mussels in captivity to another Biological Resources Division facility.
4. Attempt to determine the host(s) for *Plethobasus cooperianus* if gravid individuals become available. Alternatively, production of juvenile *P. cooperianus* may be attempted using non-host species as outlined in item 1 above.
5. Modify existing facilities to establish an indoor recirculating system to culture juvenile mussels.
6. Propagate and culture juvenile mussels.

Part 1 of this report contains a Master of Science thesis addressing task 1. Part II includes work performed under tasks 2 and 3. Attempts to identify host fishes and culture juvenile *Plethobasus cooperianus* (task 4) were unsuccessful, because gravid individuals were never encountered. Part III includes all research conducted under tasks 5 and 6.

Part I

Investigations on Host Specificity of Freshwater Mussels

AN ABSTRACT OF A THESIS

INVESTIGATIONS ON HOST SPECIFICITY OF FRESHWATER MUSSELS

Jason R. Khym

Master of Science in Biology

Host specificity of glochidia is believed to be immunologically based. Cortisol is a corticosteriod produced by teleost fish during stress. Prolonged elevations of cortisol can have an immunosuppressive effect on fish. Results from recent research have demonstrated the ability to induce glochidial metamorphosis on certain cortisol-treated nonhost fish. The objective of this study was to test the efficacy of administering cortisol to induce glochidial metamorphosis on a readily obtainable fish, largemouth bass Micropterus salmoides. Cortisol-injected night crawlers Lubricus terrestris were fed to largemouth bass prior to infesting the fish with glochidia of Potamilus alatus or Medionidus conradicus. Although this treatment successfully elevated plasma cortisol levels (≥21μg/100ml), glochidial metamorphosis did not occur on these nonhost fish in two trials. Further, cortisol treatment did not have a significant effect ($P>0.05$) on how long glochidia remained attached to the gills. In a third trial, cortisol was orally administered to largemouth bass, a reported host of the black sandshell, Ligumia recta. Metamorphosis of L. recta glochidia occurred on two of four cortisol-treated largemouth bass, and on two of six untreated fish. Juvenile metamorphosis was highly variable within and among treatments. Plasma cortisol levels of largemouth bass were not correlated ($P>0.05$) with juvenile mussel production. These results suggest that cortisol treatment is not an effective means to induce glochidia metamorphosis on largemouth bass.

In the early 1900's, several fish species were identified as hosts for L. recta. Recent attempts to propagate L. recta with two of the reported hosts (bluegill Lepomis macrochirus and largemouth bass) have produced inconsistent results and few juveniles. This study was conducted to determine which of the reported hosts were the most suitable for metamorphosing juvenile L. recta. Reported hosts were artificially infested with glochidia of L. recta and placed into 38 or 95 liter aquaria depending on fish size. Juvenile metamorphosis occurred after 15 to 170 days and varied according to season and water temperature. Bluegills and largemouth bass produced relatively few or no juveniles in three trials. Although similar numbers of glochidia encysted on all hosts, >10X more juveniles metamorphosed on sauger Stizostedion canadense compared to other hosts tested.

Part I

Investigations on Host Specificity of Freshwater Mussels

INVESTIGATIONS ON HOST SPECIFICTY OF FRESHWATER MUSSELS

A Thesis

Presented to

the Faculty of the Graduate School

Tennessee Technological University

by

Jason R. Khym

In Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

Biology

August 1998

CERTIFICATE OF APPROVAL OF THESIS

INVESTIGATIONS ON HOST SPECIFICITY

OF FRESHWATER MUSSELS

by

Jason R. Khym

Graduate Advisory Committee:

Chairman — date 7/7/98

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DEDICATION

This thesis is dedicated to my grandfathers, Joe X. Khym and E.E. Rivers.

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INTRODUCTION

The life cycle of freshwater mussels includes a parasitic larval stage. Most unionids have larvae (glochidia) that parasitize fish. Glochidial attachment most commonly occurs on either the gills or fins of fish. After attaching to a fish, glochidia are rapidly encysted by epithelial cells (Lefevre and Curtis 1910; Meyers et al. 1980; Young et al. 1987; Nezlin et al. 1994). Encystment can last anywhere from two weeks to several months depending on the mussel species and water temperature. Once metamorphosis is complete, the juvenile mussel excysts and becomes a free living benthic organism.

Turgeon et al. (1988) recognized 297 unionid taxa endemic to North America; about one-half of these taxa are either extinct or in danger of extinction (Bogan 1993). The greatest decline of unionids has occurred among species endemic to the southeastern United States, especially in the Tennessee and Cumberland river drainages and the Mobile Basin (Bogan 1993). Pollution and habitat destruction are thought to be the main causes for this decline. Industrial and non-point domestic pollution lowers water quality (Stansberry 1971; Bogan 1993). Pesticides, used in many agricultural practices, leach into waterways causing damage to aquatic ecosystems. Anderson et al. (1991) documented that strip-mining caused a decline in freshwater mussel populations of the Little South Fork of the Cumberland River in southern Kentucky.

Unionids are filtering organisms that cannot tolerate large quantities of silt. Prolonged filtration of silt particles inhibits respiration and leads to suffocation (Bogan 1993). Road construction, coal mines, agricultural runoff, and clearcuts cause the majority of siltation (Bogan 1993). Prolonged suspension of solids in the water inhibits feeding of freshwater mussels. Dams reduce habitat quality by increasing silt deposition and reducing temperature and dissolved oxygen levels (Stansberry 1971). Host fish movements are the major means of unionid dispersal; therefore, distributional barriers that limit movements of fish may also limit unionid distribution (Watters 1996).

Zebra mussel (Dreissena polymorpha) invasions have also had a negative impact on native unionid populations. Zebra mussels were accidentally introduced into Lake St. Clair in the mid-1980's (Hebert et al. 1989). Since then, they have rapidly colonized areas of the Great Lakes as well as the Ohio and Mississippi river systems, including the Cumberland and Tennessee Rivers. Zebra mussel colonization on freshwater bivalves inhibits unionid filtration leading to high mortality rates and reduced fitness (Haag et al. 1993).

In the early 1900's, the development of a pearl-button industry greatly increased the commercial mussel harvest. During this time period, mussel propagation studies began in an attempt to conserve freshwater pearly mussels. These studies explored the basis of mussel reproduction, including the glochidia-host fish relationship and processes such as ovulation and fertilization. As knowledge of unionid reproduction increased, an effort was made to artificially propogate commercial mussel species (Coker et al. 1922; Lefevre and Curtis 1910).

Unionid declines caused by anthropogenic disturbances may be mitigated by sustaining viable mussel populations through propagation. Both artificial infestations in the laboratory and natural infestation in hatchery raceways can serve as a means of glochidial encystment. Propagating endangered mussel species can be troublesome because host fishes for many of these species are unknown or would be difficult to identify (Isom and Hudson 1982). In fact, host fish have been reported for only 25% of the unionids in North America (Hoggarth 1992).

The glochidia-host relationship involves both a cellular and humoral factor. Waller and Mitchell (1989) compared host and nonhost gill tissue responses to glochidial infestations of Lampilis siliquoidea. The host (walleye Stizostedion vitreum) rapidly encapsulated glochidia, whereas the nonhost (common carp Cyprinus carpio) lost the majority of its glochidial load before encapsulation. Chemicals in the mucus of the nonhost were thought to contribute to the rapid loss of glochidia. Other factors contributing to the rejection of glochidia by common carp include: necrotic cells in the inner zones of the capsules, the presence of eosinophilic granuocytes in capsules, and pronounced hyperplasia in surrounding gill tissue. Fustish and Millemann (1978) observed that artificial infestation of Oncorhynchus kisutch (nonhost) with glochidia from Margaritifera margaritifera caused extreme hyperplasia and ultimately mortality of glochidia. The common host, Atlantic salmon (Salmo salar), encapsulated ≥95% of M. margaritifera glochidia within 9 to 12 hrs. During encystment, no hyperplasia and minimal mucual secretions were observed (Nezlin et al. 1994). Meyers et al. (1980) observed that infesting coho salmon with glochidia of M. margaritifera caused the epithelial cells to either fail

in forming a cyst or form a thickened stalk-like cyst in an attempt to isolate the parasite from the gill. Margaritifera margaritifera glochidia remained viable longer in mucus and plasma of chinook salmon compared to coho salmon (Meyers et al. 1980).

Humoral responses (i.e. those involving the formation of antibodies) of fishes play a major role in the acceptance or rejection of glochidia (Arey 1932; Meyers et al. 1980). A measurable humoral respone was evident in both host (rockbass Amblopites rupestris) and nonhost (common carp Cyprinus carpio) fishes seven days after infestation with glochidia of Villosa iris (O'Connell 1991). Following glochidial infestation, fish produce a serum factor that might be a specific parasite antibody (Bauer and Vogel 1987; Meyers et al. 1980). Glochidial antigens and juvenile antigens react differently to anti-glochidia fish sera. Glochidia antibodies produced by the fish do not react with juvenile antigens (O'Connell 1991). Susceptibility to glochidial parasitism declines as an acquired immunity is developed from prior infections (Reuling 1919; Fustish and Milleman 1978; O'Connell 1991). However, Young et al. (1987) discovered that, at least in the laboratory, older brown trout could be successfully reinfested with glochidia of M. margaritifera.

All fish species are not equally susceptible to glochidia infestations because they produce unequal amounts of parasite antibodies (Bauer and Vogel 1987). O'Connell (1991) suggested that the production or lack of certain antibodies/leukocytes could be a primary factor in host specificity. Although certain unionid species are very host specific, the components in fish blood needed for successful juvenile transformation can be found in the blood of all fish

tested (Isom and Hudson 1982). Kirk and Layzer (1997) found that nonhost fishes treated with a cortisol implant could transform glochidia.

Cortisol is a corticosteroid released from the interrenal tissue of teleost fish during stress (Pickering et al. 1989). Pickering and Pottinger (1983) reported seasonal variations in diel plasma cortisol levels for brown trout, Salmo trutta. The diel cortisol rhythm had a nocturnal peak in all seasons except winter (Pickering and Pottinger 1983). An increase in plasma cortisol was also evident 15 minutes after food consumption (Pickering and Pottinger 1983). Oral administration of cortisol (50 and 100 μg cortisol g^{-1} food) lowered the liposomatic index, hepatosomatic index, and condition factor of channel catfish, Ictalurus punctatus, compared to controls (Davis et al. 1985). Catfish fed cortisol had elevated rates of protein and lipid catabolism and tyrosine aminotransferase activity. Furthermore, liberated amino acids were transported to the liver, where glucose synthesis occurred via gluconeogenesis.

The mean plasma cortisol level for unconfined largemouth bass Micropterus salmoides in earthen ponds, concrete raceways, and fiberglass tanks was 1.5 ug/100ml, while largemouth bass exposed to various types of stress (low dissolved oxygen, confinement, and poor water quality) had plasma cortisol levels between 5 and 12 ug/100ml (Carmicheal et al. 1984a). Following in vitro cortisol injection, a decrease in circulating lymphocytes and an increase in circulating neutrophils was observed for channel catfish (Ellsaesser and Clem 1987). Following cortisol administation, lymphocytopenia occurred in brown trout and coho salmon (McLeay

1973; Pickering 1984). A reduction in lymphocytes suppresses the immune system and makes fish more susceptible to parasites and diseases (Pickering et al. 1989; Pickering and Pottinger 1985; Pickering and Duston 1983); therefore, cortisol-induced lymphocytopenia should suppress the immune response of nonhost fish enabling glochidia metamorphosis. Although Kirk and Layzer (1997) successfully used cortisol implants in nonhost fish to produce juvenile mussels, results were inconsistent. Kirk and Layzer (1997) suggested that variation in transformation rates between experiments may have been due to differences in glochidial maturity, seasonal changes in plasma cortisol levels, and the inability of cortisol implants to consistently induce immuno-suppression.

The objectives of this study were to: determine the concentration and frequency of cortisol administration needed to maintain plasma cortisol levels >5 ug/100ml in nonhost fish; determine a feasible method of orally administering cortisol to largemouth bass; and develop the methodology needed to consistently produce mass quantities of juvenile mussels from immuno-suppressed nonhost fish.

METHODS

Four lampsiline mussels (Potamilus alatus, Medionidus conradicus, Villosa taeniata, and Ligumia recta) were used in this study. Freshwater drum Aplodinotus grunniens is the only known host for Potamilus alatus. Rock bass Ambloplites rupestris is the only known host for Villosa taeniata. Rainbow darters Etheostoma caeruleum and fantail darters E. flabellare were used as host fish for Medionidus conradicus. Sauger Stizostedion canadense were used as hosts for Ligumia recta. The non-host fish utilized in this study was largemouth bass.

Fish Collection

Largemouth bass were collected by electrofishing Old Hickory Reservior, Wilson County, Center Hill Lake, Dekalb County, Falling Water River, Putnam County, and Cane Creek Lake, Putnam County, Tennessee. All of these water bodies are either void of mussels or have low mussel densities.

Host fish were also collected from water bodies with low densities of mussels to ensure no current or prior glochidia infestations. Rainbow darters and fantail darters were collected from Blackburn Fork, Jackson County and Martins Creek, Putnam County, Tennessee with backpack electro-fishing units. A boat mounted direct-current electro-fishing unit was used to collect freshwater drum from Old Hickory and Cheatham reservoirs on the Cumberland River,

Tennessee. Sauger were collected by electrofishing boats and setting gill nets in the tailwaters of Center Hill and Cordel Hull dams.

All fish were transported back to the laboratory in an aerated fish hauler or in coolers supplied with salt (10 ppt), antibiotics (10 mg/l Furacin), and mild anesthetics (15 to 25 mg/l MS-222) as recommended by Carmichael et al. (1984b). Fish were acclimated to water temperatures in the laboratory and placed into 38-liter aquaria. Individual largemouth bass were placed into separate aquaria to eliminate aggressive behavoir. All fish were acclimated to laboratory conditions for two weeks before they were used in an experiment.

Mussel Collection

Gravid P. alatus were collected from the New Johnsonville area of Kentucky Lake at Tennessee River kilometer (TRK) 164.7; gravid L. recta were collected from the Tennesssee River below Pickwick Dam (TRK 318); gravid V. taeniata were collected from the Duck River, Marshall County, Tennessee; gravid M. conradicus were collected from Horse Lick Creek, Jackson County, Kentucky. All mussels were collected by hand with the aid of self-contained underwater breathing apparatus (SCUBA) or plexiglass-bottomed view buckets. Gravid mussels were transported back to the laboratory in coolers and chilled to 10° C to prevent glochidial abortion.

Infestation and Cortisol Administration

Cortisol was orally administered to largemouth bass on a per weight basis. Cortisol was dissolved in ethanol (5% by volume) and then mixed with a 10 ppt NaCl solution. The cortisol solution was injected into nightcrawlers with a hyperdermic syringe. Fish were fed cortisol-injected nightcrawlers beginning one to six days before glochidial infestation; following infestation, fish were fed cortisol-injected nightcrawlers daily or at 3-day intervals. All fish were infested by either pipetting glochidia onto the gills or placing them into coolers containing glochidia.

Cortisol Radioimmunoassay

A subsample of largemouth bass from each experimental group was bled prior to infestation and again at the end of the infestation period. Approximately 200 µl of blood was taken from the caudal vein of each fish. Blood samples were centrifuged at 2900 rpm at 4° C for approximately 10 min to isolate the plasma. Plasma samples were stored in a freezer until assayed for cortisol. Plasma cortisol levels were measured with a radioimmunoassay kit (Coat-A-Count, Diagnostic Products Corporation), and the results were used to monitor plasma cortisol levels in experimental groups and determine cortisol administration procedures and concentrations.

Pilot Studies

Initially, several pilot studies were conducted on different fish species to estimate cortisol concentrations following different methods of administration and different concentrations. Results of these studies are presented in Appendices A-F and will not be discussed otherwise.

Experimental Design

For each trial, three groups of fish were used: host, control, and treatment. Host fish were infested with glochidia to verify metamorphosis capability. A control group was used to verify that untreated largemouth bass would not serve as a host. The treatment group was fed cortisol-injected nightcrawlers daily. Results of pilot studies indicated that a cortisol administration rate of 500µg/kg/24 hrs would elevate plasma cortisol levels >5µg/100ml (Appendix G).

Trial 1 was conducted from April 7, 1997, to May 5, 1997. Glochidia of Potamilus alatus were used to infest largemouth bass and freshwater drum. Ten largemouth bass received a daily cortisol dosage of 500µg/kg/24hrs. Cortisol administration was initiated three days before infestation. Three largemouth bass comprised the control group; the host group consisted of two freshwater drum.

Trial 2 was conducted from July 1,1997, to August 9, 1997. Largemouth bass, fantail darters, and rainbow darters were infested with glochidia from Medionidus conradicus. The treatment group was divided into three cortisol administration rates: 500 µg/kg/24hrs, 1000µg/kg/24hrs, and 1000 µg/kg/72hrs. Cortisol treatment was initiated six days prior to infestation. The treatment and control groups consisted of 14 and 4 largemouth bass, respectively.

Trial 3 was conducted from August 12, 1997, to October 6, 1997. In this trial, largemouth bass (a marginal host, see Part II) was used for the treatment group to test the ability of cortisol to enhance juvenile production. Cortisol was administered to four largemouth bass at a rate of 3000 µg/kg/24hrs. Cortisol treatment began 24 hrs prior to glochidial infestation. Sauger, a good host for glochidia of L. recta, were infested to verify that glochidia were capable of metamorphosis. A control group of six largemouth bass received no cortisol treatment.

Siphoning Procedure

All tanks were siphoned daily during the first five days of the experiment. For the remainder of the experiment, tanks were siphoned every other day. A flexible, 18-mm diameter hose was used to siphon approximately 20L of water from the bottom of each tank. The siphonate was passed through a 100µm mesh screen to capture glochidia and juvenile

mussels. Cross-polarized light microscopy (10X-40X) was used to examine the siphonate (Johnson 1995). All juveniles and glochidia were counted. Juveniles were characterized by two developed adductor muscles, closed valves, and movement within 24 hours. Each experiment was terminated when no juvenile mussels were recovered at least one week after the last juvenile mussel was found, or examination of the gills revealed no glochidial encystment. The response of host and nonhost fish to glochidial infestation was measured by counting the number of glochidia sloughed off and the number of juveniles produced.

Statistical Analysis

Differences in mean numbers of glochidia recovered among groups were compared using one-way analysis of variance (ANOVA). The percentage of the total glochidial load sloughed each day by individual fish was arcsine-transformed and compared using analysis of covariance (ANCOVA) to determine if cortisol treatment had any effect on how many days glochidia remained attached to the gills.

RESULTS

Trial 1

Metamorphosis of glochidia did not occur on any of the 10 cortisol-treated largemouth bass nor on any of the control fish (Table 1). However, glochidia were capable of metamorphosis because freshwater drum transformed a mean of 76 juveniles/fish (Table1). There was no significant difference in the number of glochidia recovered among treatment groups ($P \geq 0.05$, ANOVA). Analysis of covariance revealed that treatment did not have a significant effect on how long glochidia remained attached to the gills. Mean plasma cortisol levels of largemouth bass were 14.7μg/100ml at the beginning of the trial and 21.7μg/100ml at the end of the trial (Table 2). These levels were slightly higher than those observed by Carmicheal et al. (1984a) for stressed largemouth bass.

Trial 2

Glochidial metamorphosis did not occur on any of the 14 cortisol-treated largemouth bass (Table 1). Glochidia were capable of metamorphosis because a mean of 9 juveniles/fish metamorphosed from fantail darters and a mean of 18 juveniles/fish metamorphosed from rainbow darters (Table 1). The mean number of glochidia recovered from largemouth bass in each treatment was not significantly different ($P > 0.05$, ANOVA); however several

Table 1. Mean number ± S.D. of glochidia and juveniles collected from various treatment groups.

Trial	Fish Species	N	Treatment	Mussel Species	Mean (±S.D.) # of	
					Glochidia	Juveniles
1	Largemouth bass	10	500µg/kg/24hrs	*Potamilus alatus*	2042±881	0
1	Largemouth bass	3	Control	*Potamilus alatus*	2018±679	0
1	Freshwater drum	2	Host	*Potamilus alatus*	571±372	76±6
2	Largemouth bass	5	1000µg/kg/24hrs	*Medionidus conradicus*	1084±698	0
2	Largemouth bass	4	1000µg/kg/72hrs	*Medionidus conradicus*	794±340	0
2	Largemouth bass	5	500µg/kg/24hrs	*Medionidus conradicus*	484±186	0
2	Largemouth bass	4	Control	*Medionidus conradicus*	881±531	0
2	Rainbow darters	7	Host	*Medionidus conradicus*	91	18
2	Faintail darters	7	Host	*Medionidus conradicus*	60	9

Table 2. Mean plasma cortisol concentrations ± S.E. of largemouth bass Micropterus salmoides at the beginning and end of infestation periods for three trials.

Trial	Treatment (µg/kg/hr)	Number of days fish were treated before glochidia infestation	Cortisol concentrations ±S.E. (µg/100ml)				Infestation Period (days)
			N	Beginning*	N	Ending	
1	500µg/kg/24hrs	3	3	14.6 ± 5.8	4	21.7 ± 4.6	15
2	500µg/kg/24hrs	6	3	17.7 ± 9.3	3	32.4 ± 4.1	29
2	1000µg/kg/24hrs	6	3	9.1 ± 4.5	3	50.6 ± 15.7	29
2	1000µg/kg/72hrs	6	1	1.45	3	43.7 ± 16	29
2	Control	N/A	-	-	1	7.59	29
3	3000µg/kg/24hrs	1	3	117.8 ± 39.2	-	-	31
3	Control	N/A	4	11.8 ± 3.6	-	-	26

* Beginning concentrations were measured on day of glochidia infestation

times more glochidia were recovered from largemouth bass compared to the host fish. Cortisol treatment had no effect on the length of time glochidia remained attached to the gills ($P>0.05$, ANCOVA).

Ending plasma cortisol levels of cortisol-treated fish were elevated above control fish (Table 2). Ending plasma cortisol levels of fish treated with a dosage of 1000µg/kg/24hrs were positively correlated ($r = 0.99$, $P<0.05$) with the number of days fish refused to eat. The weights of cortisol-treated fish (500µg/kg/24hrs) were negatively correlated with ending ($r = 0.99$, $P<0.05$) plasma cortisol levels.

Trial 3

Juvenile metamorphosis was highly variable within and among treatment and control groups of largemouth bass (Table 3). In contrast, sauger produced a mean of 688 juveniles/fish. Two of four cortisol-treated (3000µg/kg/24hrs) largemouth bass produced juvenile mussels; however, two of the six largemouth bass in the control group produced juveniles. All cortisol-treated largemouth bass died prior to the end of the trial. Upon death, the gills of each fish were examined and no encysted glochidia were found. The mean number of glochidia sloughed among treated and control groups were not significantly different ($P\geq0.05$, ANOVA). Cortisol treatment had no effect on how long glochidia remained encysted or the number of juveniles that metamorphosed ($P\geq0.05$, ANCOVA).

Table 3. Plasma cortisol levels (µg/100ml) of largemouth bass, and numbers of Ligumia recta glochidia and juveniles collected from largemouth bass and sauger.

Species	Treatment (µg/kg/24hrs)	Total number of glochidia	Total number of juveniles	Beginning* plasma cortisol concentration (µg/100ml)
Largemouth bass	3000	1852	0	154.2
Largemouth bass	3000	5180	87	39.5
Largemouth bass	3000	1747	0	159.6
Largemouth bass	3000	1008	1	N/A
Largemouth bass	Control	1561	61	5.5
Largemouth bass	Control	1248	0	N/A
Largemouth bass	Control	3155	0	8.3
Largemouth bass	Control	1617	0	N/A
Largemouth bass	Control	3837	2	11.4
Largemouth bass	Control	3467	0	22.1
Sauger	Host	-	878	N/A
Sauger	Host	-	697	N/A
Sauger	Host	-	489	N/A

* Beginning concentrations were measured on day of glochidia infestation

At the beginning of the infestation period, cortisol-treated largemouth bass had a mean plasma cortisol level of 117.8 μg/100ml. Largemouth bass in the control group had a mean plasma cortisol level of 11.8 μg/100ml (Table 2). Plasma cortisol levels on the first day of glochidia infestation (i.e. 24hrs after cortisol treatment) were negatively correlated with the weight of cortisol-treated fish ($r=0.99$, $P<0.10$) and significantly elevated above those of control fish ($P<0.05$, ANCOVA).

DICUSSION

Although cortisol levels for treated fish equaled or exceeded those observed by Carmicheal (1984a) for stressed largemouth bass, juvenile metamorphosis was not induced by cortisol treatment. Possibly, cortisol treatment did not reduce the number of circulating lymphocytes. McLeay (1975) demonstrated that elevated cortisol level had no effect on lymphocyte count but increased the susceptibility to diseases. Pickering and Pottinger (1985) also observed that elevating cortisol levels in brown trout increased their susceptibility to infections without reducing the lymphocyte count. Seven days of cortisol treatment ranging from 2μg/g/day to 200μg/g /day decreased the percentage of small lymphocytes but increased the percentage of large lymphocytes and neutrophils of juvenile coho salmon Oncorhynchus kisutch (McLeay 1973). Although Atlantic salmon Salmo salar treated with cortisol for 1 day showed a clear reduction in lymphocytes or granulo-/monocytes, there was no significant difference between treated and control fish after four days of treatment (Wiik et al. 1989).

Plasma cortisol levels varied throughout all trials. Previously, Wiik et al. (1989) reported variable cortisol levels (5.9 to 29.8 μg/100ml) for control fish and (0.4 to 40 μg/100ml) for treated Atlantic salmon. In the present study, it is unlikely that variation in plasma cortisol levels resulted from measurement error (Appendix H). Plasma cortisol levels of

cortisol-treated largemouth bass (500µg/kg/24hrs and 3000µg/kg/24hrs) were negatively correlated with the weight of largemouth bass. Larger fish may have metabolized cortisol more rapidly than smaller ones. In Trial 3, cortisol treatment did not enhance juvenile metamorphosis on a reported host, largemouth bass. On the contrary, treated fish having the lowest plasma cortisol level transformed the most juveniles. Similarly, control fish with the lowest plasma cortisol levels produced more juveniles than other control fish.

In the present study cortisol was orally administered; whereas, Kirk and Layzer (1997) implanted cortisol in a suspension of cocoa butter. To determine the approximate plasma cortisol levels in the implanted fish used by Kirk and Layzer (1997), their procedure was used to inject cortisol implants into banded sculpins. The plasma cortisol levels in these fish ranged from 2.9 to 9.5 µg/100ml over a five week period (Appendix I).

The effects of cortisol treatment on glochidial metamorphosis may be species specific. Although Kirk and Layzer (1997) successfully produced juveniles from cortisol-implanted banded sculpins Cottus carolinae and orangethroat darters Etheostoma spectabile, no juveniles metamorphosed on creek chubs Semotilus atromaculatus during any trial. Cortisol treatment may be lowering lymphocyte numbers in some species and not in others. Pickering et al. (1989) found that cortisol implants lowered lymphocyte counts in immature brown trout but not in immature rainbow trout. The effects of cortisol administration on lymphocyte counts in largemouth bass is unknown.

If efforts to refine this technique are pursued, changes in the methodology of the current study should be implemented. Blood samples should be collected from at least five fish per treatment group to compensate for plasma samples lost as a result of gelling. Gelling of plasma commonly occurs when fish are sick. Blood samples should also be collected at least three times during the infestation period and the relationship between lymphocyte counts and plasma cortisol concentration should be determined. Long-term survival of juveniles produced by this technique should be monitored and compared to survival of juveniles produced from host fish. Further research is needed to better understand the host-glochidia relationship and identify the basis for host specificity.

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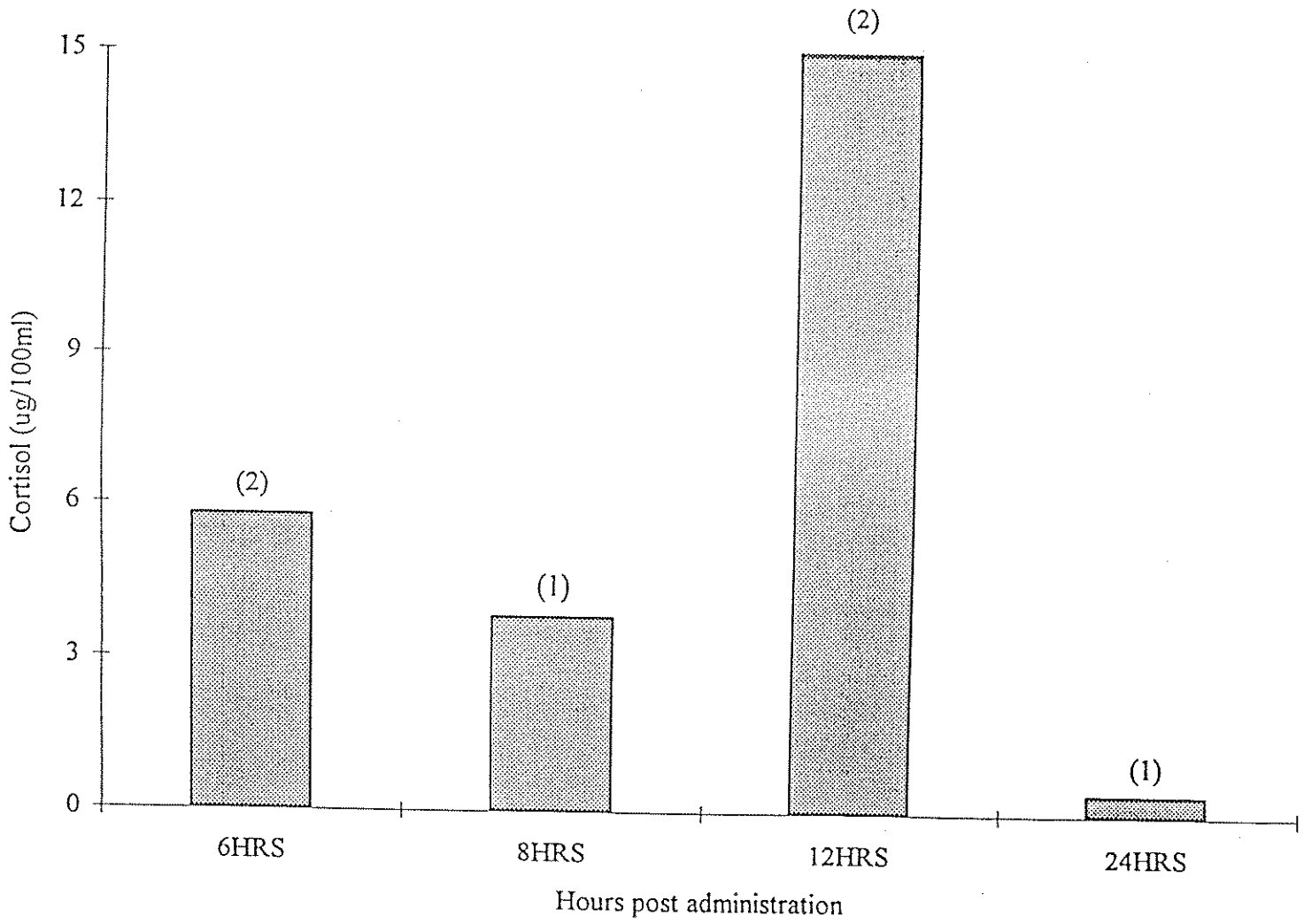
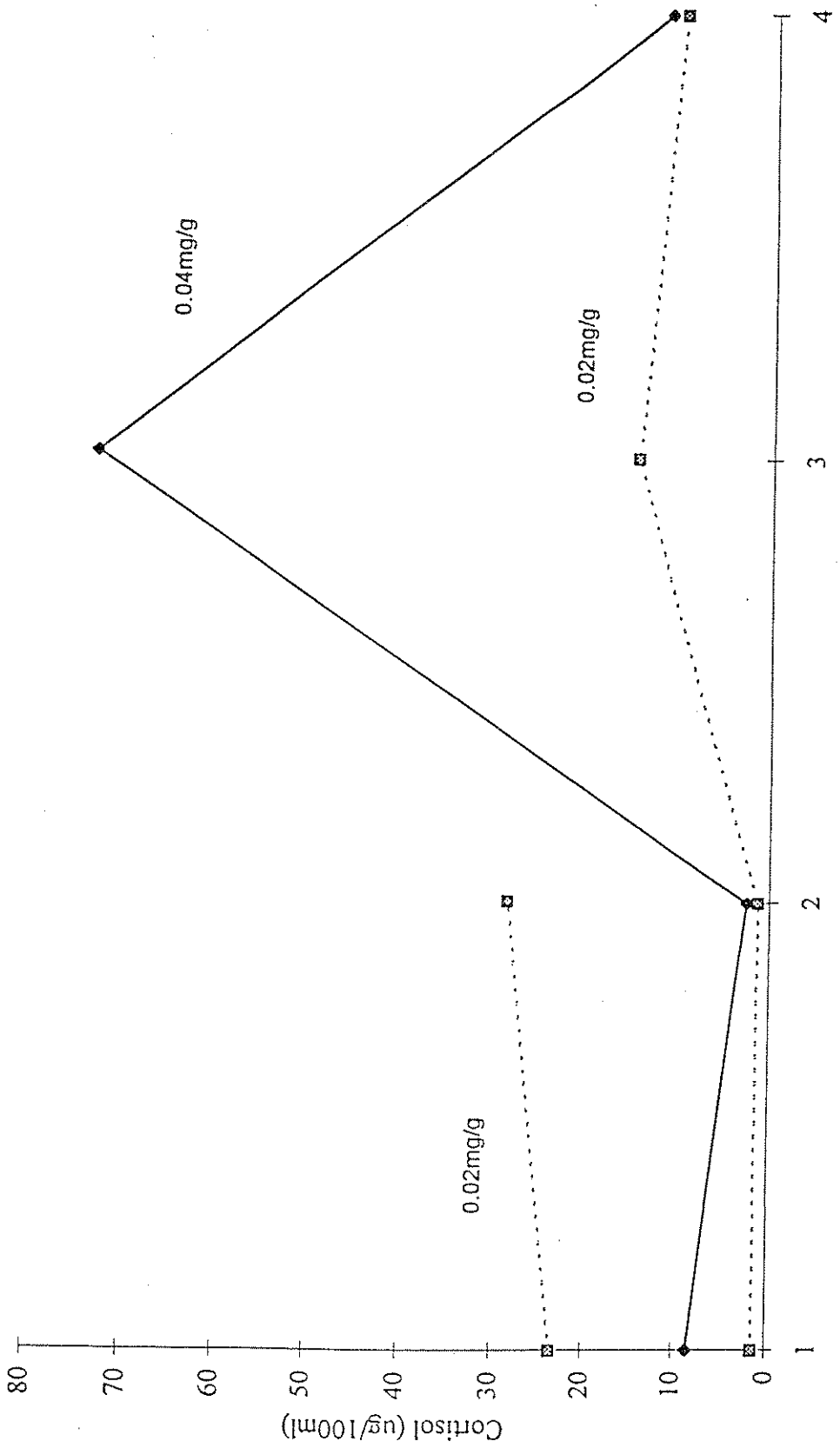
APPENDICES



Appendix A. Mean plasma cortisol levels of banded sculpin Cottus carolinae after a single oral cortisol administration (500 ug/kg). Sample sizes are in parentheses.

Appendix B. Plasma cortisol levels of individual banded sculpin Cottus carolinae following a single cortisol administration. Two types of cortisol administration were investigated.

Cortisol concentration	Type of cortisol administration	Time interval after administration	Plasma cortisol levels (µg/100ml)
500µg/kg	oral	6 hrs	7.3
500µg/kg	oral	6 hrs	4.4
500µg/kg	oral	8 hrs	3.8
500µg/kg	oral	12 hrs	3.4
500µg/kg	oral	12 hrs	26.9
500µg/kg	oral	24 hrs	0.4
0.02mg/g	implant	1 wk	3.5
0.02mg/g	implant	1 wk	22.3
0.02mg/g	implant	1 wk	2.6
0.02mg/g	implant	2 wk	6.7
0.02mg/g	implant	3 wk	2.5
0.02mg/g	implant	3 wk	17.3
0.02mg/g	implant	4 wk	8.1
0.02mg/g	implant	5 wk	2.9
Sham	implant	N/A	1.1



Appendix C. Plasma cortisol concentrations of three largemouth bass following intraperitoneal implants of cortisol.

Appendix D. Plasma cortisol levels of individual largemouth bass Micropterus salmoides following a single cortisol administration (500 µg/kg).

Cortisol Concentration Administered (µg/kg)	Time interval after cortisol administration	Plasma cortisol level (µg/100ml)
N/A	0*	0.5
N/A	0*	0.8
500	6	26.5
500	6	21.0
500	6	39.9
500	8	11.0
500	8	6.7
500	8	9.6
500	12	13.7
500	12	12.9
500	12	10.0
500	24	29.9
500	24	1.18
500	24	1.72
500	96	11.4

* Control fish

Appendix E. Plasma cortisol levels (µg/100ml) of individual largemouth bass Micropterus salmoides in three trials.

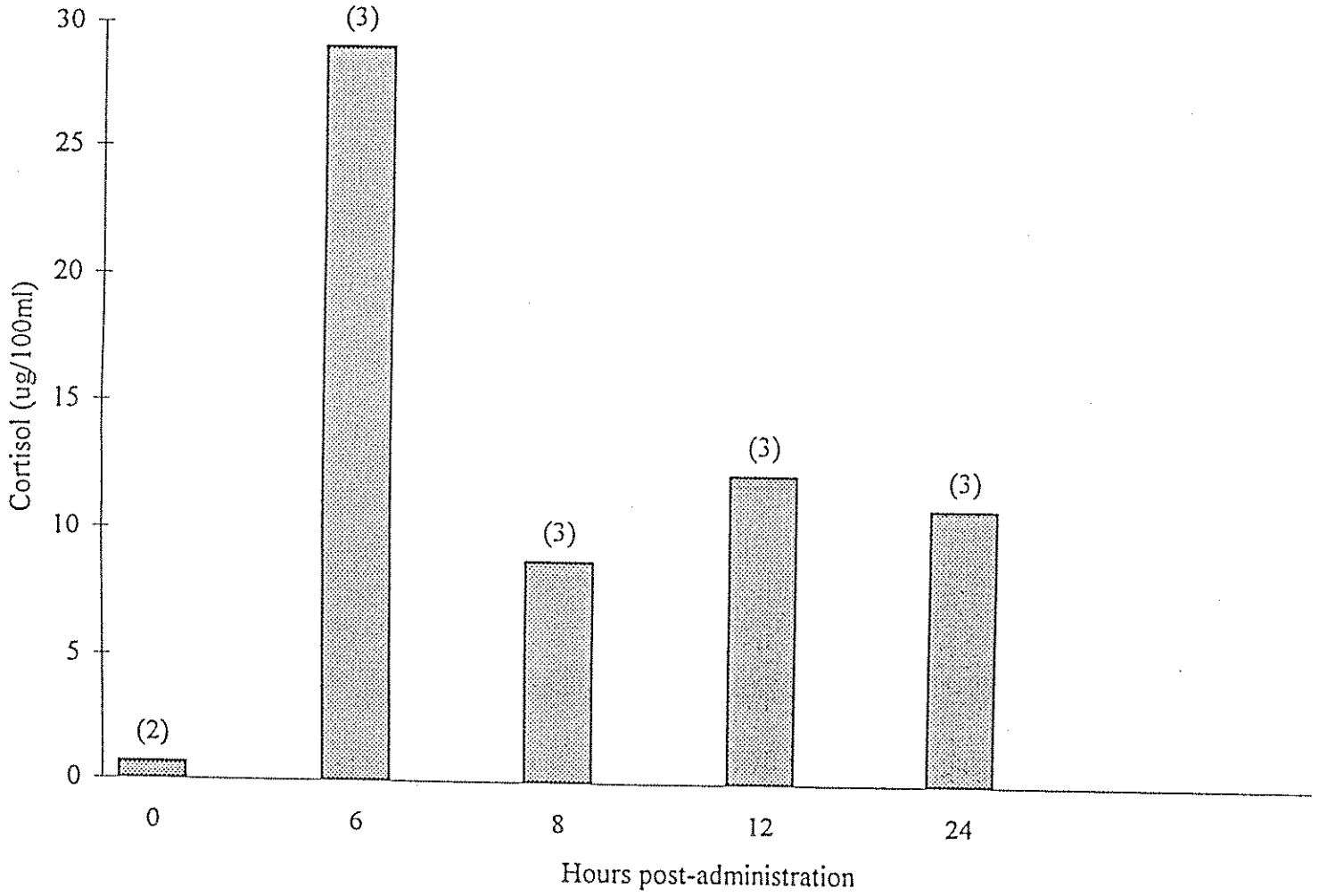
Trial	Treatment (µg/kg/hr)	Part of infestation period blood sample was collected	Plasma cortisol level (µg/100ml)
1	500µg/kg/24hrs	Beginning	9.8
1	500µg/kg/24hrs	Beginning	26.1
1	500µg/kg/24hrs	Beginning	8.0
1	500µg/kg/24hrs	End	19.1
1	500µg/kg/24hrs	End	11.5
1	500µg/kg/24hrs	End	33.5
1	500µg/kg/24hrs	End	22.6
2	500µg/kg/24hrs	Beginning	35.6
2	500µg/kg/24hrs	Beginning	4.5
2	500µg/kg/24hrs	Beginning	13.2
2	500µg/kg/24hrs	End	40.1
2	500µg/kg/24hrs	End	31.2
2	500µg/kg/24hrs	End	26.1
2	1000µg/kg/24hrs	Beginning	0.5
2	1000µg/kg/24hrs	Beginning	11.3
2	1000µg/kg/24hrs	Beginning	15.5
2	1000µg/kg/24hrs	End	55.2
2	1000µg/kg/24hrs	End	75.2

Appendix E. continued

2	1000µg/kg/24hrs	End	21.3
2	1000µg/kg/72hrs	Beginning	1.5
2	1000µg/kg/72hrs	End	19.7
2	1000µg/kg/72hrs	End	73.9
2	1000µg/kg/72hrs	End	37.5
2	Control	End	7.59
3	3000µg/kg/24hrs	Beginning	39.5
3	3000µg/kg/24hrs	Beginning	154.3
3	3000µg/kg/24hrs	Beginning	159.6
3	Control	Beginning	5.5
3	Control	Beginning	8.3
3	Control	Beginning	11.4
3	Control	Beginning	22.1

Appendix F. Mean number of glochidia and juveniles collected from bluegill Lepomis macrochirus fed cortisol-injected nightcrawlers.

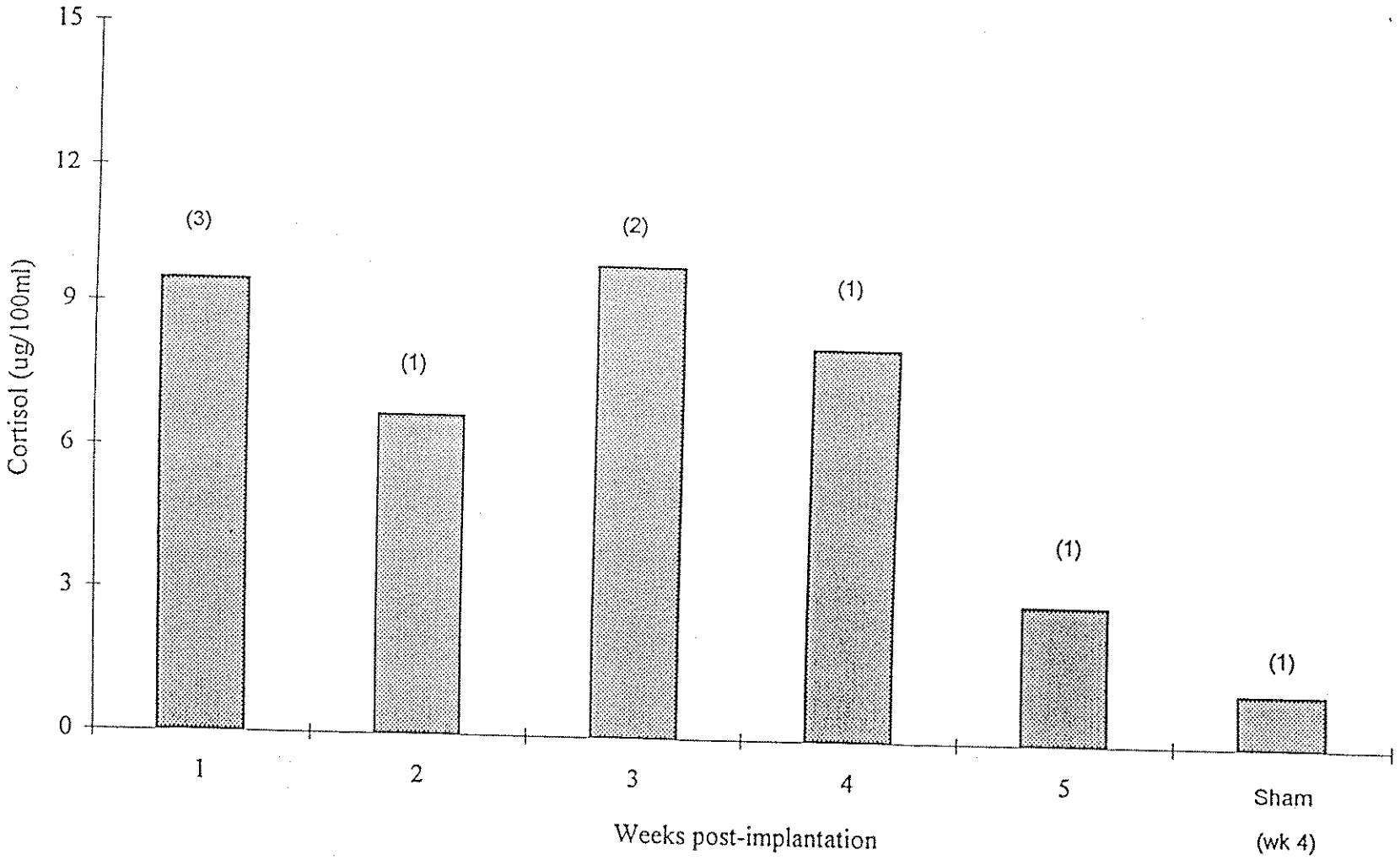
Cortisol Treatment	N	Mean number of		Attachment Period (Days)
		Glochidia	Juveniles	
Control	3	672	0	39
1000µg/kg/24hrs	3	1340	0	39
1000µg/kg/72hrs	3	2329	0	43



Appendix G. Changes in mean plasma cortisol levels of largemouth bass Micropterus salmoides after a single cortisol administration (500ug/kg). Sample sizes are given in parentheses.

Appendix H. Mean measurements (± S. E.) of cortisol standards determined by radioimmunoassay.

Cortisol standard (µg/100ml)	N	Mean (± S.E.) measured cortisol concentration (µg/100ml)
1	10	1.0±0.1
10	10	11.2±0.4
50	9	46.9±1.1



Appendix I. Mean plasma cortisol levels of banded sculpin Cottus carolinae after a 0.02 mg/g implant. Sample sizes are in parentheses.

PART II:

HOST FISH SUITABILITY FOR

GLOCHIDIA OF *Ligumia recta*

INTRODUCTION

The black sandshell, Ligumia recta, is widely distributed but uncommon throughout much of its range (Cummings and Mayer 1992). Historically, L. recta occurred throughout much of the Great Lakes, Ohio, and Mississippi river drainages (Wilson and Clark 1914; Boepple and Coker 1912; Coker et al. 1921; Ortmann 1925). During the past 50 years, populations of Ligumia recta have declined; it is now considered a species of special concern and is listed as threatened in Virginia and Ohio (Williams et al. 1993). Cochran (1993), Weiss and Layzer (1995), and Laudermilk (1993) found few L. recta in the Green, Barren, and Licking Rivers in Kentucky. In the Clinch and Powell Rivers, L. recta was rarely collected in quadrat samples (Ahlstedt and Tuberville 1997). Due to its low abundance and seemingly declining status, the black sandshell is a species in need of management.

In the early 1900's, identification of glochidia on fish was often done to determine host species. Moreover, many reported hosts were not verified with laboratory infestations. Hosts identified for L. recta are: banded killifish Fundulus diaphanus, black bass Micropterus spp., largemouth bass M. salmoides, bluegill Lepomis macrochirus, crappie Pomoxis spp., white crappie Pomoxis annularis, green sunfish Lepomis cyanellus, orangespotted sunfish Lepomis humilis, and sauger Stizostedion canadense (Young 1911; Surber 1913; Lefevre and Curtis 1912; Wilson 1916; Coker et al. 1921; Pearse 1924).

Recent attempts to propagate L. recta with reported hosts have produced inconsistent results and few juveniles. For instance, few juveniles were recovered from 40 infested fingerling largemouth bass (O. Westbrook, Tennessee Technological University, personal communication). In one trial, Hove et al. (1994a) reported juvenile metamorphosis on largemouth bass but not on bluegill; in a second trial, juveniles metamorphosed on bluegill but not on largemouth bass (Hove et al. 1994b). Juvenile metamorphosis did not occur on green sunfish in either trial. In contrast, Steg and Neves (1997) noted juvenile metamorphosis on green sunfish. The results of these studies suggest that host suitability for glochidia of L. recta varies among fish species and trial. Therefore, the objective of this study was to determine which of the reported hosts were the most suitable for juvenile metamorphosis.

METHODS

Fish were collected by electrofishing and gill netting in bodies of waters containing few or no mussels to minimize current or prior glochidial infestations. Sauger were collected in the tailwaters of Center Hill and Cordell Hull Dams. Largemouth bass, bluegill, white crappie, and green sunfish were collected from Old City Lake, Putnam Co.; Cane Creek Lake, Putnam Co.; and Normandy Reservoir, Coffee Co., TN. Northern studfish Fundulus catenatus were collected from Blackburn Fork, Jackson Co., TN. Fingerling black crappie Pomoxis nigromaculatus were obtained from the Normandy Hatchery.

Gravid female L. recta were collected from Kentucky Lake below Pickwick Dam (Tennessee River Kilometer 318). All mussels were collected by hand with the aid of self-contained underwater breathing apparatus (SCUBA). Mussels were examined in the field and gravid individuals were transported to the laboratory in insulated coolers. In the laboratory, females were held in a living stream at 10°C to prevent glochidial abortion.

Glochidia were obtained by anaesthetizing mussels with 250 ppm of MS-222 and then flushing water through each water tube of the marsupial gills with a hypodermic syringe. Glochidial viability was checked by introducing a NaCl solution to a subsample. Glochidia were considered viable if ≥ 95% of the subsample snapped shut. The remaining glochidia were poured into a cooler containing test fish and the water was stirred frequently to keep glochidia in suspension. Glochidia were allowed approximately 10 minutes to attach to the fish. Infested fish were placed into either 39L or 95L aquaria supplied with aeration and filtration. In Trial 1, survival of sauger was low in static 38 L

aquaria. Therefore, in Trial 2, sauger were placed into recirculating aquaculture systems for the first 13 days post-infestation. On day 14, fish were placed into separate 95L aquaria. In Trial 3, sauger were placed directly into separate 95L aquaria following glochidial infestation.

Aquaria were siphoned with a flexible 18-mm hose every day for the first five days following infestation and every other day for the remainder of the trial. The siphonate, collected on a 100μm mesh screen, was washed out into a grided petri-dish and then examined with cross-polarized microscopy; all juveniles and glochidia were counted. Juveniles were characterized by the presence of two adductor muscles, closed valves and movement within 24 hours. Trials were terminated one week after the last juvenile was recovered or examination of the gills revealed no encystment. The total number of glochidia and juveniles recovered throughout the trial from an individual tank was considered to be the initial infestation intensity. To facilitate comparisons among trials, data was normalized by assigning a value of 1.0 to the modal number of glochidia sloughed in one day and normalized values for all other days were determined as a proportion of the modal number. Means of the normalized data were then analyzed.

RESULTS

Although individuals of each species were simultaneously exposed to glochidia in the same cooler, infestation intensities varied among species (Table 1). In Trial 1, bluegill and largemouth bass sloughed the majority of their glochidial load 2 to 3 days post-infestation (Figure 1). In Trial 2, glochidia sloughing from bluegill and largemouth bass was nearly complete 9 days postinfestation.

In Trial 3, all hosts experienced high glochidial sloughing rates during the first three days postinfestation (Figure 2). A second peak in glochidial sloughing was evident from largemouth bass and bluegill at 5 to 12 days postinfestation. A third peak in glochidial sloughing 16-19 days post-infestation was distinct for bluegill (Figure 2). These secondary and tertiary peaks were not observed from sauger.

The mean number of juveniles metamorphosed from sauger was more than an order of magnitude greater than any other species tested (Table 1). Both Pomoxis species were marginally suitable as hosts. Largemouth bass and bluegill produced ≤ 4 juveniles per fish. Metamorphosis did not occur on northern studfish or green sunfish (Table 1). Glochidial metamorphosis occurred on 19% of the largemouth bass and 6% of the bluegill infested (Table 4). In contrast, juvenile transformation occurred on all sauger infested. Less than 5% of the glochidia on largemouth bass, bluegill, and crappie species metamorphosed into juveniles. In contrast, 53% of the glochidia on sauger transformed into juveniles.

Table 1. Number of fish that juveniles metamorphosed on, mean infestation intensity(glochidia + juveniles), and mean number of juveniles/fish for each species tested in three trials.

Species	N	Number of fish that juveniles metamorphosed on	Mean infestation intensity	Mean # of juveniles/ fish
Largemouth bass	16	3	3118	4
Bluegill	17	1	1301	0.8
Sauger	4	4	5467	2904
"	3	3	*	881
White crappie	1	1	3540	85
Black crappie	6	N/A**	605	20
N. Studfish	7	0	232	0
Green sunfish	3	0	608	0

* Glochidia were not counted for sauger in Trial 2
** All black crappie fingerlings were keep in a single aquarium

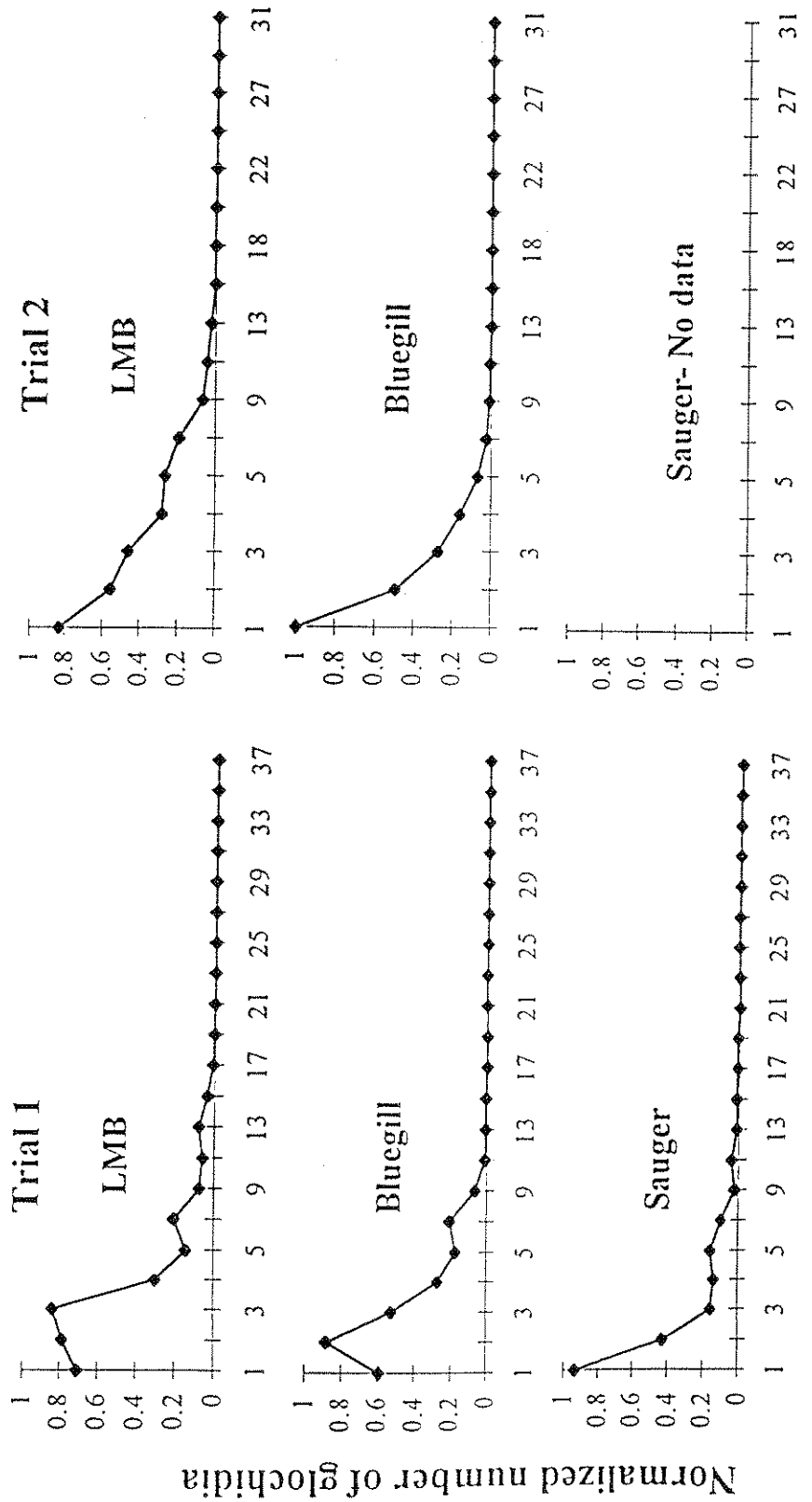


Figure 1. Mean normalized numbers of glochidia sloughed each day in two trials.

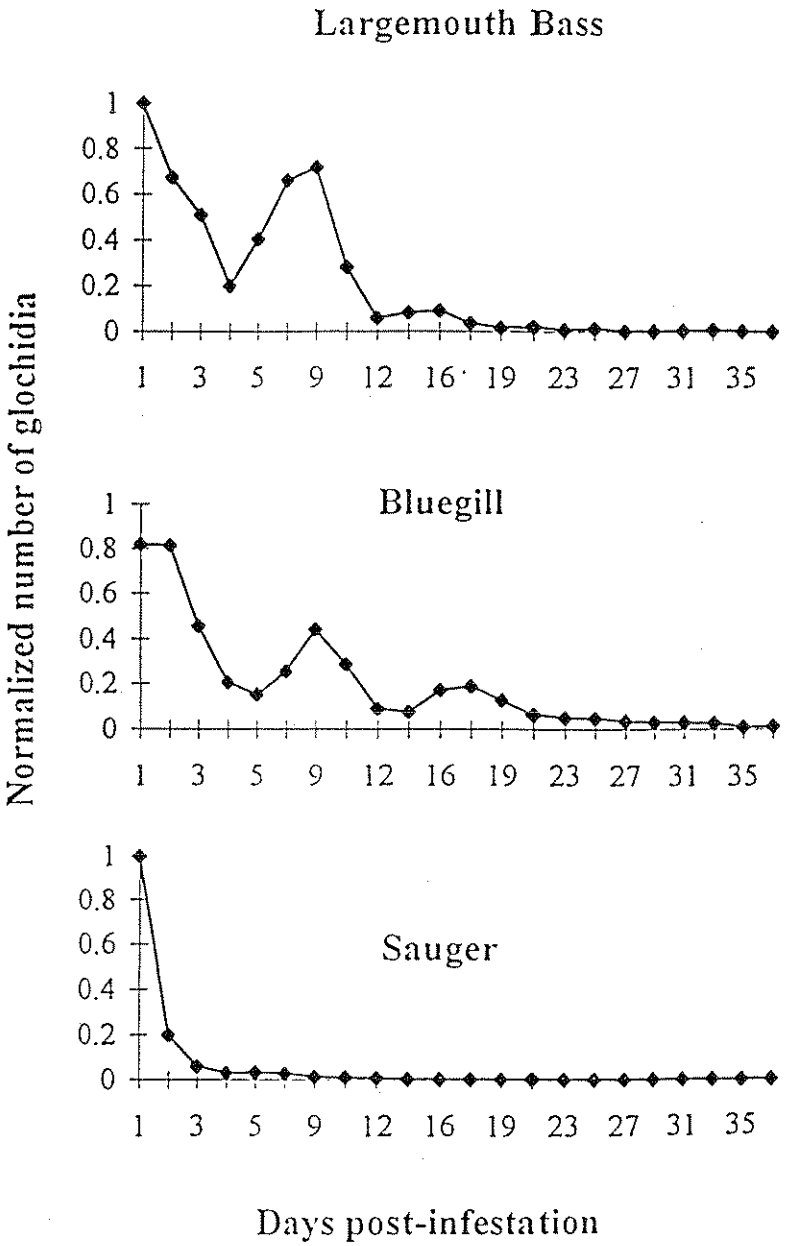


Figure 2. Mean normalized numbers of glochidia sloughed each day during Trial 3.

Temporal patterns in juvenile metamorphosis on sauger differed among trials (Figure 3). The modal day of juvenile metamorphosis varied among trials conducted from spring 1997 to fall 1997 (Table 2). Mean water temperature ranged from 18.2°C to 22.0°C among trials. Although mean water temperatures were not significantly different between Trial 1 and Trial 3 ($P>0.05$), mean water temperature in Trial 2 was significantly higher ($P<0.05$) than those of Trial 1 and Trial 3. In Trial 1, metamorphosis occurred sooner and over a shorter time period than in Trial 3 (Figure 3). Juvenile metamorphosis was complete by day 48 in Trial 1, whereas in Trial 3 <0.002% of the juveniles had dropped off by day 48. A lower percentage of juveniles transformed in Trial 1 than Trial 3 (Table 2).

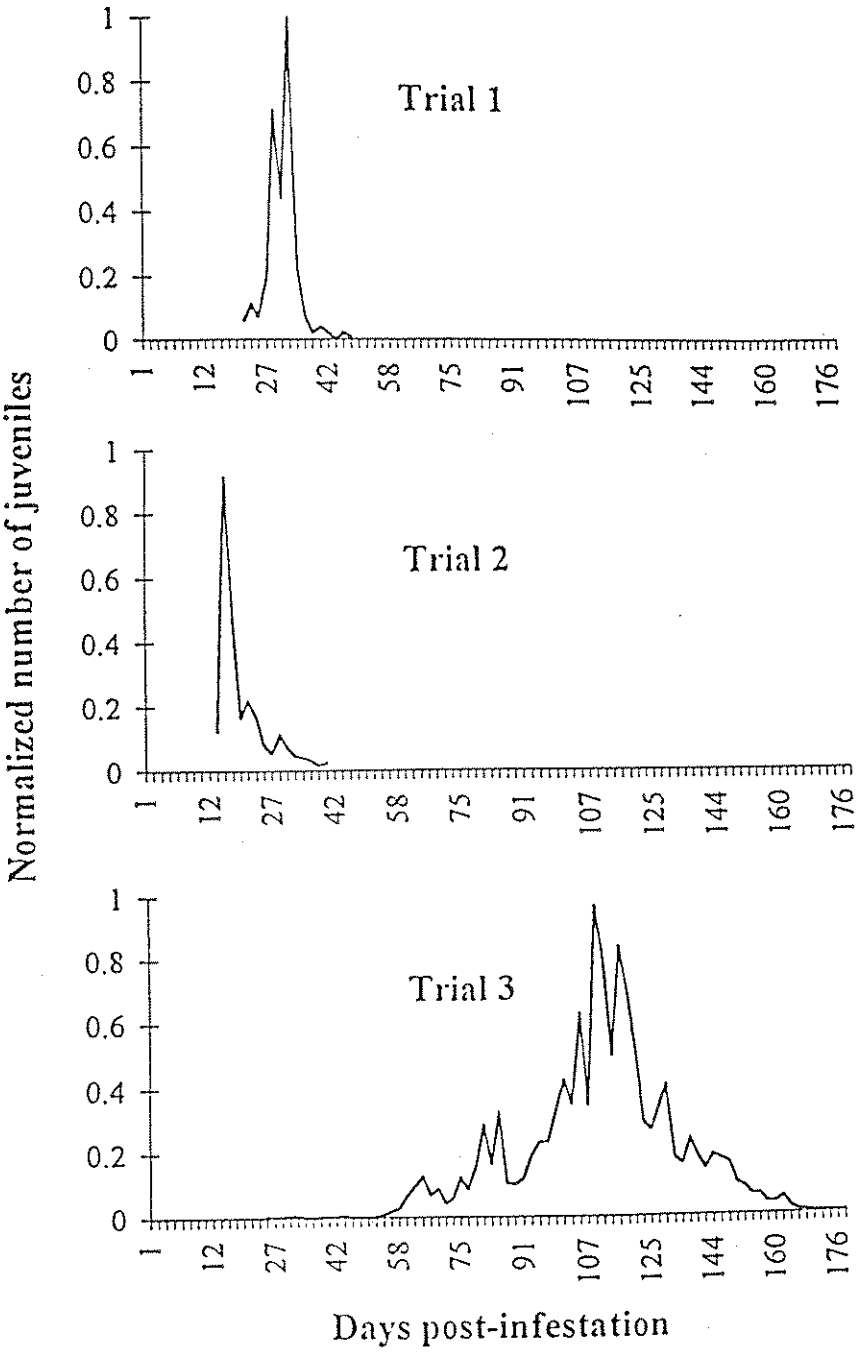


Figure 3. Mean normalized numbers of juveniles metamorphosed each day on sauger in three trials.

Table 2. Dates of mussel collection and fish infestation, mean water temperatures, and juvenile metamorphosis data for sauger in each trial.

Trial	Date collected	Date infested	Mean water temperature (±S.D.)	N	Mean # of juveniles/ fish	% Juvenile transformation	Metamorphosis Period	
							Modal Day	Range (Days)
1	4-15-97	4-23-97	19.0(1.0)	1	689	26	31	21-48
2	8-11-97	8-12-97	21.9(0.5)	3	882	N/A*	16	15-44
3	10-16-97	11-13-97	18.2(1.4)	3	3978	61	109	25-180

* Sloughed glochidia not counted

DICUSSION

All reported hosts for L. recta tested were not equally suitable; clearly, sauger was the most suitable host. Glochidial metamorphosis from other reported hosts was inconsistent and few juveniles were produced. Glochidial sloughing patterns suggest that glochidia elicited an immune response in some fish species and not others. In Trial 3, all hosts had a high sloughing rate from Day 1 to Day 3, possibly due to glochidia not becoming encysted. A second peak in glochidial sloughing was observed in largemouth bass and bluegill. Perhaps, this second peak was caused by a specific immune response to the glochidia. No second peak occurred in sauger. In Trial 1, an immune response 2 to 3 days post-infestation from bluegill and largemouth bass may have caused the ascending limb. In Trial 2, a second peak in glochidial sloughing did not exist for largemouth bass and bluegill. Perhaps, the higher water temperature shortened the activation time of an immune response and thus hid the second peak in the initial sloughing of glochidia.

The mean infestation intensity was variable among fish species despite infesting fish simultaneously. Some differences in infestation intensity were probably due to fish size; however infestation intensity also varied between similar sized fish (largemouth bass and sauger, bluegill and white crappie). Due to these variations in glochidial attachment, the number of glochidia sloughed from each fish should be included in host suitability studies. Juvenile production from hosts is most useful for comparative purposes when the infestation intensity is known. For example, sauger in Trial 3 were exposed to twice as

many glochidia as sauger from Trial 1 even though glochidial exposure times were similar. Determining the percentage of glochidia that transform into juveniles is likely the most valid way of determining the suitability of potential host fishes. If sloughed glochidia are counted, the percentage of glochidia that metamorphosed into juveniles can be assessed and comparisons among trials, season, and other suitability tests can be made.

In Trial 3, more juveniles metamorphosed per individual sauger than in Trials 1 and 2 combined. Nearly twice as many glochidia attached to sauger in Trial 3 compared to sauger in other trials. Season may be a factor not only affecting the number of glochidia that metamorphose into juveniles but also glochidial infestation rates. Future studies should investigate seasonal differences in juvenile transformation and determine the survival of juveniles in relation to the length of time to metamorphosis. Survival of juveniles may be related to the length of the encystment period.

Temperature was not the only factor regulating the length of time to metamorphosis on sauger. The duration of glochidial metamorphosis periods has often been attributed to water temperature (Zale and Neves 1982; Yeager and Saylor 1995). However, in Trial 1 and Trial 3 water temperatures were similar but the modal day of metamorphosis differed by 78 days. The only quantifiable difference between Trial 1 and 3 was season. Black sandshells spawn in the late summer and become gravid in early fall (Lea 1842; Sterki 1895; Ortmann 1909; Leferve and Curtis 1912; personal observation). Therefore, glochidia used in Trial 1 were in the marsupial gills four to five months longer than those glochidia used in Trial 3. Glochidia used in Trial 2 were in the marsupial gills seven to eight months longer than those used in Trial 3. Thus, there seems to be a negative relationship between the length of time glochidia are in the marsupial gills and the

time required to metamorphose into juveniles. Glochidia may be undergoing further maturation in the marsupial gills and the longer they remain in the marsupial gills the shorter the time required to metamorphose. Corwin (1920) and Tedla and Fernando (1969) also noticed that the season glochidia were obtained from two lampsiline mussels, Lampsilis luteola and L. radiata affected the duration of the attachment period. The length of time glochidia are in the marsupial gills also seems to be negatively related to the duration of the glochidial metamorphosis period.

The decline and relatively low abundance of Ligumia recta may be related to a decline in sauger runs. Many reservoirs were constructed across the United States from the 1940's through the 1960's. The closure of dams inhibits upstream migration and reduces spawning habitat for sauger. These two factors can lead to a decline in sauger populations (Nelson 1968; Nelson and Walburg 1977; Alexander 1987). Ahlstedt and Tuberville (1997) found a relatively low number of L. recta in the Clinch River above Norris Reservoir. Sauger are also rare in the upper Clinch River (Charles Saylor, Tennessee Valley Authority, personal communication). In the Licking and Barren Rivers, sauger and L. recta are present but in low numbers (Kornman 1989; Laudermilk 1993; Weiss and Layzer 1995). In contrast, sauger are relatively abundant and black sandshells are common below Pickwick Dam (Pegg et al. 1997; personal observation). Thus, the abundance of L. recta is seemingly related to the abundance of its most suitable host, sauger.

To effectively manage some mussel populations, it may be important to identify the most suitable host. Once the most suitable host is identified, artificial propagation using this host fish will be the most efficient means of producing mass quantities of juveniles for

supplemental stockings. An alternative to artificial propagation is to direct management efforts toward the conservation of suitable hosts through harvest regulations and habitat restoration. Additionally, many host species are also sportfish that are commonly stocked by management agencies. These stockings could provide a great opportunity to enhance some mussel species such as L. recta by infesting hatchery fish prior to release.

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APPENDIX

Appendix A. Modal day and number of glochidia sloughed from fish infested with glochidia of Ligumia recta.

Trial	Species	Total number of glochidia sloughed	Modal day of glochidial sloughing	Modal # of sloughed glochidia
1	Bluegill	690	1	307
1	Bluegill	1050	2	416
1	Bluegill	524	2	201
1	Bluegill	1214	4	355
1	Bluegill	1799	7	450
1	Bluegill	1401	2	565
1	Bluegill	678	2	262
1	Largemouth bass	977	2	377
1	Largemouth bass	1241	3	334
1	Largemouth bass	1321	3	627
1	Largemouth bass	1852	1	338
1	Largemouth bass	1281	2	566
1	Sauger	599*	2	328
1	Sauger	444*	1	239
1	Sauger	998*	1	563
1	Sauger	2001	1	1210
1	Sauger	1035*	1	499
1	Sauger	1316*	1	515
2	Bluegill	627	1	200
2	Bluegill	1663	1	1097
2	Bluegill	1502	1	1124
2	Bluegill	1802	1	1132
2	Bluegill	880	2	346
2	Largemouth bass	3155	1	1722
2	Largemouth bass	1617	1	808
2	Largemouth bass	3837	1	1328
2	Largemouth bass	3466	1	1942
2	Largemouth bass	1561	5	326
2	Largemouth bass	1248	3	414
3	Bluegill	2086	1	618
3	Bluegill	1533	1	570
3	Bluegill	2270	9	545
3	Bluegill	1225	1	249
3	Bluegill	1171	5	201
3	Largemouth bass	1936	2	336
3	Largemouth bass	6525	1	1865

Appendix A. continued

3	Largemouth bass	3835	2	784
3	Largemouth bass	8325	1	1989
3	Largemouth bass	7499	1	2187
3	Sauger	2250	1	1148
3	Sauger	2966	1	1596
3	Sauger	2029	1	798

* Died before end of infestation period

VITA

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Part II

Evaluation of Quarantining and Maintaining Adult Mussels from the Ohio River

INTRODUCTION

Zebra mussels (*Dreissena polymorpha*) were accidentally introduced into the Laurentian Great Lakes in the mid-1980's. This exotic species has devastated mussel populations in Lake St. Clair and western Lake Erie. The zebra mussel negatively impacts native unionids by inhibiting filtration and normal metabolic functions (Haag et al. 1993; Mackie 1993). The rapid spread of zebra mussels down the Mississippi River and into many of its major tributaries poses a significant threat to the most diverse mussel fauna in the world. Zebra mussels were first reported from the lower Ohio River in 1991. Since then they have spread upstream and have reached densities >3,600/m^2 in some areas. Captive populations of native mussels may need to be established to insure their survival. Khym et al. (1999) evaluated the feasibility of transporting and quarantining mussels collected from the Ohio River to establish a captive population. This report presents results of long-term maintenance of this captive population.

METHODS

In August 1996, mussels were collected by TVA divers while sampling below the Olmstead Lock and Dam on the Ohio River. On site, mussels were cleaned of zebra mussels, packed into 48 qt. coolers, covered with wet burlap, and held overnight in an air-conditioned room (temperature ~ 21°C). The following day the mussels were transported to the quarantine facility at

Tennessee Technological University (TTU). In all, aerial exposure of mussels lasted 18 hours. Upon arrival at the quarantine facility, each mussel was scrubbed with a brass bristle brush to dislodge any attached zebra mussel veligers. Mussels where then placed into stock tanks filled with 760 liters of water obtained from Center Hill Reservoir. Minnow agitators (1500 R.P.M.; 1/20 hp) were used in each tank to provide aeration.

During the quarantine, survival of all mussel species was monitored and all dead individuals were removed daily. The following water quality parameters were measured daily during the 30-day quarantine: water temperature, pH, conductivity, and dissolved oxygen.

At the end of the quarantine period, mussels were visually inspected for zebra mussels and then transported to an embayment in Center Hill Reservoir for long-term holding. At Center Hill Reservoir, mussels were suspended in pocket nets one meter below the surface.

RESULTS AND DISCUSSION

Overall, 91% of the mussels quarantined survived (Table 1). Although all individuals of some species such as *Ellipsaria lineolata* survived the quarantine, mortality was moderately high during the first year in captivity. However, relatively little mortality of most species occurred during the next 32 months in captivity (Table 1). All *Ligumia recta* and *Obliquaria reflexa* not only survived the quarantine, but also survived the 44 months in captivity. Moreover, both female

Ligumia recta were gravid when they were removed from Center Hill Reservoir in April, 2000.

The success of maintaining captive populations of mussels from the Ohio River over the long-term varied among species. This variation in survival rates suggests that energy reserves, nutritional needs or physical habitat requirements differ among species. Some species may naturally contain more energy reserves, and therefore have higher survival rates during quarantine and after the quarantine. For some species the holding facilities at Center Hill may be suitable in terms of nutritional requirements; for other species, it may not be suitable. In April, 2000, all surviving mussels were transferred to the Leetown Science Center.

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Table 1. Percent survival of mussels after a 30-day quarantine and over 44 months in captivity. Mussels were collected on August 15, 1996 from the Ohio River.

		% Survival			
Species	Number	Quarantine	12 months	24 months	44 months
Amblema plicata	3	100	66	66	66
Ellipsaria lineolata	13	100	54	54	54
Elliptio crassidens	9	77	77	66	44
Fusconaia ebena	255	92	55	44	33
Ligumia recta	6	100	100	100	100
Megalonaias nervosa	8	100	75	75	38
Obliquaria reflexa	4	100	100	100	100
Obovaria olivaria	14	71	50	43	43
Potamilus alatus	4	75	50	25	25
Quadrula metanevra	63	89	87	83	81
Quadrula nodulata	4	100	50	25	25
Quadrula pustulosa	29	93	79	68	62
Quadrula quadrula	16	100	69	56	56

Part III

A Design and Test of an Indoor Recirculating System for Culturing Juvenile Mussels

INTRODUCTION

Freshwater mussel populations in North America are in a state of serious decline. It is believed that approximately 67% of the nearly 300 mussel species residing in North America are either extinct or vulnerable to extinction (Williams et al. 1993) Construction of dams, poor land-use practices, and industrial and municipal pollution are all detrimental to benthic fauna (Bogan 1993). Other factors, such as the introduction of non-native species and the loss of host fish, are also negatively impacting freshwater mussels (Williams et al. 1993). Species in the Ohio River, such as the federally endangered *Plethobasus cooperianus*, are especially threatened by the invasion of the exotic zebra mussel *Dreissena polymorpha*. The National Native Mussel Conservation Committee (NNMCC) has drafted a strategy for the conservation of these species (NNMCC 1997).

This plan includes artificial propagation and culture of juvenile mussels (NNMCC 1997). In certain cases where reproduction in a population has ceased, hatchery and laboratory production of juveniles may be the only viable method for preventing losses. The Tennessee Cooperative Fishery Research Unit has successfully reared mussels in hatchery situations (Westbrook 1999; Brady 2000), but a short growing season has limited production. The development of an indoor culture system would allow culturing to take place year-round.

Methods

System Design -

Two identical indoor recirculating systems were constructed for culture of juvenile mussels. Each system is comprised of four cascading series of vinyl troughs with each series made up of four troughs 2.7 m long and 10 cm wide (Figure 1). Water is pumped by a magnetic drive pump from a 38-l reservoir to the top level of each series and returned by gravity flow at a rate of approximately 60 ml/s. Total volume of one series, including its reservoir, is 78 l. Water is aerated by falling action between levels and by an airstone in each reservoir. Water used in culture is City of Cookeville municipal water filtered using a carbon canister filter from US Filter®. Each of the four levels is illuminated by a 40 watt white, fluorescent light fixture which is set on a 13:11 h light:dark cycle.

Juvenile Mussel Propagation -

Lampsilis cardium and *Actinonaias pectorosa* were artificially propagated in the laboratory at Tennessee Technological University. Glochidia were extracted from a gravid adult mussel by pricking the water tubes with a syringe and flushing them with water. Largemouth bass *Micropterus salmoides* and Spotted bass *Micropterus punctulatas* were used as host fish. Host fish were anesthetized using MS-222 and glochidia were directly pipetted on their gills and were maintained in aquaria until excystment of the juvenile mussels. Each aquarium was siphoned routinely through a 105 μm sieve to collect newly

metamorphosed juveniles. The contents of the sieve were washed into a petri dish where juveniles were counted and pipetted into another container of fresh water.

Mussel culture -

In Trial 1, a layer of sediment 5-8 mm in depth covered the bottom of one trough. Sediment particle size was a mix of 2-4 mm and sediment less than 250 microns. Beginning 9 February 1999, a total of 4044 juvenile *L. cardium* (<24 h old) were put into the sediment over a 3 day period. Mussels were then cultured for one month.

Maintenance of the culture system included changing water, removing excess algae and feeding the mussels. Approximately half of the water in the system was changed each week by emptying the reservoir and refilling with fresh water. Excess algal growth in troughs was mechanically removed as needed. A microalgal culture of the genus *Ankistrodesmus* (250 ml) was fed to each series as a food source three times per week.

At the end of one month, the bottom of the trough was siphoned and sediment sieved to harvest the juveniles. Surviving juveniles were placed into a petri dish where they were counted, and length was measured on a subsample of 40 individuals. Juveniles were then divided into 5 groups of approximately 80 individuals each. Each group was placed into a round 250 micron mesh basket and put back in the culture system. In the bottom of each basket was a 5-7 mm layer of sediment with a particle size of 2-4 mm. Baskets were cleaned with a

wash bottle as needed and were replaced with 500 micron mesh baskets after 3 months. Growth and survival were checked monthly.

To assess growth and survival, the contents of one basket were placed into a modified McDonald's egg hatching jar. Juveniles were separated from sediment by pumping water into the jar forcing the mussels out of the jar into a collecting basket. Mussels were then placed into a petri dish, counted and measured with an ocular micrometer under a dissecting microscope. After each assessment, juveniles and sediment were placed back in their respective basket and returned to the culture system.

Trial 2 began 12 July 1999 when 1515 1-day-old *L. cardium* juveniles were placed into one of the culture troughs over a period of three days. There was a 5-8 mm layer of sediment on the bottom of the trough with a particle size of 2-4 mm. Juveniles were fed with 500 - 1000 ml of the algal culture three times weekly, but other maintenance was the same as mentioned for Trial 1. After culturing the mussels for 2 months, the trough was siphoned, and juveniles were separated from the sediment using the modified egg hatching jar. Live mussels were counted, and length was measured on a random subsample of 30 individuals using an ocular micrometer.

Beginning 10 March 1999, a total of 1700 *Actinonaias pectorosa* were stocked into the culture system at 1-day-old over a period of 1 week. A layer of sediment lined the bottom of the trough with a mixed particle size of 2-4 mm and less than 250 microns. Juveniles were cultured for three months with the feeding and maintenance schedule as mentioned for Trial 2.

On 1 June 1999, the trough was siphoned and juveniles were taken to the hatchery. Length was measured on a small number of individuals using a micrometer. Surviving juveniles were counted after one month at the hatchery, and a rough estimate was made of survival at the end of the indoor culture.

Results

In all, 2200 *L. cardium* (54.4%) were alive after one month during Trial 1. After five months in the recirculating culture system, 24.8% of the mussels were alive (Figure 2). The average length of mussels increased about 250% during the first month of culture (Figure 3). After five months of culture, mean length of the juveniles was 2.3 mm – a 10-fold increase in size. During our second trial, 32% of the mussels were alive after two months, and they averaged 1.2 mm long. About 20% of the *Actinonaias pectorosa* cultured for nearly three months survived and ranged in length from 0.8 to 1.0 mm.

Discussion

Survival and growth rates of newly-metamorphosed juveniles in our trials were similar or exceeded those reported for other culture systems (Yang 1996; Gatenby et al. 1997). Juvenile survival in our trials was comparable to that seen in two studies using floating cage culture in the Minor E. Clark hatchery raceway, but growth indoors was much slower (Westbrook 1999; Brady 2000). The slow growth indoors may be due to poor nutrition.

Although growth was slow indoors, comparable survival allows culture of juveniles to begin in February -- approximately three months before the growing season begins at the hatchery. Juveniles could be moved to the raceway in May, which is after the critical period of 30-60 days of age. They would already be 1-2 mm in length, which has advantages over stocking newly metamorphosed mussels. A cage with a larger mesh size could be used, allowing better flow of water through the cage and requiring less maintenance. Stocking larger juveniles in the hatchery in May also gives a one to two month head start on growth. Since the juveniles grow exponentially at the hatchery, this should yield substantially larger juveniles at the end of the growing season.

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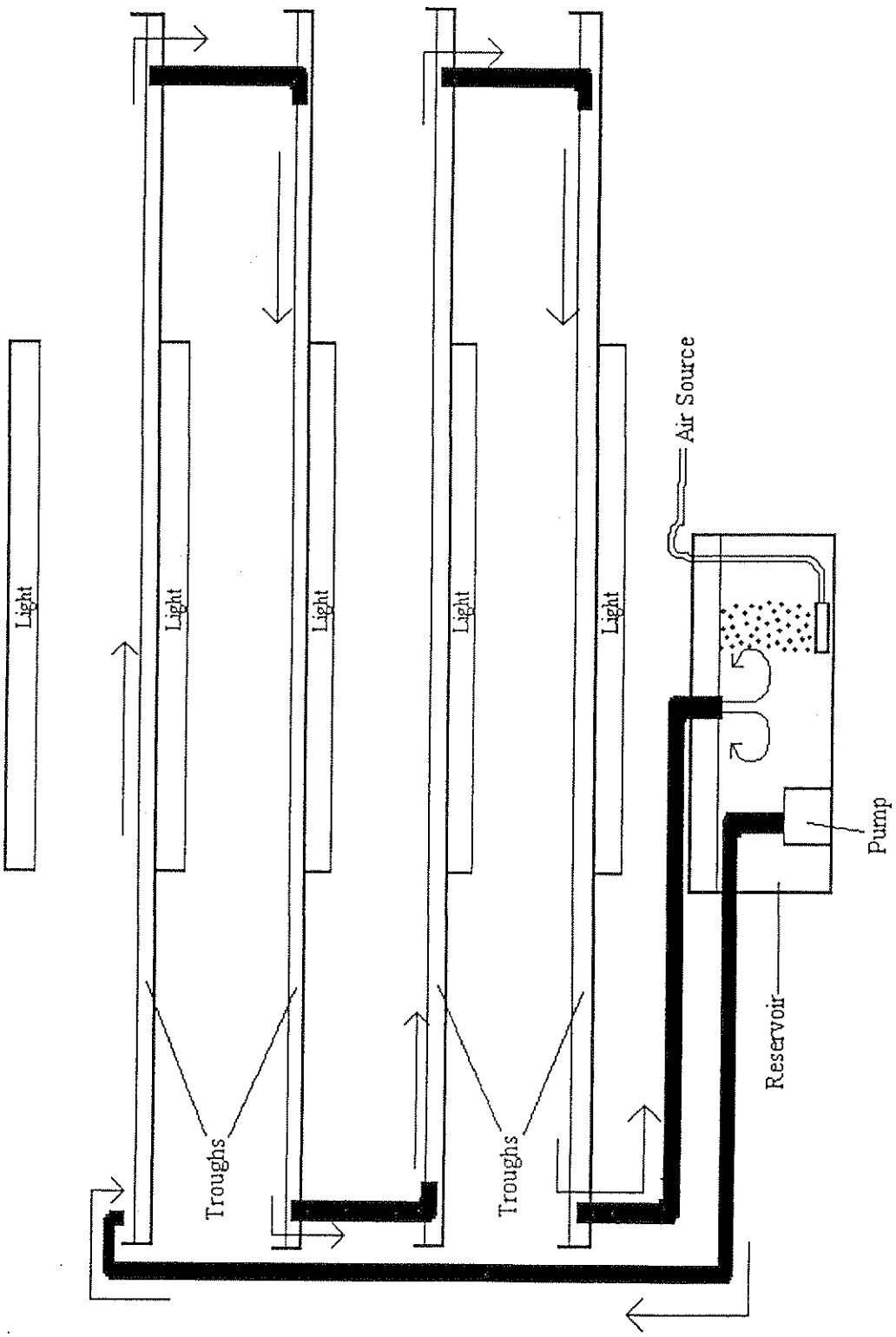

Figure 1. Diagram of sideview of one series of cascading troughs in the indoor recirculating system.

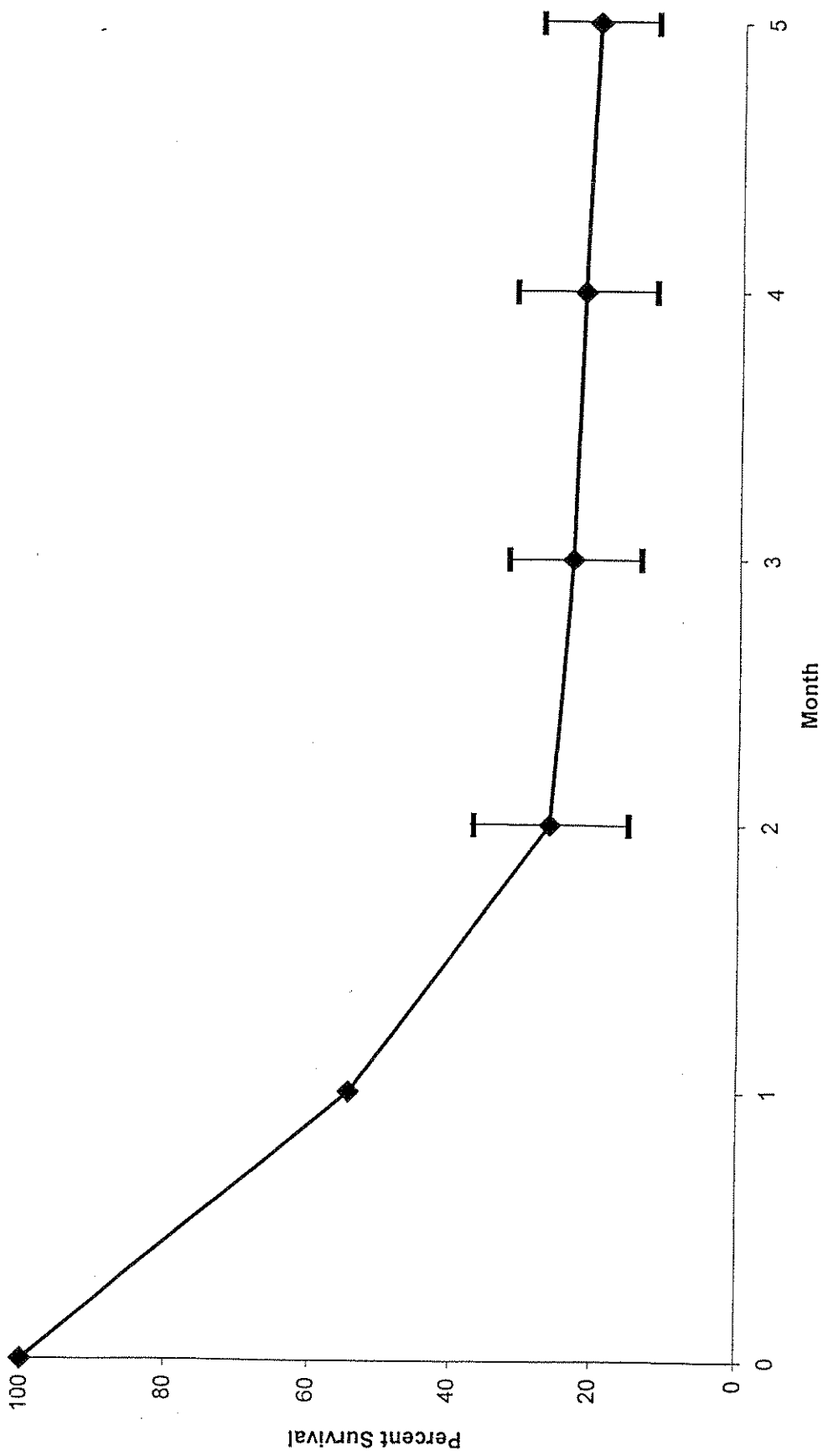

Figure 2. Survival of *Lampsilis cardium* with 95% CI in an indoor recirculating culture system (Trial 1). Juveniles were cultured in the bottom of one trough for the first month then divided into five replicates.

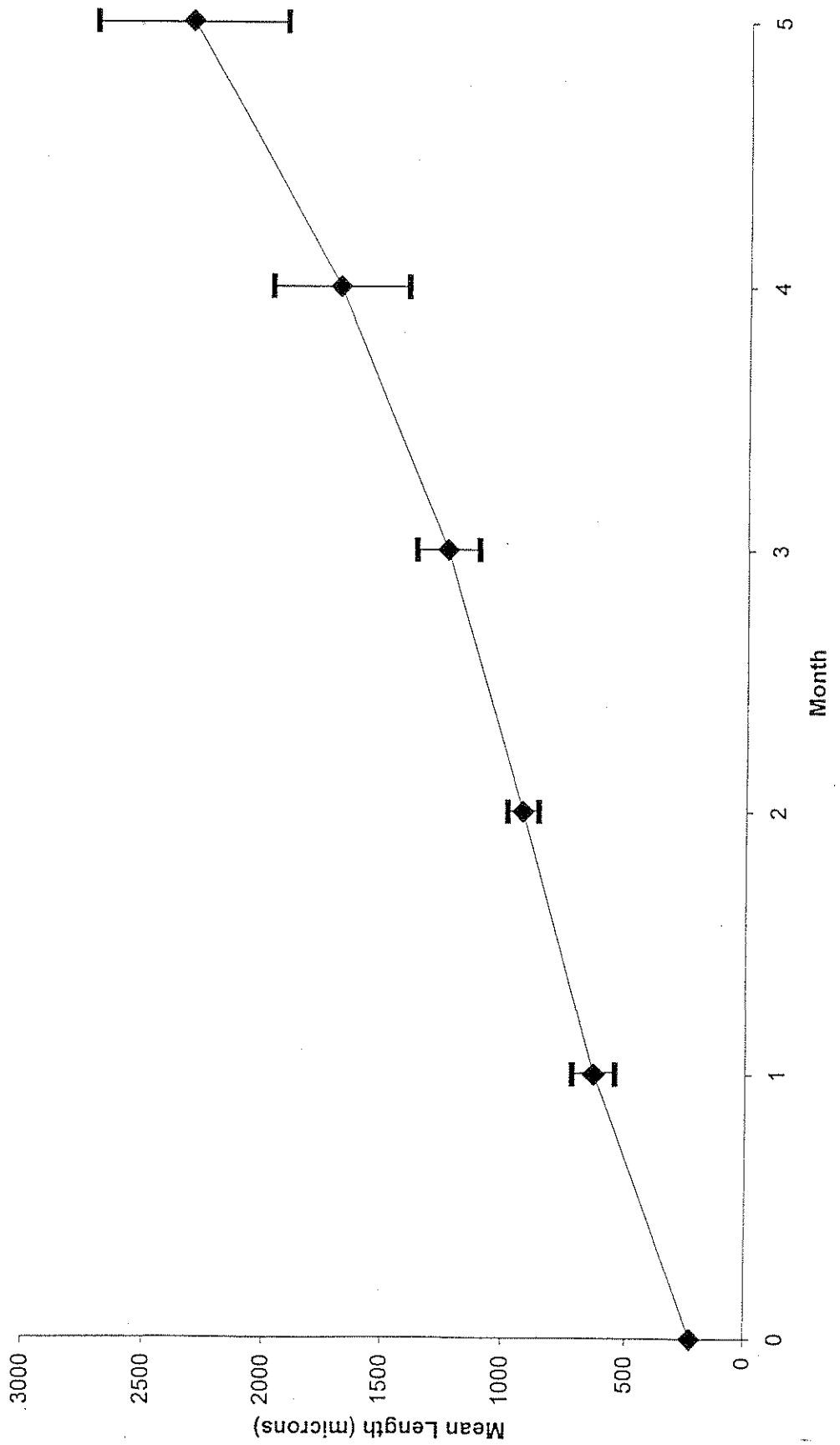

Figure 3. Growth of *Lampsilis cardium* with 95% CI in an indoor recirculating culture system (Trial 1).