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Feasibility of an Implantable Capsule for Limiting Lifespan of Grass Carp

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ABSTRACT

The grass carp (*Ctenopharyngodon idella*) is an herbivorous cyprinid stocked to control undesirable aquatic vegetation. However, stocking grass carp presents several problems including complete eradication of submersed aquatic vegetation, dispersal out of the target area, adverse effects on fish communities, and damage to waterfowl habitat and native vegetation. The purpose of this research was to consider the feasibility of an implantable capsule for limiting the lifespan of grass carp. Stainless steel dowel pins were inserted into 49 fish to identify the most appropriate site to implant the capsule. The throat region along the body's longitudinal axis was identified as the most suitable location because it resulted in minimal loss over an 8-month holding period. Rotenone solutions were injected into the ventral surface between the pelvic fins to determine the lethal dosage to 95% of the population (LD_{95}). The LD_{95} for grass carp increased curvilinearly with fish weight. Four polymers that merit further evaluation in constructing the capsule are poly[bis(*p*-carboxyphenoxy) propane anhydride], poly[bis(*p*-carboxyphenoxy) hexane anhydride], poly-l-lactide, and poly(ε-caprolactone). Implants are commonly used to deliver pharmaceutical products in medical and veterinarian applications, and have been used in fish. Developing a bioerodible capsule could increase the safety and flexibility of stocking grass carp for control of aquatic plants, and may also be applicable for management of other exotic species.

Key words: fish management, grass carp control, *Ctenopharyngodon idella*, bioerodible polymers, rotenone.

INTRODUCTION

The grass carp is an herbivorous cyprinid stocked into lentic waterbodies to control undesirable aquatic vegetation. The natural range of grass carp is in eastern China and the former Soviet Union (USSR), but it has been introduced

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worldwide into over 50 countries including the United States in 1963 (Shireman and Smith 1983). Grass carp are highly adaptable with fry withstanding salinities of 7 to 12‰ (Singh et al. 1967). Their average lifespan was reported to be about ten years in a South Carolina reservoir system (Kirk et al. 2000, Kirk and Socha 2003).

Adult grass carp are voracious consumers of preferred vegetation (Hickling 1966), and a relatively inexpensive, long-term solution to controlling nuisance aquatic vegetation (Allen and Wattendorf 1987). Triploid grass carp are generally preferred for vegetation control due to their sterility and nearly identical feeding habits to diploid grass carp (Wattendorf and Anderson 1984, Sutton 1985). However, stocking grass carp to manage aquatic vegetation presents potential risks including complete eradication of submersed aquatic vegetation (Sutton 1977, Leslie et al. 1987, Santha et al. 1991, Kirk 1992), elimination of nontarget aquatic plants, dispersal out of the target area, adverse effects on fish communities, damage to waterfowl habitat, and damage to native vegetation after fleeing the target control area, including vegetation in estuarine nursery areas (Bain 1993). Moreover, once the target vegetation has been controlled grass carp are difficult to remove with typical gears (Schramm and Jirka 1986, Leslie et al. 1987, Bonar et al. 1993, Mallison et al. 1994, 1994a), which makes it more imperative to find alternative methods for removal.

The purpose of this research was to consider the feasibility of an implantable capsule for limiting the lifespan of grass carp to control the density and dispersal of triploid grass carp released into target systems. The implantable capsule should be capable of delivering a release of toxicant within a prescribed period, say 1-3 years, to promptly euthanize the fish. The timeframe could be adjusted depending on latitude (i.e., control is expected to be faster in lower latitudes, so a short timeframe might be best); level of control desired (i.e., complete eradication may take longer, so a long timeframe might be best), and number of fish stocked (i.e., the target level of control will be achieved sooner with many fish, so a short timeframe might be best). The three main objectives of this research were to (1) establish a suitable subcutaneous position to implant a toxicant-filled capsule; (2) approximate the volume of toxicant required to euthanize grass carp; and (3) identify bioerodible polymers potentially suitable for constructing the delivery device.

MATERIALS AND METHODS

Implant Positioning

Identifying an appropriate location to implant the capsule was the first element of this study. Ease of application was considered a priority due to the large number of fish that may potentially have to be implanted with the capsule. Fish tend to encapsulate implanted objects and excrete them, so the location must prevent or show only a restricted defensive reaction by the immune system. Also, grass carp are occasionally captured by anglers so the capsule must be preferably located in a section discarded prior to consumption. Locations that were tested included the throat region anterior to the pectoral fins on the ventral surface (parallel and perpendicular to the longitudinal axis of the body), near the caudal fin, and near the anal fin (Figure 1).

Stainless steel dowel pins (McMaster-Carr, Atlanta, Georgia) 0.16 cm diameter and 1.27 cm long were inserted into anaesthetized grass carp (mean = 0.32 kg; range = 0.14 – 0.64 kg) using a pit tag applicator (AVID Identification Systems, Inc., Norco, California). Steel dowel pins represent an approximation of the expected size of the capsule. To limit the number of experimental fish, multiple capsules, typically three, were implanted in different sites of the same fish. At implantation, fish were tagged with numbered tags to facilitate identification of individuals.

In all, 49 grass carp were implanted in all three anatomical locations during two trials initiated 43 days apart. The 17 fish in trial 1 received the pin longitudinally in the throat region, whereas the 32 fish in trial 2 were split relative to throat implantation between longitudinal and perpendicular. Thus, replications for the caudal and anal region implantations were 48 each, and for the longitudinal and perpendicular throat region implantations were 27 and 22, respectively.

Fish were held indoors for 9 to 10 months in two 1,325 L tanks supplied with water via a flow-through system that exchanged the entire volume approximately three times per day. To monitor migration or expulsion of the implants, fish were X-rayed monthly with a portable machine (Model VR 8020, Vet X-ray, Inc., Boise, Idaho) adjusted to 10/80 MA/KVP 0.12 s. During X-raying, fish were anaesthetized with a 5% quinaldine solution (Sigma, St. Louis, Missouri) and examined for disease or malnutrition.

Plots of retention against time suggested that some pin losses occurred immediately after implantation, but that losses approached an asymptote as time progressed. Three models were considered to estimate the asymptote, which was of primary interest because it represents the percentage of fish that retain the implant over the long term. Model 1 (Régnière and Beilhartz 1983) considered retention (Y_R) as:

$$Y_R = P_1 + P_2 e^{P_3 T} \quad (1)$$

where P_1 is the asymptote (retention rate as time approaches infinity); P_2 a parameter that changes the magnitude of Y_R; P_3 a parameter that changes the curvature of the relationship by scaling the value of T; and T is the number of days since pin implantation. Because P_2 changes the magnitude of Y_R, P_2 and P_1 determine the intercept of the relationship (Régnière and Beilhartz 1983). Model 2 represented a Johnson type curve (Ricker 1979):

$$Y_R = P_1 e^{\left(\frac{1}{P_3 T}\right)} \quad (2)$$

where P_1, P_3, and T are as described for Model 1. Model 3 was represented by a von Bertalanffy type curve (Ricker 1979):

$$Y_R = P_1 (1 + e^{-P_3 T}) \quad (3)$$

where P_1, P_3, and T are as described for Model 1. These models were fit using nonlinear regression (PROC NLIN; SAS In-

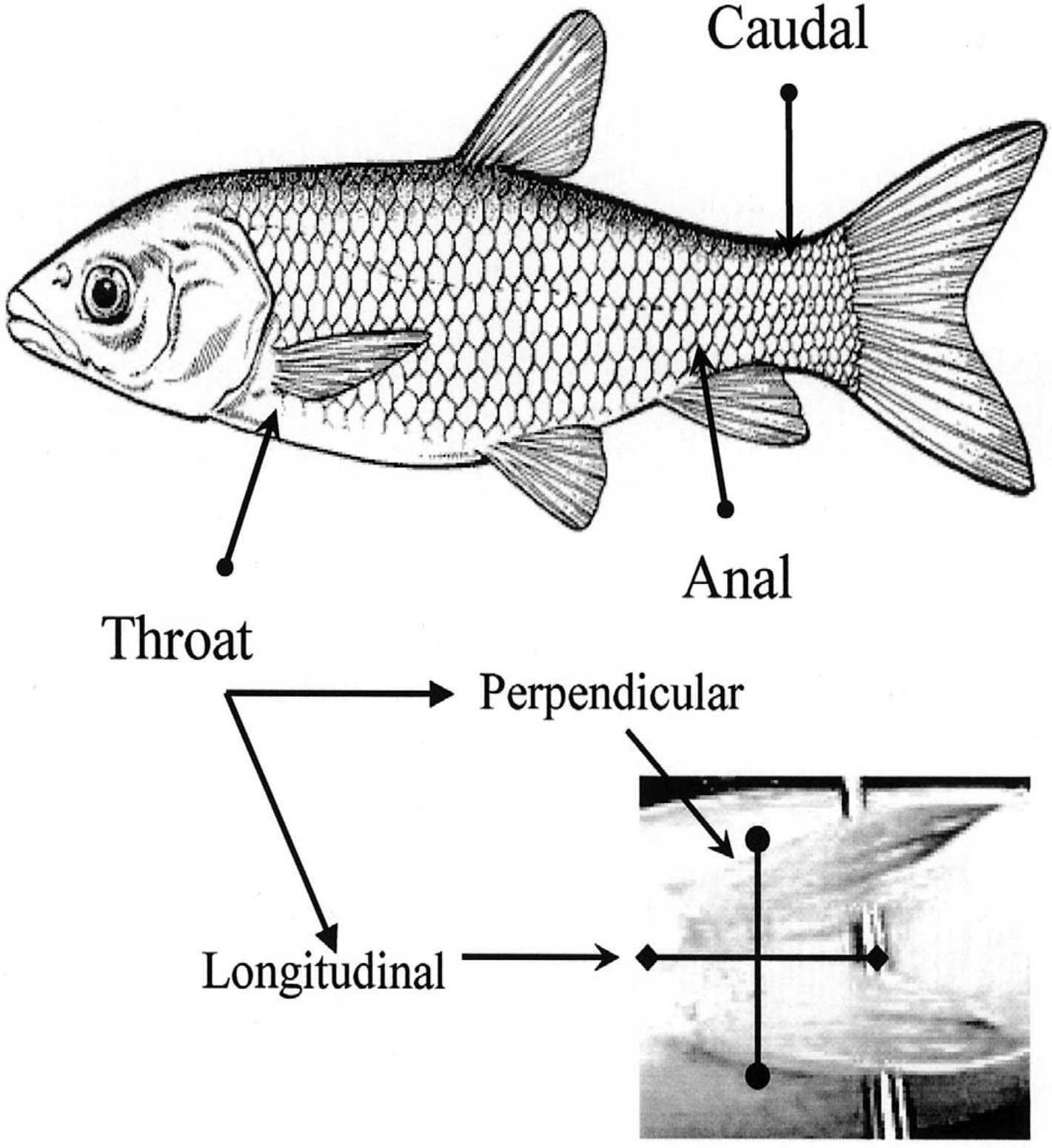

Figure 1. Pin locations tested for grass carp implanted with steel dowel pins. The throat region received pins implanted either longitudinal or perpendicular to the main axis of the body. The inset differentiates the two pin orientations corresponding to the throat-longitudinal and throat-perpendicular sites. This portrait of a grass carp was obtained from http://www.dpi.qld.gov.au/fishweb/2373.html.

stitute 1996). The most suitable model for each trial/implant location combination was assessed with the coefficient of determination (R^2) computed as (Neher and Campbell 1997):

$$R^2 = 1 - \frac{SSE}{SSCT} \quad (3)$$

where SSE is the sum of squares for the error and SSCT is the corrected total sum of squares. Values of the asymptote P_1 were compared to identify the most suitable implant location. Steel pin retention was analyzed using 80% confidence limits rather than the traditional 95% because there were no cost differences associated with different implantation locations, so identifying even small differences in retention was important.

Lethal Doses

Preliminary trials to identify potentially suitable toxicants and starting dosages were performed on channel catfish (*Ictalurus punctatus*) because they attain weights similar to grass carp and were readily available. Because the trials were conducted on channel catfish, they were not intended to identify precisely the toxicant that would most effectively euthanize grass carp. Instead, the intent was to identify a toxicant suitable for assessing the feasibility of an implantable capsule to euthanize grass carp. Rotenone (97%; Acros Organics, Fair Lawn, New Jersey), antimycin (Aquabiotics Corporation, Bainbridge Island, Washington), esfenvalerate (a pyrethroid; Sigma, Steinheim, Germany), and balyuscide (Sigma, St. Louis, Missouri) were pre-tested on channel catfish to identify which required the least volume to euthanize the fish. Each of the toxicants was dissolved in an appropriate reagent, except antimycin that was already in solution.

Initial trials with catfish identified rotenone as a promising toxicant (details in Results). Thus, in subsequent trials with grass carp we used powder rotenone (97%) dissolved in a mixture of acetone (Acros Organics, Fair Lawn, New Jersey) and the synergist piperonyl butoxide (Aldrich, Milwaukee, Wisconsin). Piperonyl butoxide was added at a 10% concentration as suggested by B. Finlayson (California Department of Fish and Game, Cordova, California, personal communication). Maximum solution of rotenone allowed by the solvent before saturation was 74.9 mg per 1 mL acetone at 24°C, consistent with Hartley and Kidd (1987). Injections were given in the ventral surface between the pelvic fins using a 1 mL needle.

Varying concentrations of rotenone were injected into grass carp ranging in body weight from 0.14 to 9.98 kg. Small grass carp (<2.5 kg) were subjected to a wide range of concentrations to identify the levels (i.e., weight of active toxicant per weight of fish) required to euthanize them within 4 hours. Once euthanizing concentrations were established for the small fish, large grass carp were subjected to dosages roughly equivalent to those effective on small fish. These large grass carp corresponded to sizes expected several years after release (Morrow et al. 1997).

Standard procedures were followed to reduce experimental error. Control fish were injected with a deionized water placebo to determine if handling and insertion procedures caused mortality. A 4-hour holding period was used to assess mortality, and fish that survived the 4-hour holding period were not used in further experiments.

The response of grass carp to the toxicant formulations was analyzed with logistic regression. The model allowed estimation of the lethal dose required to euthanize 95% of fish (LD_{95}), and accounted for differences in volume of toxicant injected as (SAS Institute 1996):

$$y = \beta_o + \beta_1 d_i + \beta_2 w_i + \beta_3 v_i \quad (5)$$

where y represented the logit of the probability of mortality; β_o the intercept; β_1 the slope parameter for d_i, the $\log_e$ of dose (milligrams of active chemical) administered to the i^{th} fish; β_2 the slope parameter for w_i, the $\log_e$ of fish weight (kg); and β_3 the slope parameter for v_i, the volume (mL) of formulation injected into the i^{th} fish. Volume injected was selected randomly to investigate if differences in volume may account for differences in responses to fixed concentrations of toxicant by possibly facilitating dispersal of the solution. Logistic regression parameters and differences in volume were considered significant at $P \leq 0.05$. Once the logistic regression model was fit, the probability of mortality (p_i) was estimated as:

$$p_i = 1 - \frac{e^y}{1 + e^y} \quad (6)$$

The dosage that resulted in a 95% probability of mortality was estimated by setting p_i to 0.95 and solving for y in equation 6, and subsequently for d_i in equation 5.

Polymer Selection

Stainless steel capsules plugged in one end with a bioerodible polymer, or a bioerodible polymer capsule, will eventually be tested as a delivery mechanism for a toxicant. An ideal polymer, aside from being biocompatible, nontoxic, and hydrophobic, is expected to contain the toxicant without leakage and bioerode or biodegrade through surface or bulk erosion to release the toxicant after a prescribed period. The latter time constraint eliminates many of the products developed for pharmaceutical use where often the objective is a continuous delivery. A literature search was conducted to identify potentially suitable polymers to construct the delivery device. The search, conducted in April and May 2003, explored the PubMed, MEDLINE®, EBSCO, CAB Abstracts, American Chemical Society Publications, and Cambridge Scientific Abstracts databases. Keywords used in search included *bioerodible polymers*, *bulk erosion + polymers*, *surface erosion + polymers*, *drug release*, *polymer erosion rates*, *crystallinity + polymers*, and *hydrophobicity + polymers*. Often, references in literature identified by the search uncovered additional literature.

RESULTS

Implant Positioning

Pin losses were recorded in both trials and all anatomical locations (Figure 2). However, rate of loss varied, and fish in

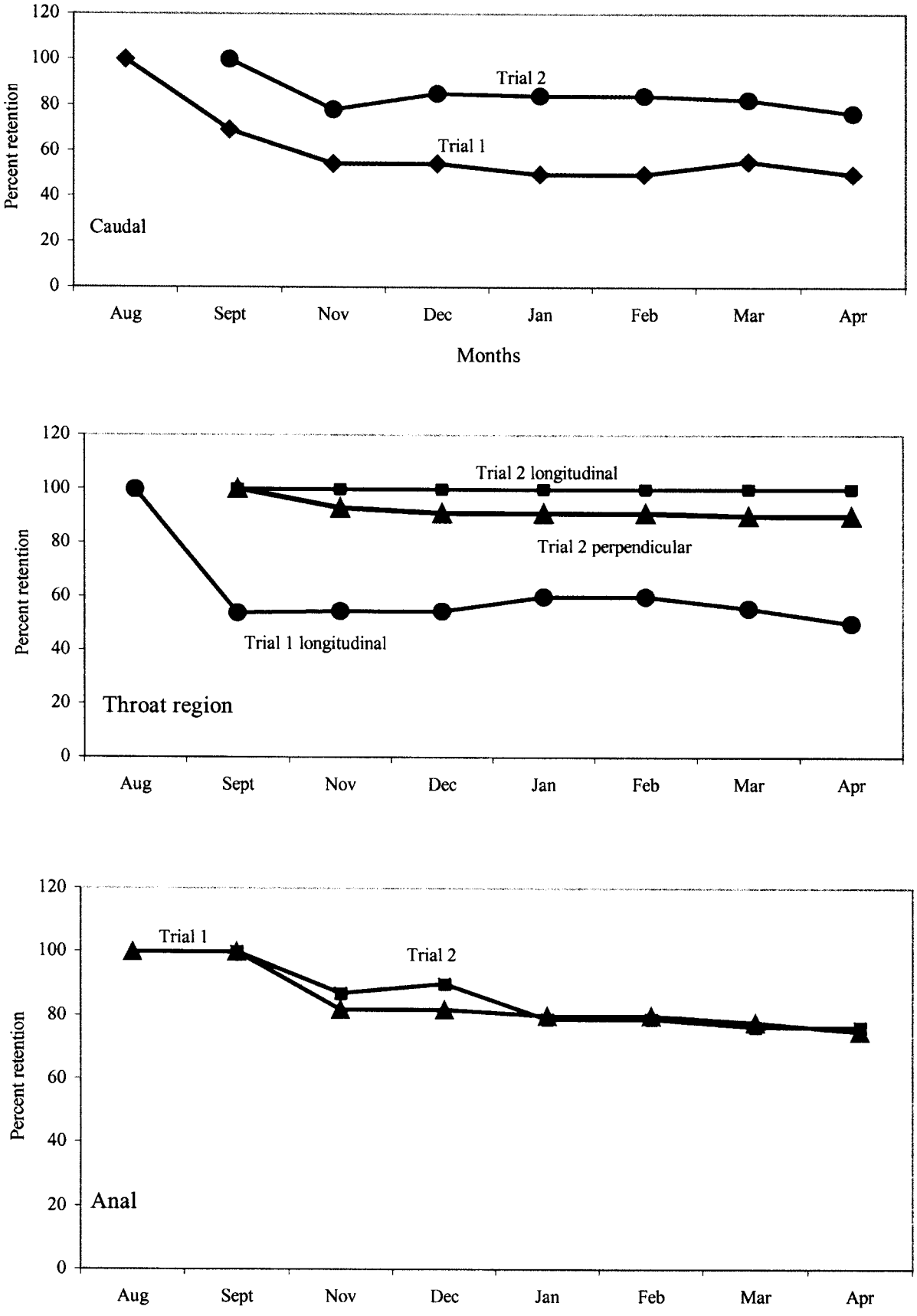

Figure 2. Retention of steel dowels implanted on grass carp at the locations shown in Figure 1.

trial 2 did not lose pins implanted longitudinally in the throat region. There generally was an initial loss of pins, and losses diminished as time progressed. Mortality during the first month after implantation was 24% for trial 1 and 19% for trial 2, and over the length of the holding period 53% for trial 1 (8 months) and 47% for trial 2 (7 months).

Pin losses were best described by models 1 and 3. These models had R^2 values ranging from 82 to 99% and were all statistically significant ($P \leq 0.005$; Table 1). Estimated asymptotes were 72% for trial 1 and 71% for trial 2 at the anal site, 52% for trial 1 and 82% for trial 2 at the caudal site, 56% for trial 1 and 100% for trial 2 at the throat site with longitudinal implantation, and 90% for trial 2 at the throat site with perpendicular implantation. Thus, pins implanted in the throat region tended to have higher retention rates, followed by those implanted in the anal region, and those in the caudal region. Statistical comparisons based on overlap of 80% confidence limits (a conservative comparison, Schenker and Gentleman 2001) indicated trial 2 pins implanted in the throat region, longitudinally or perpendicularly, had significantly greater retention than any of the other site/trial combinations.

Lethal Doses

Preliminary trials with injections of feasible volumes of several toxicants in channel catfish identified rotenone as a plausible candidate for further trials with grass carp. Trials with balyuscide and esfenvalerate indicated that the concentrated test volumes were not large enough to euthanize the test channel catfish (Table 2). Conversely, applied volumes of antimycin and rotenone produced various levels of mortality, although rotenone appeared to be most effective. Despite antimycin's reported greater toxicity when in solution (Marking and Bills 1981), it did not perform as well as rotenone when injected into the catfish's tissue. Thus, rotenone was selected for further tests with grass carp; because of the preliminary nature of these trials, they did not eliminate any of the test toxicants as unsuitable for euthanizing grass carp.

In all, 104 grass carp ranging in weight from 0.14 to 9.98 kg (mean = 2.00 kg) were injected with rotenone solutions to determine lethal doses. Lethal dosages required to euthanize grass carp ranged from 0.5 to 107.4 mg/kg (N = 73), whereas tolerated dosages ranged from 0.2 to 20.1 mg/kg (N = 20). These dosages were injected using volumes ranging from 0.10 to 2.00 mL (mean = 0.90 mL), although most fish received 1.0 mL injections. All control fish injected with the distilled water placebo (N = 11) survived the injection procedure. The relationship between dosage, fish weight, and mortality (y) was as formulated by equation 5:

$$y = 1.321 - 1.852(\log_e \text{dose in mg}) + 2.945(\log_e \text{weight in kg}) \quad (7)$$

Both slopes were significantly different from zero ($P < 0.001$). Volume was not statistically significant ($P = 0.91$) when incorporated into equation 7 along with dose and weight. Interactions between dose, fish weight, and injected volume were also not significant ($P \geq 0.45$). Lethal doses ranged from 6.6 mg/kg for 0.5 kg fish, to 38.9 mg/kg for 10.0 kg fish (Table 3). Assuming average weights of 7-9 kg by age 3 (Morrow et al. 1997), equation 7 predicts LD_{95} values of 221-329 mg, and assuming rotenone can be diluted at a rate of 74.9 mg/mL, a capsule would have to hold at least 2.9-4.4 mL of solution.

Polymer Selection

Four polymers including poly[bis(p-carboxyphenoxy) propane anhydride] (PCPP), poly[bis(p-carboxyphenoxy) hexane anhydride] (PCPH), poly-l-lactide (PLLA), and poly(ε-caprolactone) (PCL) were identified as potentially suitable for constructing a bioerodible capsule or plug for a stainless steel capsule. All four polymers were found to be biocompatible and hydrophobic, and have no or low toxicity. All exhibited surface erosion, except for PLLA that exhibited bulk erosion. Additional properties, advantages, and disadvantages of each of the polymers are summarized below; details are reported by Thomas (2004). Further selection within these four classes of polymers would require additional research.

PCPP

PCPP is a crystalline polymer with low porosity and permeability (Leong et al. 1985). The hydrophobic nature is bestowed by the presence of phenylene groups (Leong et al.

TABLE 1. ASYMPTOTES (RETENTION RATE AS TIME APPROACHES INFINITY, P_1) FOR EACH TRIAL/IMPLANT LOCATION COMBINATION. MONTHS IS THE NUMBER OF MONTHS FISH WERE HELD; MODEL IS THE BEST FIT MODEL OUT OF THREE MODELS DESCRIBED IN THE METHODS SECTION; R^2 IS THE PERCENTAGE OF ERROR EXPLAINED BY THE MODEL; F-VALUE IS THE TEST STATISTIC, AND P-VALUE IS THE PROBABILITY OF OBTAINING A LARGER F-VALUE. THE VALUES IN PARENTHESES REPRESENT THE 80% CONFIDENCE INTERVALS. P_1 VALUES FOLLOWED BY DIFFERENT LOWER CASE LETTERS ARE SIGNIFICANTLY DIFFERENT.

Trial	Implant location	Months	Model	R^2	F-value	P-value	P_1
1	Anal	8	1	0.92	27.1	0.002	72 (63-82) dc
1	Caudal	8	1	0.99	188.1	<0.001	52 (50-53) f
1	Throat-longitudinal	8	3	0.99	1256.7	<0.001	56 (54-57) e
2	Anal	7	1	0.94	28.9	0.004	71 (60-82) dc
2	Caudal	7	3	0.82	22.6	0.005	82 (80-84) c
2	Throat-longitudinal[a]	7	1				100 a
2	Throat-perpendicular	7	1	0.99	184.2	<0.001	90 (90-91) b

[a]The throat-longitudinal location implanted during trial 2 had no implant losses.

TABLE 2. DEATHS OF CHANNEL CATFISH IN PRELIMINARY TESTING TO IDENTIFY SUITABLE TOXICANTS. PARENTHETICAL VALUES REPRESENT THE RANGE OF THE PARAMETER.

Toxicant	Reagents	Catfish weight (kg)	Number of test fish	Deaths (%)	Mean dose and range (mg toxicant)	Mean dosage (mg toxicant/kg fish)	Mean volume (mL)
Antimycin	[a]	0.10-1.45	25	16	82.0 (20.0-200.0)	136.3 (14.1-518.7)	0.41 (0.10-1.00)
Balyuscide	Acetone, methanol[b]	0.20-1.45	18	0	6.3 (1.2-16.4)	12.5 (1.6-45.1)	0.62 (0.15-1.00)
Esfenvalerate	Acetone	0.60-0.90	5	0	0.9 (0.9-0.9)	1.3 (1.0-1.5)	0.20 (0.20-0.20)
Rotenone	Acetone	0.25-1.15	24	50	23.4 (1.7-73.8)	63.1 (4.1-222.6)	0.59 (0.05-1.00)
Rotenone	Ethyl acetate	0.30-0.90	9	67	14.6 (3.5-53.1)	24.4 (5.9-62.8)	0.84 (0.65-1.00)

[a]Antimycin is marketed in solution.
[b]Mixed as a 50/50 ratio.

1985), and causes the polymer to undergo surface erosion (Leong et al. 1986). Erosion rate of PCPP increases with pH, with acidic conditions stabilizing the polymer (Leong et al. 1985). Microsphere size is inversely related to erosion rate (Mathiowitz and Langer 1987). The time required to completely degrade a PCPP capsule (140 to 160 mg, 14 mm diameter, and 0.9 to 1.1 mm thick) was estimated to exceed three years (Leong et al. 1985). Diffusional escape of a test drug was negligible when the polymer was prepared via injection molding, whereas compression-molded samples demonstrated greater diffusional release (Leong et al. 1985).

PCPH

PCPH is an aromatic polymer that degrades over several years through surface erosion (Leong et al. 1985, Kipper et al. 2002). Increasing the number of methylene groups in the polymer backbone decreased reactivity and increased the hydrophobicity of the polymer (Leong et al. 1985). Mathiowitz et al. (1990) measured the structure of PCPH and found it to be about 20% crystalline. PCPH microspheres had smooth external surfaces, whereas the internal structures were solid and non-porous (Kipper et al. 2002).

TABLE 3. PREDICTED LETHAL DOSAGE TO 95% OF THE POPULATION (LD_{95}) BASED ON THE LOGISTIC REGRESSION MODEL DERIVED FOR GRASS CARP (EQUATION 7). ALSO SHOWN ARE THE 95% CONFIDENCE INTERVALS AND THE VOLUME OF SOLUTION NEEDED TO EUTHANIZE FISH.

Fish weight (kg)	LD_{95} estimate (mg rotenone/ kg fish)	95% confidence interval for LD_{95}	Volume required to euthanize (mL)[a]
0.5	6.6	(6.6-6.6)	0.1
1.0	10.1	(10.1-10.1)	0.1
1.5	12.7	(12.7-12.7)	0.3
2.0	15.1	(15.1-15.1)	0.4
3.0	19.1	(19.1-19.1)	0.8
4.0	22.7	(22.6-22.7)	1.2
5.0	25.9	(25.7-26.0)	1.7
6.0	28.8	(28.5-29.1)	2.3
7.0	31.5	(31.0-32.0)	2.9
8.0	34.1	(33.4-34.9)	3.6
9.0	36.6	(35.5-37.7)	4.4
10.0	38.9	(37.3-40.5)	5.2

[a]The solubility of rotenone for this calculation is considered to be 74.9 mg/ mL because that amount of rotenone was dissolved in acetone during a solubility trial conducted at 24°C.

PLLA

Biodegradation of PLLA and other aliphatic polyesters occurs by bulk erosion (Lewis 1990). Aliphatic polyesters are the most widely investigated and advanced polymers in terms of available toxicological and clinical data, have highly predictable degradation kinetics, and are easy to fabricate (Lewis 1990). Altering the amount of catalyst used during fabrication can control the intrinsic viscosity, a measure of molecular weight and degradation capacity (Kulkarni et al. 1966). Degradation of PLLA implants fabricated using a variety of techniques occurred in 2-8 years (Mainil-Varlet et al. 1997), illustrating the influence of chemical composition, fabrication parameters, and geometrical dimensions on degradation characteristics (Dürselen et al. 2001).

Drug stability is a particular concern in polymer matrices composed of PLLA. PLLA microspheres that undergo hydrolysis eventually break down into lactic acid, which causes pH around the microsphere to continually decrease as the polymer degrades (Shao and Bailey 2000). Creating an acidic environment might adversely affect the stability of encapsulated toxicants. Johansen et al. (1999) speculated that adding BSA (bovine serum albumine) to the polymer matrix might prevent interaction of the toxicant with the polymer or polymer solvent, which reduces the chance of toxicant degradation.

PCL

Poly(ε-caprolactone) is a semicrystalline polymer (Pitt 1990). The bulk crystalline phase is inaccessible to water and other permeants, but increasing crystallinity results in a decrease in permeability (Pitt et al. 1981a). Reducing the accessible ester bonds decreases PCL's biodegradation rate (Pitt et al. 1981a). The role of crystallinity in degradation and permeability can be used to control the degradation rate of a drug-delivery device.

Degradation of PCL involves a two-stage process. The first stage is characterized by non-enzymatic, random hydrolytic ester cleavage; the initial molecular weight and the polymer's chemical structure determine degradation time (Pitt et al. 1981b). The beginning of the second stage is characterized by a loss of weight and mechanical strength (Pitt et al. 1981b). According to Yakabe and Tadokoro (1993), the non-enzymatic cleavage reaction is the rate determining process in the biodegradation of PCL. This dependence on the cleavage reaction is attributed to the surface area of the polymer,

which is varied by the particle size of the test substance (Yakabe and Tadokoro 1993). The biodegradation rate of PCL is slow over time. The time for complete degradation of a PCL block film was reported to be approximately three years (Feng et al. 1983, Song et al. 1993).

There are a few concerns with using PCL for developing an implant for grass carp. PCL is brittle and is very permeable. PCL polymer films exposed to progesterone, testosterone, and three steroids had high drug diffusion (Pitt et al. 1979). Brittleness of implanted films and capsules is due to an increase in polymer crystallinity, associated with biodegradation (Pitt et al. 1981a). Also, enzymatic degradation has the ability to alter the degradation kinetics of PCL. Because the proposed capsule will be implanted subcutaneously in the grass carp, it will be exposed to non-enzymatic and enzymatic degradation kinetics. Gan et al. (1997) reported a decrease in crystallinity throughout the degradation process when exposed to an enzymatic, a decrease that resulted from degradation occurring throughout the polymer matrix. During the enzymatic degradation, the extent of erosion increased slowly, and pores were exposed during the later stages of the degradation process (Gan et al. 1997). We observed degradation in a magnified scale model of a tubular capsule plugged in one end with PCL and filled with a dye - after 5 to 6 months, the dye had began to stain throughout the plug, although leakage was not evident.

DISCUSSION

Pin retention was characterized by a period of initial loss followed by relatively constant retention. This pattern is similar to studies evaluating retention of implanted microtags such as integrated transponder tags and coded-wire tags (Clugston 1996, Buzby and Deegan 1999, Fries 2001). Because most of the losses occurred within the first month, it is possible that the dowel pins were forced out of an entry wound due to their shallow insertion and stiffness. Other authors have reported that tags were forced back out in this manner (Bailey et al. 1998, Buzby and Deegan 1999), and that those inserted close to the surface of the skin were more easily shed (Clugston 1996). In our study, implant location affected retention rate with throat-longitudinal implantation exhibiting the greatest retention rates. A potential advantage of this location is its proximity to the heart, which may facilitate uptake and distribution of the toxicant solution. Conceivably, additional research could identify other suitable or better-suited locations. However, it is unlikely that 100% retention could ever be achieved other than in experimental settings given the propensity of biological tissue to reject foreign objects and the occasional difficulties associated with precisely following an implantation protocol. Before stocking, implanted fish could be held (e.g., 1 month) and examined for tag loss before release, to increase the certainty that the fish released have a capsule to ensure a higher percentage of kill.

The rotenone dosages required to euthanize adult grass carp would require a large implant. Alternatives for keeping the implant small might be to increase the concentration of rotenone in solution, increasing the effectiveness of rotenone, or identifying more powerful toxicants. Reagents other than acetone might dissolve a higher rotenone concentration. According to the U.S. Environmental Protection Agency (1988) acetone is capable of dissolving 66 mg rotenone/ml at 20°C, whereas chloroform dissolves 472 mg/ml, ethylene dichloride 330 mg/ml, benzene 80 mg/ml, and chlorobenzene 135 mg/ml. The effectiveness of rotenone as a toxicant is enhanced by piperonyl butoxide, a synergist that inhibits detoxification by binding with cytochrome P450 enzymes to limit their detoxifying capabilities (Hodgson and Levi 1998). Increasing the concentration of this synergist (10% used in our study) in the rotenone solution may increase the lethality of rotenone. While piperonyl butoxide is itself moderately toxic to fish (LC_{50} = 4-6 mg/L, Osmitz and Hobson 1998), increased lethality of solutions with piperonyl butoxide are likely associated with the interaction between rotenone and the synergist (Boogard et al. 1996). Although rotenone was identified as the most suitable toxicant in preliminary tests with channel catfish, other toxicants have been shown to be more lethal. Marking and Bills (1981) measured the lethality of several toxicants to four species of carp (common carp *Cyprinus carpio*, grass carp, bighead carp *Aristichthys nobilis*, and silver carp *Hypophthalmichthys molitrix*) immersed in solutions. Antimycin was the most lethal toxicant in their study with a 96-hour LC_{50} ranging from 0.57-1.00 µg/L, followed by salicylanilide (LC_{50} 1.50-9.35 µg/L), Noxfish® (5% rotenone; LC_{50} 0.05-0.08 mg/L), and GD-174 (LC_{50} 0.05-0.55 mg/L). Grass carp was the most resistant of the four species to antimycin, salicylanilide, and rotenone (Marking and Bills 1981).

Using an implant to control the lifespan of fish is a plausible approach because implants are commonly used to deliver pharmaceutical products in medical and veterinarian applications (Kanjickal and Lopina 2004, Varde and Pack 2004), and have been used in fish. Shelton (1982) produced monosex populations of grass carp by releasing testosterone for extended periods of time through a silastic capsule implanted in the fish. Development of testes was induced in rainbow trout (*Oncorhynchus mykiss*) and pink salmon (*O. gorbuscha*) through cholesterol pelleted formulations of pituitary extract (Robertson and Rinfret 1957) and salmon gonadotropic hormone (MacKinnon and Donaldson 1978), respectively. Moreover, the reproductive cycle of migratory and landlocked Atlantic salmon (*Salmo salar*, Crim et al. 1983, Crim and Glebe 1984) has been successfully accelerated through hormonal implants made from either cholesterol or silastic elastomer, an approach determined to be as effective as frequent injections of hormones (Weil and Crim 1983).

An important concern when implanting a toxicant-filled capsule into a fish is the threat that the fish might be caught and ingested by humans. This scenario appears unlikely because grass carp are rarely caught with traditional angling tackle, but established procedures for implementing fish-consumption advisories and raising public awareness might need to be applied. Capture with commercial fishing gear is more likely, and therefore a program to inform commercial anglers and buyers would be necessary. Another safeguard would be to place the implant in a part of the carcass normally discarded if prepared for consumption, while through consumption advisories inform the public about the need to discard the parts of the carcass potentially carrying the implant. Grass carp are generally consumed as fillets or steaks, with both of these dressing procedures discarding the head, tail, and viscera. An

external tag could identify the fish as carrier of an internal tag, and warn against consumption. Perhaps the last and strongest safeguard would be designing an implant that would not be ruptured when preparing the fish for consumption.

Development of an implant to control the lifespan of grass carp is in its early stages. With current and developing technology, and proper funding, we have no doubt that our design or an alternative delayed-delivery implant design can be manufactured. Rapid progress can be achieved through collaboration with pharmaceutical scientists and bioengineers, and by conducting preliminary degradation and release tests *in vitro* before *in vivo* performance assessments. A bioerodible capsule could increase the safety and flexibility of stocking grass carp for control of aquatic plants, and may also be applicable for management of other exotic species.

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