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Evaluation of Substitute Diets for Live Algae in the Captive Maintenance of Adult and subadult Freshwater Unionidae.

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ABSTRACT

Ten non-live algal diets were evaluated as potential broodstock diets for adult and subadult unionids. These diets varied significantly in their ability to support growth, reproduction and survival. Growth, increase in glycogen stores, and limited glochidial formation were seen in most unionid species on two of the diets. However, long-term survival (>3 years) remained problematic, and the cause of mortality in these animals could not be determined. While two of the diets tested are potentially useful for supplemental feeding of adult unionids to increase glycogen levels during quarantine, or during short-term captive maintenance in the laboratory, none can be recommended without reservation for long-term maintenance because of the lack of survival after three years during this study.

INTRODUCTION

Nearly 70% of the freshwater Unionids in North America are currently facing extinction (Williams et al. 1993). Conservation efforts have focused on relocation of endangered populations, and aquaculture of recently transformed larvae. Captive maintenance of endangered animals is a common technique used to enhance and preserve species-at-risk, however freshwater unionids have proved difficult to maintain in captivity or to relocate into new habitats (Cope and Waller 1995). Most aquaculture efforts have concentrated on developing live algal diets that will support the growth and survival of larvae ($\leq$ 1year of age). A tri-algal diet has recently been produced that appears to support survival of a few species for about one year after larval transformation (see Gatenby, et al 1994; Gatenby, et al 1996; Gatenby, et al 1997). Adult unionids have rarely been kept alive for more than three years even in hatchery ponds or raceways that are fertilized to maintain algal production and seeded with the tri-algal species that have been used successfully with larvae. One problem in maintaining adult unionids is the lack of information regarding actual nutritional requirements. Recent studies have indicated that while live algae may be supplying certain key nutrients, detritus and bacteria are an important part of unionid diets (Nichols and Garling 2000).

Marine aquaculturists have experienced similar difficulties in long-term feeding of adult oysters and clams, and usually rely on natural food supplies found in offshore grow-out areas. Hatchery production of live algae is used to rear seed bivalves to planting size (1-2 mm), but is rarely used as a sole food source for adult animals due to the difficulties and costs associated with maintaining sufficient year-round supplies. In recent years

attempts have been made to replace live algae with artificial diets in order to reduce the need for expensive algal production. These artificial feeds have been successful, but this success varies with both feed type and farm operator (Coutteau and Sorgeloos, 1993). The objective of our study was to determine if similar types of non-live algal feeds could be developed to support adult unionid survival, growth, and reproduction under captive conditions.

MATERIALS AND METHODS

We tested commercial and laboratory-prepared (experimental) feeds from 1994 to 1998 on eight unionid species *Amblema plicata* (Say, 1817), *Cyclonais tuberculata* (Rafinesque, 1820), *Lampsilis fasciola* (Rafinesque, 1820), *Lampsilis ovata* (Say, 1817), *Lampsilis siliquoidea* (Barnes, 1823), *Leptodea fragilis* (Rafinesque, 1820), *Pyganodon grandis* (Say, 1829), and *Quadrula quadrula* (Rafinesque, 1820). The size and age of the animals varied and both adult and subadult animals were tested. Adult animals in our experiments were at least 5 years of age according to external annuli. Subadults animals were <3- but >1-years old based on external annuli. Shell morphometrics for all animals were measured upon arrival in the laboratory and individual tracking numbers etched onto one of the shell valves.

Unionids were held at the Great Lakes Science Center in a flow-through system of 8L rectangular aquaria, containing 10 cm of coarse gravel, with a water replacement rate of 4L/h. Baseline water quality parameters were: $CaCO_3$ of ~ 100mg/L (EDTA titrimetric method, APHA 1989); dissolved oxygen 8.0ppm (Winkler method, APHA 1989); dissolved ammonia of <0.5ppm (phenate method, APHA 1989); and a pH of 7.8 (Fisher Scientific Accumet pH meter model #AB15). Water quality parameters were

measured weekly and during any die-off of unionids. Water temperature averaged 15° C, and the light regime was on a 12 light/12 dark cycle.

Diet Formulations

We tested 10 diet formulas in this study (Table 1). Five were commercially available, and 5 were experimental mixtures. The diets were chosen on availability and/or prior successful use in culturing marine bivalves or zebra mussels *Dreissena polymorpha* (Pallas, 1771).

The five commercial diets were: dried *Chlorella sp.* (Earthrise Co., California, USA), marine algal paste *Thalassiosira pseudonana* ((Hust.)Hasle and Heimdal) (from Coastal Oyster Inc.), Hatchfry Encapsulon (30μ size particles, Argent Co. Washington, USA), fish flake food (tropical, various retailers), and a manipulated yeast diet (Artemia Reference Center, Ghent Belgium).

The five experimental diets were a combination of bacterial/ciliate cultures grown in the laboratory, commercially available products, and eggs.

Three of these diets were formulated from a bacterial/ciliate slurry based on Stuart's (1982) work on the marine bivalve *Aulacomya ater* (Molina, 1782). This was made by soaking finely ground vegetation in water for three days to encourage the growth of bacteria and ciliates and the breakdown of cellulose. Stuart used kelp as a base and we used freshwater marsh grass (*Phalaris* spp.). This formula is referred to as bacterial/slurry #1 in this paper. Bacterial slurry #2 was a mixture of 50% #1 and 50% dried *Chlorella*. Bacterial slurry #3 was a mixture of 30% #1, 30% dried *Chlorella* 10% Rich Advanced (a liquid mixture of lipids and algal growth enhancers from Sanders

Corp. Ogden Utah) and 10% Sanders Black Gold (a flake similar in composition to Rich Advanced).

We also tested a diet that we produced based on the analysis of the nutrient proportions of freshwater bivalves (Secor et al., 1993). This formula, termed "egg chow", was a mixture of 60% dried egg, 30% dextrin, 9% safflower oil, and a vitamin supplement mixed and finely ground prior to feeding.

The final experimental diet, "Langdon's recipe", was a microencapsulated feed with food particles imbedded in a gel matrix concocted according to Langdon's work on marine bivalves (Langdon and Levine, 1983; Langdon and Bolton, 1984; Buchal and Langdon, 1995).

The feeding rate of all diets was maintained at 5-8 mg diet dry weight/L of aquarium water for at least 15 hours out of the day. This rate is based on the average total organic particulate matter values found in the Huron River near a large free-living unionid bed as described in Nichols and Garling (2000).

Measuring Success of Diet Formulations

Growth and survival were the critical criteria for assessing diet success for subadult unionids; reproduction (glochidia formation) and survival were the criteria used for adult unionids, although changes in maximum shell length were recorded. In tests using adult unionids, 10 randomly selected clams were assigned to each of the ten diets for six of the eight species- *A. plicata C. tuberculata*, *L. siliquoidea*, *L. ovata*, *L. fragilis*, *Pyganodon grandis*, and *Quadrula quadrula.* Fewer *C. tuberculata* and *L. fasciola* were available

(15 individuals each) thus, only 5 adults each species were randomly assigned to three diets (egg chow, bacterial/ciliate slurry #2 and #3).

The number of subadult unionids available varied by species; *A. plicata* (75), *C. tuberculata* (15), *L. fasciola* (2), *L. ovata* (10), *L. siliquoidea* (2), *L. fragilis* (100), *P. grandis* (100), and *Q. quadrula* (50). Due to the unequal sample size, the number of individuals and number of diets tested was limited. Only two diets were tested, egg chow and slurry #3 for a period of 280 days. The distribution of individuals was as follows: egg chow *A. plicata* (27), *C. tuberculata* (7), *L. fasciola* (1), *L. ovata* (5), *L. siliquoidea* (1), *L. fragilis* (50), *P. grandis* (50), and *Q. quadrula* (18); in slurry #1 and slurry #2, *A. plicata* (5) and *Q. quadrula* (3); the remaining subadults were fed the slurry #3.

Adult unionids were measured monthly (maximum shell length to the nearest mm) and survival checked daily. Each individual subadult unionid was measured every two weeks (maximum shell length to the nearest mm) and survival checked daily. Autopsies were performed on any animals that died and tissues dissected and examined for flukes, fungus, or bacteria, or gross structural changes in appearance. Reproductive efforts were monitored by the development of glochidia in the marsupium, mantle lure behavior, and glochidial release in the test aquaria. Marsupia were examined by gently prying open the animal and visually examining the gills for obvious swelling on a monthly basis.

Changes in glycogen content were measured in an additional group of animals from January-December 1998. Twenty adult *P. grandis* were placed on egg chow and bacterial slurry #3 (10 animals each diet) in order to determine the glycogen status of all soft tissues as a measure of fitness. Glycogen content based on wet soft tissue weights was obtained using the homogenized mantle tissue and the phenol-sulfuric acid method

used by Haag et al. (1993) to assess clam fitness and reported as mg/g wet tissue. This was a whole body analysis, using 3 randomly selected animals from each diet at the beginning of the (January 2) and at the end (December 28) of the 1997 test year. All ten animals were from the same age class based on external age lines (10 years old) and had a shell length of 135-140 mm.

The statistical relationship between differences in growth rates, survival rates and glycogen concentrations of adult and subadult unionids on the various diets was determined using t-tests. Differences in growth rates between subadults of different species on different diets were analyzed using analysis of covariance (ANCOVA), a sequential analysis of the slopes and t-tests, and Bonferroni tests because of unequal sample size. Growth rate statistics were based on changes in length, not in length at T=0. Results were considered different at the $p \leq 0.05$ level. Treatments without survivors were not included in any of the analyses.

RESULTS

Adults

Of the ten diets tested on adult unionids, none can be recommended without reservation, although at least two do show potential for use in captive maintenance. Initially, all of the ten diet formulations were cleared from the water column, and directly ingested (based on fecal production) by all species of unionids. However, two of the diets caused severe stress in the animals and failed to support growth or survival for more than 30 days; six diets supported growth and survival for at least one year; three diets supported glochidia formation, and; one supported growth, reproduction, and survival but the animals died after the third year (Table 2). All diet formulations caused problems

with water quality and some mortality was more directly related to rapid changes in water quality than to diet.

The commercial marine algal paste caused severe stress in all adult animals tested with excessive mucous formation occurring which was detrimental to water quality. Within 24 hours after feeding long strands of mucus were drifting through the water column, clouding the water, and causing a sharp rise in ammonia levels to 8ppm within 48 hrs. Twenty-five percent of the unionids on this feed died within the first month, due to water quality problems. This diet was dropped from the tests after 30 days.

A second commercial diet, Hatchfry Encapsulon, was dropped from the tests after eight months although this commercial rotifer-replacement feed was initially very successful. All adult unionid species fed well on it, and growth was seen in the first three months on the diet in some species. Five of the ten *Q. quadrula* grew an average of 2 mm in the first three months, with one individual adding 4 mm of shell in that time period. Two of the ten *L. siliquoidea* grew 2 mm each and seven of the ten *Leptodea. fragilis* grew an average of 3 mm each in the first three months. No growth was seen in any animal after this time period. After the fifth month when we began using feed from a different batch, the unionids refused to eat the new feed. The feed was not ingested (no fecal matter produced). We reordered the feed to see if the problem was batch related, but the clams did not feed on the next batch either. Since the unionids continued to refuse this diet, it was dropped from the tests after an additional sixty days.

Of the three remaining commercial diets, the fish flake food and the manipulated yeast could support survival of any adult unionid species for longer than 13 months (Tables 3 and 4). None of the unionids showed any shell growth at all while on these diets.

Survival and growth was better on the last commercial feed the dried *Chlorella spp.* Unionids survived at least 15 months and showed shell deposition during the first four months, but not afterwards. Shell growth was limited to *P. grandis* and *L. fragilis*. Seven of the twenty animals showed shell growth, but less than 2 mm each over the first four months on the diet. Shell growth then ceased.

Four of the five experimental diets were successful in supporting growth and survival, but long-term survival (>3.5 years) was still problematic (Tables 2 and 4). The exception was the encapsulated feed, Langdon's recipe. This encapsulated feed (Langdon's recipe) was ingested by the clams, but caused severe stress, with animals gaping and non-responsive within 12 hours of feeding. We dropped this feed from the diet tests after 30 days and 60% mortality.

The best diet for supporting survival, growth and reproduction of adults of most species, at least up to year three, was the high-protein egg chow. The one exception was *L. fasciola*, all of whom died. At the beginning of year three, the survival rates of the adult unionids feeding on egg chow were: *A plicata* 81%, *C. tuberculata* 80%, *L. ovata* 72%, *L. siliquoidea* 65%, *Leptodea. fragilis* 64%, *P. grandis* 71%, and *Q. quadrula* 69%. Twenty-one percent of the *P. grandis* and 15% of the *L. fragilis* females formed glochidia during year 2, on this diet. However, during the third year adult unionids began to die, and by the beginning of year four all had perished. The body weight and glycogen levels (discussed below) of these animals was high, indicating that starvation was not a factor. At times soft tissue growth was so rapid that they could not completely close their shells. Autopsies showed no signs of parasitism or other disease factor, but all of these animals had greatly enlarged kidneys.

The series of bacterial/ciliate slurries differed in their ability to support adult unionid growth and survival. The bacterial/ciliate slurry #1 proved an acceptable feed for species such as *A. plicata* and *Q. quadrula*, but killed all of the *P. grandis* within a day or two of the initial feeding and 99% of the *Lampsilis* species within a few weeks. On the other hand, *Amblema plicata* and *Q. quadrula* adults survived well on this diet during the two years it was tested, but no reproductive effort was seen (Table 3).

The bacterial/ciliate slurry formulation #2, containing dried *Chlorella* provided the same basic results regarding survival and growth as was seen with the bacterial/ciliate slurry #1. Neither adult *Pyganodon grandis* nor *Leptodea fragilis* could tolerate this feed, but once again, *A. plicata* and *Q. quadrula* did well.

The basic bacterial/ciliate slurry #3 with the addition of various lipid supplements was acceptable to adults of all species. *Pyganodon grandis* did well on this diet, as did all the other unionid species tested. Survival of the adults was at 100% after one year, with the exception of *L. fasciola*, which we were not successful at handling regardless of what they were fed or handled.

Adult unionids fed egg chow and bacterial slurry #3 showed a consistent increase in glycogen levels from January 1998 to December 1998. The glycogen levels of animals on slurry #3 rose from an average of 7.2$\pm$ 1.9 mg/g in January 1998, to an average of 9.7 $\pm$2.4 mg/g by December 1998. This was not a significant increase at the $p<0.05$ but was significant if we set the alpha at $p<0.10$. During the same time period, animals on the egg chow showed a significant increase ($p<0.05$) in glycogen from an average of 7.6$\pm$ 1.1 mg/g to 11.2$\pm$ 0.5 mg/g. Glycogen concentrations of *P. grandis* that had been feeding on egg chow for at least 36 months (36-45 months) were significantly higher ($p<0.05$) than

P. grandis that had been on the diet for 12 months, averaging 14.1± 2.6 mg/g. Initial glycogen concentrations are not available for these animals.

Subadults

The growth rates of subadult unionids fed on egg chow and the bacterial slurry #3 differed more by species than by diet. *Pyganadon grandis* and *L. fragilis* grew significantly faster on egg chow and on the slurry #3 than did the other species over the 350-day period, with *P. grandis* showing an average increase in shell length of 8.7 mm and *L. fragilis* , 8.1 mm. There was no significant difference in growth rates between these species on either diet. The thick-shelled species (*A. plicata, C. tuberculata*, and *Q. quadrula*) grew significantly less, averaging 6mm. *Lampsilis siliquoidea* and *L. ovata* grew even less, averaging only 3 mm during the test period. Survival during the test period was 100% for all species except *L. fasciola*. The growth equations are presented in Figure 1. Significant differences in growth rates are as follows, with species sharing a line not significantly different ($p \leq 0.05$): A=*A. plicata*, C=*C. tuberculata*, L=*L. fragilis*, P=*P. grandis*, Q= *Q. quadrula*, E= egg chow, B= bacterial slurry.

Within diet

LB PB AB CB QB LE PE AE CE QE

Between diets

AB AE CB CE LB LE PB PE QB QE

Water Quality

Water quality was difficult to maintain in the test chambers, particularly when feeding the bacterial slurries. There were 22 episodes of water quality problems during the four years of tests. During these events, dissolved oxygen levels would plummet to <1ppm

and ammonia levels rise to >3ppm often in less than 12 hours. Mortality was seen in animals being fed the marine algal paste and the encapsulated feed during these events (25% and 60% respectively). None of the other test animals died during these events, but gaping and other signs of stress were noted.

DISCUSSION

One problem in evaluating these diets is that none of the unionids survived for longer than 3.5 years, although a couple of feeds supported growth and short-term survival ($\leq$ 3.5 years). Our data indicate that unionids are capable of feeding on a wide variety of materials, and can survive and grow for months on non-live algae diets. Long-term survival as would be needed for broodstock maintenance remains problematic. Other than survival, the criteria we selected for measuring diet success, such as growth, reproduction and glycogen concentration were not capable of predicting the gradual die-off of all test animals between 36 and 48 months. Additional criteria need to be identified so that changes in diet and environmental conditions can be implemented before mortality occurs. Long-term studies (>3 years) are necessary to truly evaluate clam diets.

The two diets that were most effective in our tests differed substantially in protein, lipid, carbohydrate, and phytosterol composition, but did not differ in their ability to support unionid growth. The bacterial/ciliate slurry #3 is a low protein (~8%), low lipid (5%), high carbohydrate (87%) feed with added algal sterols. In contrast, the egg chow is a high protein (~65%)/low carbohydrate (30%) feed that is naturally is high in cholesterol, but contains no phytosterols. The bacterial/ciliate slurry #3 was the closest in

nutritive content and physical structure to the food resources utilized by wild unionids in the Huron River (Nichols and Garling 2000). It proved acceptable to all species tested, supported significantly higher growth rates in the subadults and kept 100% of the test animals alive for the entire year of the study. The inclusion of additional materials as compared to the other bacterial/ciliate formulations, thus reducing the proportion of bacteria and ciliates in this diet, supported the survival of *P. grandis* and *L. fragilis*. The addition of extra lipids, increased the growth rate from that seen with bacterial/ciliate slurries #1 & #2. However, while promising results were obtained, the problem in whole-heartedly recommending this diet is that it was tested for only one year. It is possible that this diet, like the egg chow cannot support long-term (>3 year) survival.

Up until year three of our study, we thought the egg chow diet was a successful diet formula, it was easy to make in the laboratory, was readily ingested by all species, and supported growth, survival, glycogen storage, and limited reproduction, at least until year three. The question remains unanswered as to why long-term survival was not supported. This is a high protein feed (~65% protein), and high protein is apparently not a dietary requirement of unionids since these bivalves grew as well, or even significantly faster, on the low protein (8%) levels found in the bacterial/ciliate slurry #3 as they did on the high protein egg chow (Figs. 1 & 2). Our work on Huron River unionids indicates that they preferentially utilize a lower protein food (the <28μ fine particulate organic matter,~6.7% protein) as their main food source (Nichols and Garling 2000).

Feeding a high-protein feed such as the egg chow as a sole diet at the rate used in this study may have overfed the animals. Glycogen levels in animals fed on egg chow were far higher than those recorded by Naimo et al (1998), Naimo and Monroe (1999) and

Patterson et al. (1997) for newly caught unionids. However, food quantity may not have been the only problem. Feeding excessive dietary protein to vertebrates that normally utilize low protein feeds will support rapid growth and reproduction initially, but cause high mortality due to excess nitrogen excretion causing kidney failure after a few years. This supposition is not a direct cause-and-effect relationship in unionids. While all the dying unionids on this feed did show greatly enlarged kidneys, unionid kidneys do not excrete excess nitrogen, as do vertebrate kidneys; that function is performed by the gills. The primary function of unionid kidneys is to control ionic balance of bodily fluids. Theoretically, excess protein can affect ionic balance due to alteration of blood pH through excess amine production and stress the metabolic balance of the unionid; we could not determine cause and effect from our study. We cannot recommend the long-term use of high protein feeds for unionids. However, short-term or supplemental feeding at a lower rate than used in this study might be acceptable to prevent glycogen loss during quarantine as recorded by Patterson et al. (1997 & 1999).

There is a possibility that unionid mortality after three years in captivity relates more to environment than nutrition. Even aquaculture efforts that feed their unionids live algae report incidents of poor water quality that at times does not lead to immediate mortality (Gatenby et al. 1994; 1996). However, such events may produce sublethal stress that over a period of time and after a number of incidents may eventually kill the adult unionids. One of the greatest problems in using the types of feeds we tested is that of maintaining water quality, especially with bacterial/ciliate slurries. We experienced 22 different episodes over 3.5 years where short-term water quality problems caused obvious unionid stress (gaping) and in the case of two feeds, the marine algal paste and the

encapsulated feed, high mortality in the test animals. Usually these water quality problems were related to well pump failure, or a ruptured pipe reducing water flow over the weekend.

There are a number of other environmental factors that may produce sublethal stress, including altered flow, light, and temperature regimes. Such environmental factors need further study before we can establish captive management protocols for the various unionid species, as there will be variability in environmental tolerances. Survival was to some degree species-specific. *L. fasciola* could not be kept alive under any type of environmental condition, even though we were able to keep other *Lampsilis* species alive for several years.

Unionids can survive, grow, and even reproduce for about 3-3.5 years on non-live algal diets, but long-term survival remains problematic. Health monitoring criteria using glycogen concentrations, shell growth rates, reproduction, and survival did not provide enough warning to prevent mortality. Additional criteria to judge success or failure of captive management protocols, other than death, are needed. These types of non-live algal diets may function for supplemental feeding, but at this time, maintaining adult unionid populations in captivity will require access to natural foods and water supplies from water systems that support native unionid fauna in order to increase the likelihood of long-term survival.

Table 1. Diets fed to adult and subadult unionids.

	Dried *Chlorella*	Marine algal paste	Hatchfry encapsulon	Fish Flake Food	Yeast	Langdon's Encapsulate	Egg chow	Bacterial slurry #1	Bacterial slurry #2	Bacterial slurry #3.
Adults	+	+	+	+	+	+	+	+	+	+
Subadults							+			+

Table 2. Synopsis of ability of various diets to support growth, survival, and reproduction of both adult and subadult unionids.

	Dried *Chlorella*	Marine algal paste	Hatchfry encapsulon	Fish Flake Food	Yeast	Langdon's Encapsulate	Egg chow	Bacterial slurry #1	Bacterial slurry #2	Bacterial slurry #3.
Ingested	+	+	+	+	+	+	+	+	+	+
Ingested but caused stress		+ (1)				+ (1)				
Survived at least 6 months	+		+	+	+		+	+ (2)	+	+
Survived ~ 1 year	+			+			+	+	+	+
Survived ~2 years							+	+	(4)	(3)
Survived ~3 years							+	(4)	(4)	(3)
Showed shell growth	+		+				+	+	+	+
Initiated glochidia							+ (5)			+

(1). Stopped after one month

(2). Kills *Pyganodon grandis* almost immediately and *Leptodea fragilis* within a couple of weeks..

(3). Tested only for one year.

(4). Tested only for two years.

(5). 20% of females died after glochidial release.

Table 3. Adult unionid survival at 12 months on various diets. N=10 for each species, each diet, except for *C. tuberculata* and *L. fasciola* where N=5/diet tested.

	Unfed	Dried *Chlorella*	Hatchfry Encapsulon	Fish Flake Food	Yeast	Egg chow	Bacterial slurry #1	Bacterial slurry #2	Bacterial slurry #3
A. plicata	45%	100%	51%	25%	100%	100%	100%	100%	100%
C. tuberculata	*	*	*	*	*	100%	*	100%	100%
L. fasciola	*	*	*	*	*	0%	*	0%	0%
L. ovata	21%	100%	14%	5%	2%	100%	3%	100%	100%
L. siliqouidea	15%	100%	11%	10%	1%	100%	4%	100%	100%
L. fragilis	12%	100%	6%	0%	0%	100%	0%	100%	100%
P. grandis	32%	100%	37%	0%	0%	100%	0%	100%	100%
Q. quadrula	0%	100%	54%	0%	79%	100%	100%	100%	100%

* indicates diet not fed to that species.

Table 4. Adult unionid survival at 24 months on various diets. N=10 for each species, each diet, except for *C. tuberculata* and *L. fasciola* where N=5/diet tested. The bacterial slurry #3 was not tested for longer than one year.

	Unfed	Dried *Chlorella*	Hatchfry Encapsulon	Fish Flake Food	Yeast	Egg chow	Bacterial slurry #1	Bacterial slurry #2
A. plicata	0%	0%	0%	0%	0%	87%	78%	82%
C. tuberculata	*	*	*	*	*	89%	*	0%
L. fasciola	*	*	*	*	*	0%	*	0%
L. ovata	0%	0%	0%	0%	0%	78%	0%	0%
L. siliqouidea	0%	0%	0%	0%	0%	71%	0%	0%
L. fragilis	0%	0%	0%	0%	0%	70%	0%	0%
P. grandis	0%	0%	0%	0%	0%	72%	0%	0%
Q. quadrula	0%	0%	0%	0%	0%	75%	80%	77%

* indicates diet not fed to that species.

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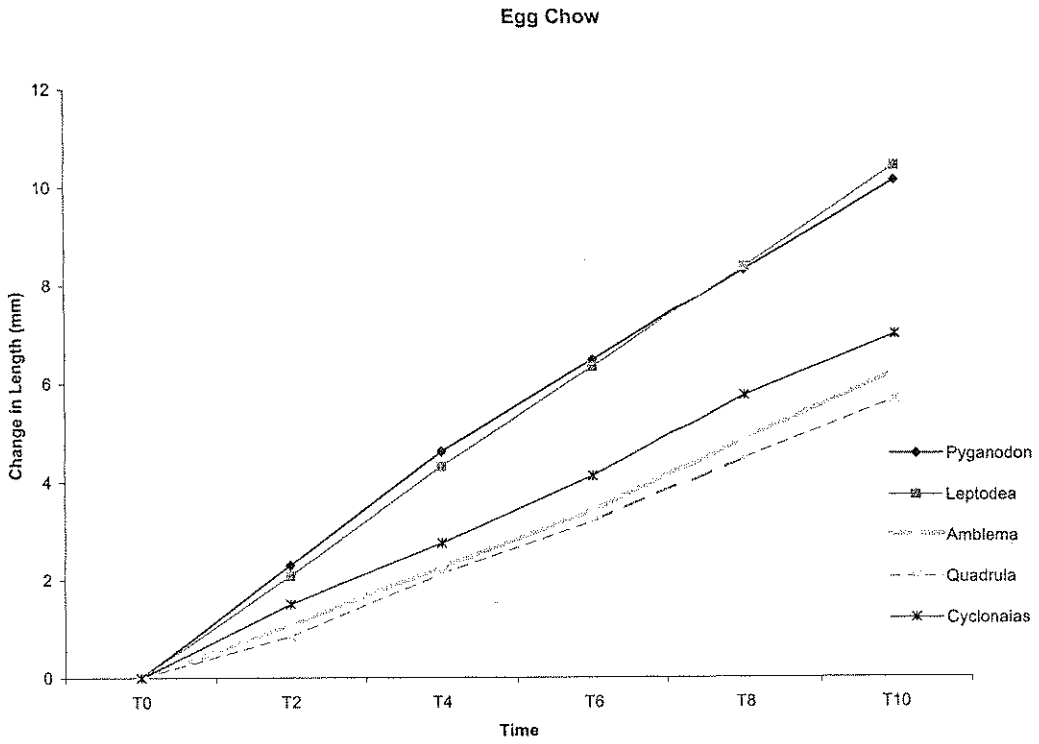
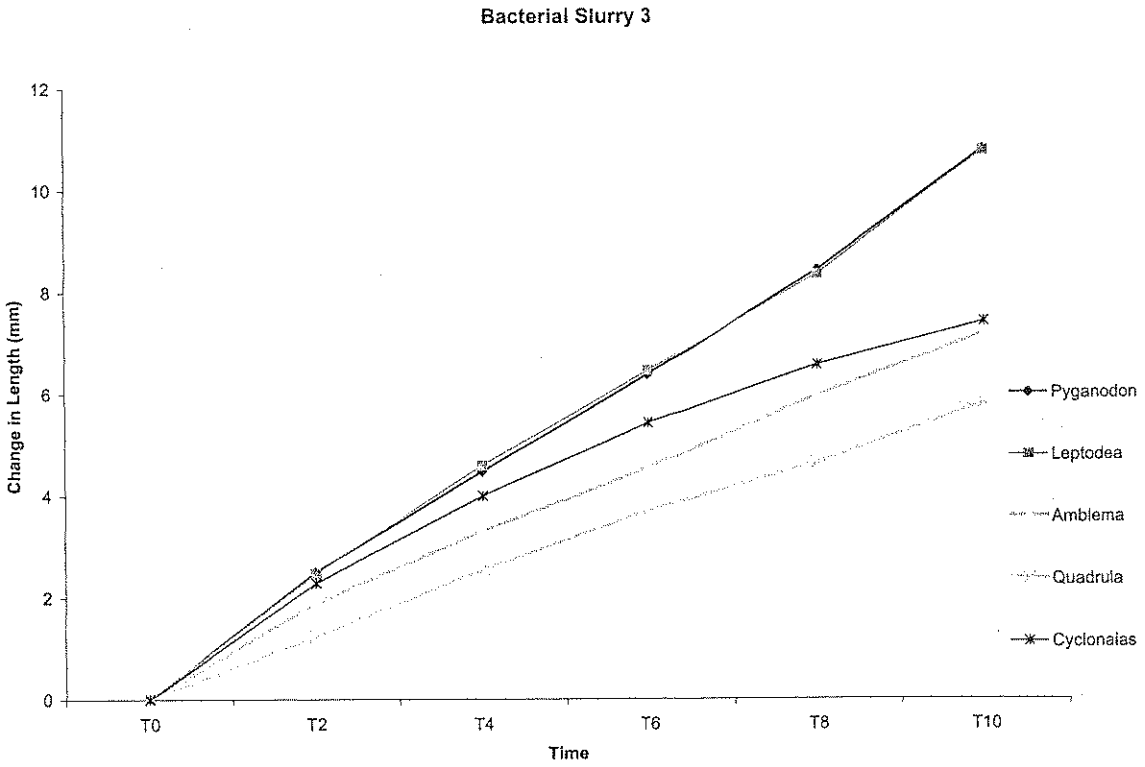
Figure 1. Comparison of growth equations of various unionid subadults fed the experimental egg chow diet over 350 days.



Ambelma plicata y=3.33677Ln(x)-0.7147 R^2=0.9095; *Cyclonais tuberculata* y=3.8123Ln(x)-0.6746 R^2=0.935; *Leptodea fragilis* y=5.7222Ln(x)-1.0147 R^2=0.941; *Pyganadon grandis* y=5.5916Ln(x)-0.8214 R^2=0.9602; *Quadrula quadrula* y=3.1152Ln(x)-0.6959 R^2=0.9112.

Figure 2. Comparison of growth equations of various unionid subadults fed the bacterial slurry #3 over 350 days.



Ambelma plicata y=3.9346Ln(x)-0.4899 R^2=0.9649; *Cyclonais tuberculata* y=4.1877Ln(x)-0.3063 R^2=0.9909; *Leptodea fragilis* y=5.5873Ln(x)-0.7751 R^2=0.9608; *Pyganadon grandis* y=5.7785Ln(x)-0.903 R^2=0.9426; *Quadrula quadrula* y=3.1988Ln(x)-0.5076 R^2=0.9533.

Abstract

- Treatments & P values?
- no results?
- conclusion?

Intro

Example of artificial diet and results in literature?
Objective is very vague

Materials & Methods

p4 - size and age of animals varied → vague - how? size?
p4 - flow-through system → water piped from where
in Marine lit, flow-through usually means water is pump from some ~~natural~~ body of water. was it a recirculating system?
p5 - combine paragraphs
- to many uses of parentheses w/in sentence
- last sentence very unclear - commas left out?

p6 - 1st paragraph - how did "egg chow" match "nutrient proportions of F10 diets"?
- Combine Ps
- 11 feeding rate of all diets"? Do diets have feeding rates?
- rate was based on $\bar{X}$ total organic particulate values in the Hudson River → describe how feeding rate and particulate matter values are linked and justify the assumption

p6 under "Measuring Success of Diet Formulations" (2)

→ "reproduction" = "glochidial formation" ?

— Last TP of p6 and 1st TP of p7

- Confusing
- were the sample sizes of n=5 big enough for adequate statistical power?

p7 1st TP — subadults

N's of some were not big enough and should be deleted from report

* what were the sample events for adults? how many were harvested? were these enough for statistical power?

Methods from p6 to p8 are very confusing

— It is hard to determine what they did and when

(3) what were the dependent variables used in the adult studies?

P8
what was used as the co-variate in the ANCOVA procedure?

"T" undefined

Results

P8 ~~onward~~ onward → were are the statistical results?

(X) the authors make results conclusions w/out support in the text

P1 "cleared from the water" — statistics?
- not mentioned in methods
- how measured?

"directly ingested" → was it in pseudofeces?

"caused severe stress" - how do they know?
- how was it measured?

P9 mortality - "directly related to rapid Δs in H_2O quality than to diet"
how do they know?

~~[redacted]~~

The above may be true, but the claims are unsubstantiated in the text → the burden of proof is on the authors.

p9 No statistical statements whatsoever!

p10 Last P → where these survival rates good? How do they know they were good?
→ No Statistical statements!

p11 1st P "no reproductive effort was seen"
→ there was a very gross-level dependent variable → Glochidial development
- if they would have looked at gametogenesis, then they probably would have obtained a very different picture

p11 3rd P → "did well on this diet"
→ substantiate?! w/statistics

p12 Statistics:
- the multiple comparisons feature should not be included in the text.

p12 under "Water quality"
- these were probably systems-based problems
i.e. - design of systems

Dick, This is a high school quality report, and should be rejected for publication in JSR.

B.

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Office of the EDITOR

Sandra E. Shumway, Ph.D., D.Sc.

February 10, 2002

Hi!

The enclosed manuscript has just been submitted to JSR. It may still be possible (if the paper passes muster!!) to include it in the upcoming issue, i.e. I need a quick turnaround on the review. If you can oblige, great. If not, not a problem, please read it when you can.

Thanks for you help,

PS Reviewer forms are with the secretary so a plain sheet or paper or email will do.

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