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# Weaning of alligator gar (*Atractosteus spatula*) larvae to artificial diets

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## Abstract

Growth performance and survival of alligator gar (*Atractosteus spatula*) larvae fed *Artemia* nauplii, two artificial diets (with different protein content and buoyancy) and a control under starvation at first feeding [5 days after hatching (5 DAH)] were studied. All larvae under starvation (C) died at 10 DAH, while survival was near 60% for the rest of the treatments at the end of the experimental period. By the end of the experimental period morphological variables (total length, snout length, wet weight and dry weight) were better in larvae fed the artificial diets compared to live food. These results were confirmed by means of nucleic acid indexes and digestive enzymatic activity. The importance of the relationship between diet size and snout gape was evidenced from these results. Differences between artificial diets were attributed to different digestibility values. Alligator gar larvae are able to grow and survive satisfactorily when fed artificial diets from the start of exogenous feeding, provided that these have an adequate size, buoyancy and quality.

**KEY WORDS**: alligator gar, artificial diets, digestibility, enzymes, nucleic acids, weaning

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## Introduction

Formulation and use of artificial diets is paramount for fish larval culture, constituting the best alternative compared to live feed (LF) which is labour intensive and expensive (Gatsoupe & Luquet 1982). However, after two decades of research on the formulation of microdiets, only limited success has been achieved due to problems related to the ingestion and digestion of these diets (Watanabe & Kiron 1994). These problems have been attributed to the incomplete functioning of the digestive system in larvae. A clear relationship has been established between the increase of digestive enzyme activity and the ability of larvae to use artificial diets; thus the replacement of LF must coincide with the maturation of the digestive system (Person-Le Ruyet 1990; Dabrowski & Culver 1991; Moyano *et al.* 1996).

The difficulty to supply high quantities of LF needed for survival and growth and the lack of adequate artificial feed has forced in some cases the premature release of the largest part of the larvae obtained from reproduction in captivity (Rosch & Appelbaum 1985). This has been a common problem during the culture of alligator gar (*Atractosteus spatula*) larvae, one of the freshwater species with the highest growth rates (Revol *et al.* 2005) and accentuated ichthyophagus behaviour from the early larval stages (Pearson *et al.* 1979). The Mexican Ministry of Agriculture's Tancol Aquaculture Center in Tamaulipas, has produced larvae since 1982; however, their rapid growth and cannibalistic behaviour have prevented the survival of gar larvae beyond the seventh day after hatching (7 DAH).

Considering this context, since 1988 a series of experiments aimed at describing different aspects of larval development have been performed. The most important results to date show that alligator gar larvae have a very fast development, characterized by a completely formed digestive tract from the beginning of exogenous feeding (5 DAH). Functional maturation of the digestive system was confirmed by different alkaline enzyme activities (trypsin, chymotrypsin, aminopeptidase) from 3 DAH, while acid (pepsin-like) activity was apparent from 5 DAH. Morphological differentiation occurs at the same time and by 15 DAH larvae acquire an adult shape. From this moment onwards growth rate becomes greater, reaching

5.6 mm day$^{-1}$ (Aguilera *et al.* 2002; Mendoza *et al.* 2002a). In this way, it was realized that gar larvae were able to ingest and digest artificial diets, provided that they are neutrally buoyant and of adequate size. This study shows the results of a series of bioassays to determine a feeding strategy suited to the particular characteristics of alligator gar larvae.

## Materials and methods

### *Experimental conditions*

Reproduction in captivity took place at the Tancol Aquaculture Center as previously described (Aguilera *et al.* 2002; Mendoza *et al.* 2002a). Just hatched larvae (1 DAH) were transported in plastic bags supplied with oxygen, to the Ecophysiology Laboratory of the Autonomous University of Nuevo Leon. Larvae were placed in 1700-L tanks until 4 DAH. At the start of the feeding trials, initial weight and total length of larvae were determined. Larvae were selected according to the uniformity of their weight and size as determined by a one-way ANOVA. At this time 6000 larvae with a mean initial weight of 27.9 ± 2 mg and a mean initial length of 15.7 ± 0.4 mm were randomly distributed into 12 500 L fibreglass tanks provided with recirculating dechlorinated freshwater in a closed system. Water flow was provided with a 1/8 hp pump and water level was adjusted by means of valves. A water volume of 30% was exchanged every third day to maintain water quality. Water temperature was maintained at 28 °C with the use of thermostats and oxygen was provided throughout the experiment by a compressor to maintain dissolved oxygen levels above 6 mg L$^{-1}$. A 12 : 12 light : dark photoperiod was maintained throughout the experimental period and light intensity at the water surface was 2000–3000 lx.

### *Diets*

A feeding experiment was conducted to determine the acceptance of artificial diets (Table 1) by gar larvae. Four treatments were tested: (i) LF consisting of recently hatched *Artemia* nauplii, as a positive control; (ii) a 453.20 g kg$^{-1}$ protein micro-extruded-marumerized (Barrows 2000) micro-spheres diet (45P) of 0.5, 0.75 and 1.25 mm diameter with variable floatability; (iii) a 518.50 g kg$^{-1}$ protein extruded diet (52P) crumbled and sieved to particles of 0.3, 0.6 and 1.18 mm diameter with variable buoyancy; and (iv) a negative control treatment (C) to which no feed was offered.

**Table 1** Composition and proximate analysis of experimental diets (45P and 52P) used

| Component (g kg$^{-1}$) | Micro-extruded-marumerized micro-spheres diet (45P) | Extruded crumbled diet (52P) |
|---|---|---|
| Moisture | 93.20 | 81.50 |
| Crude protein | 453.20 | 518.50 |
| Ether extract | 67.40 | 118.30 |
| Crude fibre | 9.90 | 7.90 |
| Ash | 116.10 | 110.80 |
| Calcium | 12.0 | 20.0 |
| Phosphorus | 10.0 | 12.0 |
| Nitrogen free extract | 238.20 | 131.0 |

### *Diet buoyancy*

Settling rate of 45P and 52P diets was evaluated by quantifying suspended solids over time (Backhurst & Harker 1988; Baskerville-Bridges & Kling 2000). Diets were previously dried in an oven at 80 °C for 12 h. A total of 2.5 g of each diet were placed in a round tank (30 cm diameter × 10 cm height) containing 5 L of freshwater at 28 °C with aeration, to simulate the turbulence commonly present in culture tanks. Aeration was discontinued after 5 min of exposure, allowing the recovery of floating feed in a filter paper, which was previously dried and weighed. The filter paper and the adhered feed particles were dried in an oven at 80 °C for 12 h. Feed weight was calculated by difference and buoyancy expressed as a percentage of feed recovered [% Buoyancy = (dry weight recovered feed*dry weight of exposed feed$^{-1}$)*100].

### *Experimental design*

Each test diet was fed to three replicate tanks with 500 alligator gar larvae per tank. *Artemia* nauplii and artificial diets were fed *ad libitum* to 5 DAH larvae (initial feeding day) in four daily rations. Adjustment of feed particle size (for both artificial diets) was made at 10 and 15 DAH. The experiment was finished after 15 days of feeding (20 DAH). A set of tanks was also assigned for the control treatment (C), to which no food was proffered.

Samples of six larvae per tank were collected at 5, 10 and 15 DAH, while 20 larvae per tank were collected at the end of the experimental period at 20 DAH. Samples were taken every morning before first feeding. Larvae were euthanized with tricaine methane sulfonate (MS222), blotted dry, and then weighed to the nearest 0.01 g. Standard length and snout length (frontal snout margin to eyes frontal margin) were recorded for each larva using a caliper rule with 0.05 mm precision. Larvae were dissected on a cold plate

(4 °C). The head was separated at the operculum, the digestive tracts removed and pooled together by treatment to determine the enzymatic activity. The remaining carcasses were individually freeze-dried and were used for further nucleic acid determination.

### Nucleic acid analysis

Nucleic acids were determined according to a modification of the Schmidt–Thanhauser–Schneider method (Ordonio 1995). All procedures were carried out at 4 °C as suggested by Nakano (1988). Each individual carcass was homogenized in 2 mL of distilled water using a 2 mL Wheaton-glass homogenizer. Nucleic acids were determined using 1 mL of the homogenate and the rest was stored at −20 °C for protein analysis. Quantification of nucleic acids was compared to a standard curve established from salmon testes type III DNA (SIGMA D1626*Lot 44H77020) and from yeast type III RNA (SIGMA D7125*Lot 102H7045).

### Protein determination

Soluble protein was determined using the Bradford (1976) protein assay with bovine serum albumin (BSA) as the standard.

### Enzymatic activities

Digestive tracts were homogenized in distilled water 1 : 5 (w/v) using a Wheaton-glass homogenizer at 4 °C. The resulting homogenates were centrifuged at 15 300 ***g*** during 15 min at 4 °C. Supernatants were stored in 0.5 mL aliquots at −20 °C to later be used as enzymatic extracts. All assays were performed in triplicate. Assays were conducted at 25 °C. The reaction mixtures consisted of 0.1 mL of enzymatic extract and 1 mL of substrate dissolved in specific buffers for each assay. Haemoglobin, dissolved in universal buffer (pH = 3), was used as substrate for measuring pepsin activity (Anson 1938). Azocasein was used to measure total alkaline proteases activity (Galgani & Nagayama 1986), while BAPNA, was used for trypsin activity, (Erlanger *et al.* 1961). Tris–HCl 50 mM, $CaCl_2$ 20 mM (pH = 8.2) buffer was used to dissolve substrates for alkaline proteases. Enzymatic activities were expressed as the increase of absorbance per minute per larva.

### Diet digestibility

Enzymatic extracts of 10 DAH larval digestive tracts were used to determine *in vitro* digestibility using the protocol described in Alarcon *et al.* (1999). Both artificial diets (45P and 52P) were homogenized in distilled water (100 mg $mL^{-1}$) and centrifuged at 21 000 ***g*** for 20 min, with the supernatant used as the substrate in the reaction mixture. Haemoglobin and casein at 1% were used as reference substrates to test acid and alkaline activities, respectively. The reaction mixture consisted on 0.5 mL substrate, 0.5 mL sodium acetate-HCl buffer (0.1 M, pH 3.0) and 0.5 mL enzymatic extract. The mixture was then incubated at 37 °C for 30 min and the enzymatic reaction was stopped with cold 12% trichloroacetic acid (TCA). A control tube to which TCA was added before incubation was used as a reference. Samples were centrifuged at 9520 g, at 4 °C for 5 min and non-digested material was discarded. Tris–HCl (50 mM, pH 8.5) buffer was used for estimating alkaline activity under the previously described conditions. The difference in absorbance at 280 nm between test tubes and the control was recorded. Digestibility, as a result of released amino acids and peptides, was expressed as a direct function of absorbance at 280 nm.

### Variables and statistical methods

Survival was evaluated at 10, 15 and 20 DAH. Morphometric variables (total length (TL), snout length (SL), wet weight (WW), dry weight (DW) and specific growth rate (SGR) = [100(ln $WW_2$ − ln $WW_1$) per no. of days]) were recorded. Different condition factors [RNA $DNA^{-1}$, DNA $larvae^{-1}$, RNA $larvae^{-1}$, DNA DW $(mg)^{-1}$ and protein larvae $(mg)^{-1}$] were determined using individual larvae of each treatment. Enzymatic activities were determined running three independent assays of the extracts for each treatment. Statistical analyses were performed using the SPSS 9.0 software, Chicago, IL, USA. Data were submitted to Kolmogorov–Smirnov normality test. Significant differences were determined by using one-way ANOVA analysis. The new Duncan multiple-range test (Steel & Torrie 1980) was used to identify differences among mean values at the 0.05 level. For comparison of mortalities among treatment values, percentage mortality was subjected to arcsin transformation (Zar 1984) and the resulting data were subjected to ANOVA as above.

## Results

### Buoyancy

Floatability of 52P diets was 64.8%, 66.4% and 65.6% for 0.3, 0.6 and 1.18 mm particles sizes, respectively. In contrast, buoyancy of 45P diets was 43.4%, 49.0% and 46.7% for 0.5, 0.75 and 1.25 mm sizes, respectively.

### *Survival*

At 10 DAH, survival was above 90% for larvae in all treatments, including the control under starvation (C). At 15 DAH, treatments 52P, 45P and LF still showed a high survival rate (near 90%), whereas all larvae had died in the control treatment. From 15 to 20 DAH cannibalism was the main cause of mortality in the rest of the treatments. Cannibalism often caused death of both, predator and prey. Predators died due to the inability to completely ingest and digest the prey. Survival at the end of the experiment (20 DAH) was near 60% for the three remaining treatments and no significant differences among them were registered.

### *Morphometric variables*

Experimental variables (TL, SL, WW, DW and SGR) reflected the differences among treatments at each sampling date (Table 2). Significant differences were evident among diet treatments at 10 DAH. A better performance could be observed in both the 45P and LF treatments, followed by 52P and C. At 15 DAH, significant differences among all treatments were observed. Treatment 45P showed the best performance, followed by 52P and LF consecutively, while the control treatment still exhibited the lowest performance. At 20 DAH, significant differences prevailed among treatments, with exception of the control in which all larvae were already dead. Larvae fed the artificial diets showed the best performance.

### *Nucleic acid indexes*

Some of the condition factors based on nucleic acids (Table 3) showed similar trends to morphometric variables, particularly those based on RNA concentration (RNA larvae$^{-1}$ and RNA DNA$^{-1}$). RNA larvae$^{-1}$, DNA larvae$^{-1}$ and protein larvae$^{-1}$ indexes allowed the observation of significant differences among treatments at 10 DAH, confirming the best performance of 45P and LF treatments at this time. It is noteworthy that performance of LF started to decline at 15 DAH, while 52P started a steady recovery. Finally, at 20 DAH, the RNA larvae$^{-1}$ and RNA DNA$^{-1}$ indexes, exposed the similar performance of both artificial diets. An exception was the index DNA DW$^{-1}$ which revealed a significant difference between artificial diets, showing a lower amount of DNA per unit of dry weight of 45P compared to 52P.

### *Digestive enzymes*

At 10DAH, total alkaline and trypsin proteolytic activities were significantly higher in LF treatment larvae, followed by those of artificial diets treatments (Table 4). Treatment 45P showed the higher total acid enzymatic activity, although it was not significantly different from that of LF. At 15 and 20 DAH, total alkaline activity became significantly higher for 52P followed by 45P and LF. From 15 DAH total acid activity was significantly higher for 45P and 52P treatments, with 45P showing the highest activity, followed by 52P and

**Table 2** Morphological variables of alligator gar larvae fed two artificial diets (45P and 52P) a live food diet and starved (control) at 5, 10, 20 days after hatching (DAH)

| DAH | Variable | 52P | 45P | Live food | Control |
|---|---|---|---|---|---|
| 5 | Total length (mm) | 15.4 ± 0.86 | 15.4 ± 0.86 | 15.4 ± 0.86 | 15.4 ± 0.86 |
| | Snout length (mm) | 1.1 ± 0.12 | 1.1 ± 0.12 | 1.1 ± 0.12 | 1.1 ± 0.12 |
| | Wet weight (mg) | 29.1 ± 2.2 | 29.1 ± 2.2 | 29.1 ± 2.2 | 29.1 ± 2.2 |
| | Dry weight (mg) | 1.1 ± 0.25 | 1.1 ± 0.25 | 1.1 ± 0.25 | 1.1 ± 0.25 |
| 10 | Total length | 22.3 ± 1.4$^{b}$ | 23.5 ± 2.0$^{a}$ | 23.6 ± 0.7$^{a}$ | 18.9 ± 1.5$^{c}$ |
| | Snout length | 2.21 ± 0.13$^{b}$ | 3.34 ± 0.37$^{a}$ | 3.35 ± 0.37$^{a}$ | 1.39 ± 0.15$^{c}$ |
| | Wet weight | 67 ± 19$^{b}$ | 84 ± 21.5$^{a}$ | 77 ± 7.6$^{a}$ | 35 ± 3.5$^{c}$ |
| | Dry weight | 3.49 ± 0.77$^{b}$ | 4.31 ± 1.26$^{a}$ | 4.27 ± 0.75$^{a}$ | 2.06 ± 0.81$^{c}$ |
| | SGR (%) | 26.2 ± 0.25$^{b}$ | 31.2 ± 7.5$^{a}$ | 31.6 ± 4.9$^{a}$ | 12.6 ± 7.04$^{c}$ |
| 15 | Total length | 31.5 ± 2.9$^{b}$ | 34.1 ± 3.6$^{a}$ | 28.2 ± 1.3$^{c}$ | 19.7 ± 0.8$^{d}$ |
| | Snout length | 4.47 ± 0.50$^{b}$ | 5.22 ± 0.63$^{a}$ | 4.0 ± 0.45$^{c}$ | 1.95 ± 0.11$^{d}$ |
| | Wet weight | 157 ± 41$^{b}$ | 247 ± 75$^{a}$ | 112 ± 21$^{c}$ | 33 ± 4$^{d}$ |
| | Dry weight | 10.1 ± 3.1$^{b}$ | 15.8 ± 6.1$^{a}$ | 7.13 ± 1.0$^{c}$ | 1.44 ± 0.24$^{d}$ |
| | SGR (%) | 20.5 ± 5.9$^{b}$ | 24.8 ± 7.17$^{a}$ | 10.0 ± 3.12$^{c}$ | −7.3 ± 3$^{d}$ |
| 20 | Total length | 42.1 ± 5.3$^{b}$ | 46.1 ± 6.3$^{a}$ | 30.5 ± 3$^{c}$ | |
| | Snout length | 6.4 ± 0.16$^{b}$ | 7.0 ± 0.18$^{a}$ | 4.33 ± 0.48$^{c}$ | |
| | Wet weight | 420 ± 178$^{b}$ | 547 ± 229$^{a}$ | 127 ± 51$^{c}$ | |
| | Dry weight | 32.8 ± 12.2$^{b}$ | 46.2 ± 24.5$^{a}$ | 7.2 ± 3.5$^{c}$ | |
| | SGR (%) | 19.0 ± 7.0$^{a}$ | 18. 8 ± 10.0$^{a}$ | −1.02 ± 7.0$^{b}$ | |

Specific growth rate (SGR) = [100(ln $WW_2$ − ln $WW_1$) per no. of days].
Values are mean ± SD of three replicates.
Mean values within a row with the same superscript are not significantly different ($P > 0.05$).

**Table 3** Condition factors based on nucleic acids of alligator gar larvae fed two artificial diets (45P and 52P) a live food diet and starved (control) at 5, 10, 20 days after hatching (DAH)

| DAH | Variable | 52P | 45P | Live food | Control |
|---|---|---|---|---|---|
| 5 | DNA larva$^{-1}$ (μg) | 8.9 ± 2.0 | 8.9 ± 2.0 | 8.9 ± 2.0 | 8.9 ± 2.0 |
| | RNA larva$^{-1}$ (μg) | 67 ± 10 | 67 ± 10 | 67 ± 10 | 67 ± 10 |
| | RNA DNA$^{-1}$ | 7.8 ± 1.4 | 7.8 ± 1.4 | 7.8 ± 1.4 | 7.8 ± 1.4 |
| | DNA DW (mg)$^{-1}$ | 8.9 ± 2.0 | 8.9 ± 2.0 | 8.9 ± 2.0 | 8.9 ± 2.0 |
| | Protein larva (mg)$^{-1}$ | 0.32 ± 0.17 | 0.32 ± 0.17 | 0.32 ± 0.17 | 0.32 ± 0.17 |
| 10 | DNA larva$^{-1}$ (μg) | 18.2 ± 5.5$^{b}$ | 24.4 ± 8.7$^{a}$ | 24.0 ± 3.0$^{a}$ | 15.0 ± 6.3$^{b}$ |
| | RNA larva$^{-1}$ (μg) | 235 ± 52$^{b}$ | 314 ± 123$^{a}$ | 293 ± 49$^{b}$ | 117 ± 31$^{c}$ |
| | RNA DNA$^{-1}$ | 13.2 ± 3.6$^{a}$ | 13.0 ± 1.9$^{a}$ | 12.5 ± 3.2$^{a}$ | 8.4 ± 2.6$^{b}$ |
| | DNA DW (mg)$^{-1}$ | 5.0 ± 1.3$^{b}$ | 5.4 ± 0.8$^{b}$ | 5.4 ± 0.9$^{b}$ | 8.0 ± 2.5$^{a}$ |
| | Protein larva (mg)$^{-1}$ | 0.41 ± 0.19$^{b}$ | 0.49 ± 0.18$^{b}$ | 0.7 ± 0.16$^{a}$ | 0.26 ± 0.13$^{c}$ |
| 15 | DNA larva$^{-1}$ (μg) | 49.8 ± 22$^{b}$ | 68.5 ± 9.7$^{a}$ | 32.9 ± 11.7$^{c}$ | 14.8 ± 5.3$^{d}$ |
| | RNA larva$^{-1}$ (μg) | 542 ± 224$^{ab}$ | 896 ± 224$^{a}$ | 408 ± 155$^{b}$ | 70 ± 24$^{c}$ |
| | RNA DNA$^{-1}$ | 12.2 ± 5.3$^{a}$ | 13.1 ± 3.0$^{a}$ | 13.3 ± 6.1$^{a}$ | 5.2 ± 2.0$^{b}$ |
| | DNA DW (mg)$^{-1}$ | 4.9 ± 1.8$^{b}$ | 4.9 ± 1.13$^{b}$ | 4.6 ± 1.7$^{b}$ | 9.7 ± 3.0$^{a}$ |
| | Protein larva (mg)$^{-1}$ | 2.7 ± 1.1$^{b}$ | 4.0 ± 1.4$^{a}$ | 2.3 ± 0.5$^{b}$ | 0.13 ± 0.07$^{c}$ |
| 20 | DNA larva$^{-1}$ (μg) | 106 ± 32$^{a}$ | 104 ± 30$^{a}$ | 31 ± 7$^{b}$ | |
| | RNA larva$^{-1}$ (μg) | 1052 ± 542$^{a}$ | 1360 ± 791$^{a}$ | 149 ± 70$^{b}$ | |
| | RNA DNA$^{-1}$ | 10.5 ± 5.6$^{a}$ | 12.7 ± 6.5$^{a}$ | 4.6 ± 1.0$^{b}$ | |
| | DNA DW (mg)$^{-1}$ | 3.1 ± 0.5$^{b}$ | 2.7 ± 0.6$^{c}$ | 4.8 ± 2.0$^{a}$ | |
| | Protein larva (mg)$^{-1}$ | 7.7 ± 2.6$^{a}$ | 7.4 ± 2.1$^{a}$ | 2.0 ± 1.14$^{b}$ | |

Values are mean ± SD of three replicates.
Mean values within a row with the same superscript are not significantly different ($P > 0.05$).

**Table 4** Total alkaline, total acid and trypsin-like enzymatic activity of alligator gar larvae fed two artificial diets (45P and 52P) a live food diet and starved (control) at 5, 10, 20 days after hatching (DAH)

| DAH | Enzymatic activity | 52P | 45P | Live food | Control |
|---|---|---|---|---|---|
| 5 | Total alkaline activity | 0.02 ± 0.25 | 0.02 ± 0.25 | 0.02 ± 0.25 | 0.02 ± 0.25 |
| | Total acid activity | 1.15 ± 0.04 | 1.15 ± 0.04 | 1.15 ± 0.04 | 1.15 ± 0.04 |
| | Trypsin like activity | 0.12 ± 0.05 | 0.12 ± 0.05 | 0.12 ± 0.05 | 0.12 ± 0.05 |
| 10 | Total alkaline activity | 0.16 ± 0.02$^{b}$ | 0.15 ± 0.04$^{b}$ | 0.30 ± 0.08$^{a}$ | 0.02 ± 0.001$^{c}$ |
| | Total acid activity | 2.6 ± 0.30$^{b}$ | 3.1 ± 0.04$^{a}$ | 3.0 ± 0.02$^{a}$ | 0.99 ± 0.06$^{c}$ |
| | Trypsin like activity | 0.46 ± 0.01$^{b}$ | 0.44 ± 0.04$^{b}$ | 0.61 ± 0.04$^{a}$ | 0.14 ± 0.02$^{c}$ |
| 15 | Total alkaline activity | 1.14 ± 0.20$^{a}$ | 0.88 ± 0.10$^{b}$ | 0.52 ± 0.001$^{c}$ | 0.02 ± 0.001$^{d}$ |
| | Total acid activity | 2.8 ± 0.03$^{b}$ | 3.3 ± 0.10$^{a}$ | 2.1 ± 0.10$^{c}$ | 0.86 ± 0.03$^{d}$ |
| | Trypsin like activity | 1.19 ± 0.02$^{a}$ | 0.85 ± 0.09$^{b}$ | 1.14 ± 0.02$^{a}$ | 0.13 ± 0.005$^{c}$ |
| 20 | Total alkaline activity | 1.45 ± 0.03$^{a}$ | 1.23 ± 0.05$^{b}$ | 0.95 ± 0.02$^{c}$ | |
| | Total acid activity | 3.7 ± 0.06$^{b}$ | 4.14 ± 0.03$^{a}$ | 3.20 ± 0.02$^{c}$ | |
| | Trypsin like activity | 2.5 ± 0.45$^{a}$ | 1.91 ± 0.20$^{b}$ | 1.62 ± 0.09$^{c}$ | |

Enzymatic activities are expressed as the increase of absorbance per minute per larva. Values are mean ± SD of three replicates.
Mean values within a row with the same superscript are not significantly different ($P > 0.05$).

LF throughout the end of the experiment. Trypsin activity levels at 10 DAH were highest for LF, while at 15 DAH the highest activity was registered for 52P and LF. However, at 20 DAH larvae belonging to the 45P treatment showed a significantly higher activity than those of the LF treatment but equal to those of the 52P treatment. The control treatment under starvation showed a significantly lesser activity for every enzyme assayed throughout the experiment.

### *Diet digestibility*

Both acid and alkaline digestion were significantly higher for diet 45P compared to 52P. It was also noticed that alkaline digestive activity values of 45P were significantly higher than those of the casein control (Fig. 1).

## Discussion

High mortality rates consistently occurred during the first attempts (1997–1999) to culture gar larvae with artificial diets and live food items restricted to *Artemia* adults and nauplii. However, after this period, different characteristics of artificial feed could be defined, simultaneously with a useful feeding strategy to promote their acceptance by larvae (Aguilera 1999; Mendoza & Aguilera 2000; Mendoza *et al.* 2002b). In this study, there was a low mortality in all

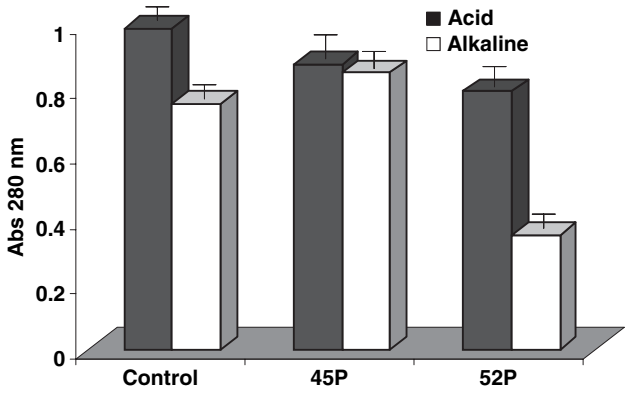


**Figure 1** *In vitro* digestibility of artificial diets (45P and 52P) using enzymatic extracts of 10 days after hatching (DAH) larval dissected digestive tracts. Haemoglobin was used as a control for acid digestion and casein for alkaline digestion. Digestibility is expressed as absorbance units at 280 nm.

treatments during the first 4 days of feeding, including starved larvae from the control treatment. This is explained by the prevailing yolk reserves in gar larvae until 8 DAH (Aguilera *et al*. 2002). Similarly to salmonids and sturgeons, eggs of lepisosteids are large and have a considerable quantity of yolk, which nourish the larvae for some days after hatching, even after the initiation of exogenous feeding. This mixed feeding period (5–8 DAH), allows a fast adaptation to available feed. During this period, the snout is not well developed, hindering larvae from ingesting live prey of their size; thus cannibalism is absent during this time. Yolk reserves availability, in addition to contribute to high survival rates, explains the high growth rates shown by larvae in those treatments where food was supplied (26–31%) and even by starved larvae (12.6%). This suggests that the weaning to artificial diets should be accomplished during this period, providing larvae with feed particles suited to the size of the snout and allowing them to identify artificial diets as a first feeding option, which may contribute to reducing their piscivory behaviour in further stages. This assumption is supported by the fact that the feeding preferences of lepisosteids seem to depend on several aspects, among which food availability is the major factor affecting the kind of food ingested by young gars (Echelle & Riggs 1972).

Starved larvae (C) did not survive beyond 15 DAH. Despite an acceptable survival rate, cannibalism was the main cause of mortality in other treatment groups. The fact that cannibalism occurred only in those treatments where larvae were fed, could be explained by the rapid development of the snout, coupled with an insufficient feeding supply.

Larvae fed live food maintained a steady growth until 10 DAH, when they were outperformed by larvae fed artificial diets (45P and 52P). At 20 DAH, it could be observed that only the latter continued growing. These aspects were evidenced by all the morphometric indexes (e.g. weight gain, TL, SL SGR) and confirmed by molecular indexes (e.g. RNA $DNA^{-1}$, protein $DNA^{-1}$; RNA $protein^{-1}$). This difference in growth could be attributed to the relationship between the snout and the feed size. With this regard Table 2 shows a marked increase in snout length in larvae belonging to the LF treatment from 1.14 to 3.35 mm, between 5 and 10 DAH, respectively. At this time, there was only a slight difference in size between *Artemia* nauplii (0.5 mm) and the 45P and 52P artificial feeds (0.5 and 0.3 mm, respectively). In fact, significant differences were only due to the lower performance of 52P diet at this time. However, after an adequate size adjustment of artificial diets at 10 and 15 DAH, an important difference in the size and weight of larvae could be observed. These results show the best performance of those treatments where larvae were fed artificial diets, contrasting with most studies where artificial diets and live food have been compared (Person-Le Ruyet 1990). The lower performance of larvae fed live food may in part be explained by the small size of the *Artemia* nauplii, in relation to the large size of the snout of gar larvae (Fig. 2). Larvae fed live food may have obtained a lower energetic reward compared to larvae fed artificial diets, due to the high energy expenditure of larvae in the search and capture of *Artemia* nauplii, even if these were highly available. On the other hand, the availability, buoyancy, attractability and increasing size of artificial feed particles may have provided a higher energetic reward. In agreement with this, Hunter (1981) mentioned that sea bream larvae selects food by size and that food size preference of sea bream increases faster than the increase in

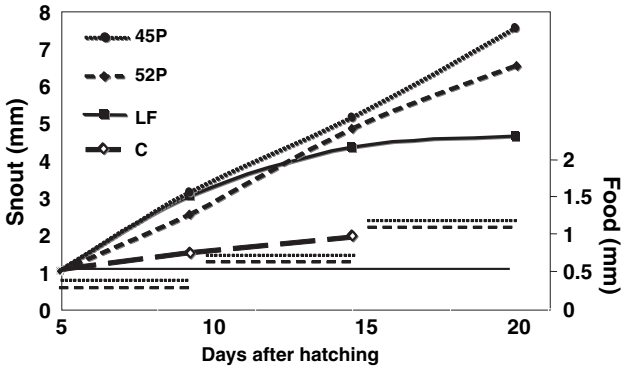


**Figure 2** Relationship between alligator gar snout size and diet size along the experimental period.

size of the food in the rearing tanks. Similarly, other authors (Dabrowski & Bardega 1984; Tabachek 1988; Enz *et al.* 2001; Petkam & Moodie 2001; Irwin *et al.* 2002) have mentioned the relationship between feed particle size and snout gape, with growth performance. Moreover, from the evolutionary standpoint, the selective advantage of individuals exhibiting cannibalistic traits is evident in situations when the cannibal benefits directly from obtaining a meal of high nutritional value (Polis 1981), e.g. during food shortage.

Total nucleic acids quantification made it possible to observe a concurrent increase in DNA and RNA with the increase in weight and/or size. This denotes cell proliferation, as well as a parallel rise in the total quantity of RNA available for protein synthesis, that is assigned for growth (Houlihan *et al.* 1993; Mathers *et al.* 1994). At the same time, the RNA/DNA ratio remained constant, suggesting that growth, as a product of protein accretion stabilizes and that larvae continue growing with this metabolic intensity. These data agree with the results of previous experiments (Mendoza *et al.* 2002c) which indicated that this time coincides with the end of metamorphosis, the start of exponential growth, the decrease of T3 levels and the stabilization of the enterocytes's cell height. On the contrary, concentrations of RNA, DNA and RNA $DNA^{-1}$ ratio in starved larvae were significantly lower (DNA) or decreased (RNA and RNA $DNA^{-1}$ ratio). These observations allowed to easily differentiate starved from fed larvae, and some indexes (eg.RNA $larvae^{-1}$ and RNA $DNA^{-1}$) even allowed to distinguish between larvae fed different diets. This was also confirmed by means of other indices (protein $DW^{-1}$, Protein $DNA^{-1}$, RNA $Protein^{-1}$, DNA $DW^{-1}$ and RNA $TL^{-1}$) (Mendoza *et al.* 2002b,c).

Larvae from the control treatment (C) showed a decrease in RNA concentration and protein content at 10 and 15 DAH. These results are in agreement with other experiments reporting the reduction of these variables when available food diminishes (Clemmesen 1987; Rooker 1997; Buckley *et al.* 1999), indicating that RNA and protein are metabolized during starving periods (Suneetha *et al.* 1999). In the same sense, larvae from the LF treatment showed a decrease in RNA concentration from 15 to 20 DAH, which was reflected in the RNA $DNA^{-1}$ ratio, while the protein content remained constant during this period. Moreover, larvae from the LF treatment showed higher levels of trypsin-like and alkaline proteases activities until 10 DAH; however, afterwards a steady decline in both activities was registered. This may be related to the low consumption of live preys during this period, which in turn would explain growth hindering. Control treatment larvae showed a very low digestive enzymatic activity as they did not have an external food source to stimulate enzymes production and secretion. These results are similar to those reported by Dabrowski *et al.* (1992), where minimal proteolytic activities were registered for trout and coregonids under irreversible starvation. Indeed, the digestive tract and associated glands are among the first tissues suffering damage as a consequence of starvation (Theilacker 1978), subsequently, cellular deterioration would be involved in the reduction of enzymatic activity. Digestive enzymes quantification has been used to evaluate nutritional condition, as they are produced and secreted just after food ingestion. Moreover, it has been reported that fish have the capacity to control enzymatic production (e.g. trypsin) in response to the quality and quantity of feed (Person-Le Ruyet 1990). In this way, enzymatic activity can be related to the nutritional condition of fish (Ferron & Leggett 1994).

The low alkaline proteolytic activity registered on 10 DAH in the 45P and 52P treatments could be related to the adaptation to artificial feed, which may had a lower digestibility than live food. An inverse relationship between pancreatic enzymes activity and feed protein digestibility has been reported (Mendoza 1993; Zambonino & Cahu 1994). The increase in proteolytic activity for the same diets on 15 and 20 DAH could be attributed to a higher feed consumption and to the progressive adaptation to the artificial feed. *In vitro* digestibility results contribute to explain growth differences observed between 45P and 52P diets. Larvae belonging to treatment 45P showed higher total acid and alkaline digestibilities than those of the 52P treatment (Fig. 1), which would be related to the higher growth of the former. In the same sense, the higher levels of trypsin-like activity registered in larvae of the 52P treatment, would be indicative of its lower digestibility according to the inverse proportionality of these variables, as stated above.

It has already been established that alligator gar larvae possess a fully functional digestive tract, including pepsin secretion, from the start of exogenous feeding (Mendoza *et al.* 2002b). Considering this, digestive capacity would not be limiting and thus nutrient availability from artificial diets would be closely related to the technological process involved in the elaboration of the diets and with the quality of the nutrients. In this case *in vitro* digestibility is suitable for evaluating nutrient quality (Bassompierre *et al.* 1998; Alarcon *et al.* 1999), mainly considering the difficulty to evaluate *in vivo* digestibility in small animals.

High protein content diets were selected for the feeding experiments taking into account the piscivorous behaviour of alligator gar larvae and considering the protein requirement determined for the tropical gar, a close related species

(U. Hernandez Vidal, pers. comm.). Differences in the performance of artificial diets 45P and 52P could be attributed to their processing. It has been mentioned that during the process of crumbled diets the individual particles tend to be irregular in shape and each particle may appear to contain single ingredients which may result in selective feeding by fish (Marr 1999). Furthermore, the survival of larval fish during weaning from live foods to formulated feeds has been shown to be higher when feeding a micro-extruded-marumerized feed compared to a crumbled feed using the same formulation for both feed types (Barrows 2000). In addition, despite the higher floatability of crumbled diets, these were less available to gar larvae because they tend to agglomerate, losing their shape and remaining on the surface. In contrast, micro-spheres had a lower floatability and the particles do not lose their shape and can be individually ingested.

Floatability is a crucial parameter of diets designated for gar larvae and this is likely related to their feeding behaviour. Indeed, gar larvae feed mostly at the water surface or in the water column, a fact that is supported by the marked abundance of pelagic organisms found in the stomach content of larvae and juveniles of lepisosteids (Echelle & Riggs 1972; Pearson *et al.* 1979). In agreement to these results, it has been reported that in the case of *Coregonus suidteri*, diet floatability is paramount for its consumption (Enz *et al.* 2001).

Equally important are feed attractants added to the diet, which are essential for an increased feed intake. It has been observed that when gar larvae approach feed particles, they stay next to them, and even have contact with them before they are ingested (Aguilera *et al.* 2005). This indicates that larvae use chemical perception to recognize the diets. Field observations of some authors (Netch & Witt 1962; Goodyear 1967) reveal a higher feeding activity during night hours, which would imply that visual identification is limited, particularly in muddy waters of swamps.

Finally, utilization of artificial diets bestows several advantages, among which is the possibility to modify the dietary composition of prepared diets according to the nutritional requirements of fish larvae (Bromley 1978). Moreover, they constitute an interesting alternative to avoid the extended use of live food, (Duray & Bagarinao 1984). The use of live food in alligator gar larvae culture is impractical as it implies the continuous and simultaneous production of high quantities of live food of different sizes to satisfy the increasing demand of fast growing larvae characterized by an allometric growth in which the snout represents 15% of the total length (Aguilera *et al.* 2002). In practice, this is complicated and requires numerous facilities and high labour cost.

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