Studies on the Physiology of *Atractosteus Spatula* Larval Development and Its Applications to Early Weaning Onto Artificial Diets

Roberto Mendoza[1], Carlos Aguilera[1], Jesús Montemayor[1] , Agnes Revol[2] and Joan Holt[3]

[1]Grupo Ecofisiología, Fac. de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Apartado F-96, Monterrey, C.P. 66450, México.
Tel. and Fax: +8183 529772, E-mail: rmendoza@ccr.dsi.uanl.mx

[2] Departamento de Bioquímica, Fac. de Medicina, Universidad Autónoma de Nuevo León.

[3] Department of Marine Science, University of Texas Marine Science Institute.

ABSTRACT

This paper describes the strategy utilized for the domestication of the alligator gar (*Atractosteus spatula*), highlighting different aspects of larval development and the weaning of larvae onto artificial diets. Developmental stages based on nutritional characteristics were established. Lecithotrophic stage was observed between 1–4 days after hatching (DAH) larvae, lecithoexotrophic stage between 5–8 DAH larvae and the exotrophic stage initiates in 9 DAH larvae. The degree of development for alligator gar larvae could be differentiated by snout length. In addition, pigmentation could be easily used to separate individuals in poor condition. Growth rate of alligator gar larvae was 1.55 mm/day during the first 10 DAH and 5.6 mm/day thereafter until 15 DAH. RIA analyses showed that T_3 levels are low during embryonic development, but increase after hatching, until 10 DAH, decreasing thereafter. The effect of thyroidian hormones and goitrogens on larval development was evaluated. Snout length was significantly reduced in larvae treated with thiourea without affecting growth, whereas in larvae treated with T3 snout development was accelerated. Histological assessment of the development of the digestive tract revealed that at the onset of exogenous feeding (5DAH) the digestive tract was completely developed. Measurement of mid-gut cell height was valuable tool for evaluating the suitability of diets. Additionally, the ontogeny of digestive enzymes was characterized. Pepsin-like acid proteolytic activity was detected from the beginning of exogenous feeding (5 DAH). Trypsin, chymiotrypsin and aminopeptidase-like alkaline proteolytic activity gradually increased between 2-9 DAH. Substrate-gel electrophoresis was utilized to confirm and further characterize digestive enzyme activity. Growth hormone coding sequence was obtained by RT-PCR and its expression was detected in embryos and in several larval stages, revealing an important role during embryogenesis and organogenesis.

RNA/DNA ratios were determined and proven to be a useful indicator of larval nutritional condition. In fed larvae RNA/DNA ratio rose until the 13 DAH, while in starved larvae the ratio RNA/DNA was low and remained constant. Three feeding experiments were carried out and some important characteristics of artificial diets were identified, namely floatability and size. The results of the feeding trials made it possible to culture larvae and juveniles using exclusively artificial diets.

Key words: alligator gar, larval development, morphology, physiology, enzymes, hormones, nutrition

INTRODUCTION

The incorporation of new species to aquaculture necessarily implies their domestication. This process requires, at least, the study of the capacity of those organisms of interest to live most of their life cycle under artificial conditions (FAO/PNUMA, 1984). Among the main characteristics desired in a species to be domesticated are: the ability to reproduce in captivity, the likelihood of spawning in captivity, the possibility of mass larval rearing, adaptability to the consumption of artificial diets and the ability to grow and being maintained at high densities (AQUACOP & Calvas, 1990). However, most of the species currently cultured hardly gather all these characteristics. In fact, the reasons to initiate the domestication of a species have been related in several occasions to different aspects, such as economical (commercial value of the species), sociocultural (traditional fisheries) and ecological (over-exploited or endangered species) (Mendoza *et al*., 2000c; Rojas & Mendoza, 2000).

Taking into account the previous considerations, we have directed our efforts to the domestication of the alligator gar (*Atractosteus spatula*). Alligator gar is the largest freshwater fish inhabiting the rivers nearby the Gulf of Mexico. These fishes have been traditionally captured in the northeast region of Mexico, where they are highly appreciated by their size and quality of their meat (Rosas, 1975; SEPESCA-INP, 1994). As a consequence of both, commercial and sport overfishing the populations have been drastically declining. A clear example is the variation of the population levels of alligator gar in the state of Tamaulipas, where the mean annual captures were estimated to be 13.2 m^3 in 1988, by 1990 this volume was reduced to 5.7 m^3 and in 1997 fishermen were able to capture only 1.1 m^3. The situation has been exasperated due to the fact that the major fishing period is coincidental with the reproduction season of this species, affecting thus the recruitment of juveniles. In addition, spawning grounds are being affected due to urban and agricultural expansion as well as by the construction of several dams. The need to develop culture techniques has emerged as a short-term alternative to preserve this species and to aid in restoring the natural populations. To accomplish this, since 1982 the Mexican Ministry of Fisheries has maintained in captivity 30 adults, whose reproduction and spawning is restrained to only one week per year, notwithstanding the government has managed to produce up to 400 thousand larvae per year. Unfortunately due to the insufficient supply of live food for the early stages high rates of cannibalism have been obtained. In addition, large differences in growth, have led to the need of releasing the

larvae very soon after hatching to avoid further cannibalism. As a result, the culture cycle has being continuously interrupted. This has impacted not only the quantity, but also the quality of larvae obtained, which has in turn affected their survival in their natural environment (Mendoza 2000; Mendoza & Aguilera, 2000b; Mendoza *et al*., 2000a, 2000b, 2002a). A similar situation has been affecting other lepisosteids such as the Cuban gar (*A. tristoechus*) in Cuba (Leon *et al*., 1978) and the tropical gar (*A. tropicus*) in Central America (Marquez, 2000).

This paper is aimed at presenting the strategy that has been developing the Ecophysiology Laboratory of the Universidad Autónoma de Nuevo León, in collaboration with different national and international institutions, in an effort to overcome some of the critical points related to the domestication of the alligator gar, particularly those concerning the larval culture and the weaning of larvae onto artificial diets. These aspects are often related and represent a bottle-neck in the domestication of most species. Indeed, the utilization of artificial diets in the culture of fish larvae constitutes a major alternative to avoid the use of live preys, which production is impractical and costly. (Gatesoupe & Luquet, 1982). Nonetheless, after two decades of formulation of microdiets for fish larvae, only limited success has been obtained due to problems related to the ingestion and digestion of diets (Watanabe & Kiron, 1994; Lazo, 2000). This has resulted, in the case of several species, that more than 90% of the larvae obtained through reproduction in captivity are immediately released after hatching. , The main reason for the use of this strategy is the difficulty to produce live feed in adequate amounts to support adequate larval growth and survival (Rosch & Appelbaum, 1985).

Thus, a multidisciplinary approach was used to study several aspects of alligator gar larval development, including their digestive morphology and physiology, to provide a basis for their weaning onto artificial diets (Fig. 1).
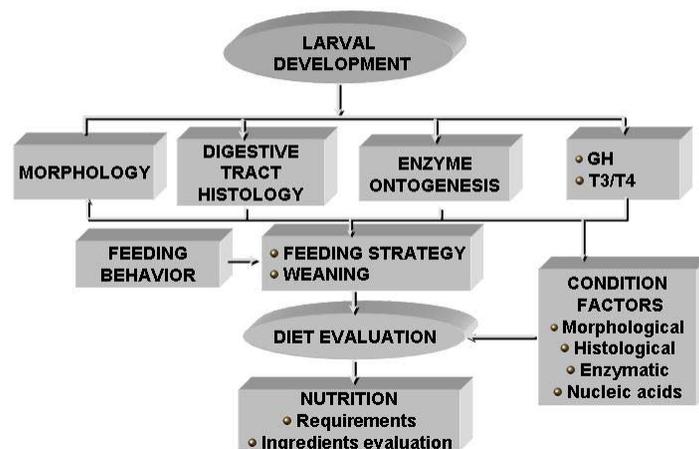

Fig. 1. Strategy adopted for the weaning of alligator gar larvae to artificial diets.

LARVAL DEVELOPMENT

The sensitivity of larvae to low food intake, especially following yolk resorption, can significantly influence their chances of survival. Therefore, in order to gauge the effect of different feeding levels, there is a need to develop criteria for evaluating larval condition and the incidence of incipient starvation (McFadzen *et al*., 1994). In relation to this, the study of morphological characteristics provides simple tools for detecting unfavorable culture conditions during larval development (Dettlaff *et al*., 1993). However, the few available studies of larval gar are limited to the description of some of their developmental stages. Dean (1895) conducted a comparative study of the embryonic development of gar and sturgeon that showed that gar has characteristics intermediate to holosteans and teleosteans. Morphological characteristics of early life stages have been described for longnose gar *Lepisosteus osseus*, shortnose gar *L. platostomus* and spotted gar *L. oculatus* (Netch & Witt, 1962; Pearson *et al*., 1979; Yeager & Bryant, 1983; Simon & Wallus, 1989; Simon & Tyberghein, 1991). However, the existing morphological and pigmentation descriptions of larval alligator gar have been completed with only 11 specimens (Suttkus, 1963; May & Echelle, 1968; Echelle & Riggs, 1972; Moore *et al*., 1973; Simon & Wallus, 1989).

We describe larval development and growth rates of alligator and tropical gar, in addition to some morphological characteristics that can be used as indicators of growth and nutritional condition during early life stages (Aguilera, 1999; Aguilera *et al*., 2002).

Gar eggs are moderately large and larvae are characterized by an extended yolk-sac period. As a consequence, larvae are relatively large and fairly developed when exogenous feeding begins. Because eggs and yolk-sac larvae remain adhered to vegetation, these stages, may incur a relatively high risk of predation. However, gars have developed a defense mechanism by incorporating cardiotoxic substances in the eggs, and among gars those of alligator gar are most toxic (Netch & Witt, 1962; Burns *et al*., 1981).

Nutritional stages for alligator and tropical gar larvae could be distinguished according to the criteria established by Beccaria *et al*. (1991). Utilizing these criteria, larvae between 6.8 and 13.5 mm TL (1–4 days after hatching - DAH) could be included in the lecithotrophic stage. At this stage larvae remained attached to vegetation and were nourished only from the yolk sac. Larvae between 12.5 and 22.5 mm TL (5–8 DAH) could be included in the lecithoexotrophic stage, when exogenous feeding began, although fed and unfed larvae did not show differences in growth, indicating that yolk reserves were still present. Finally, exotrophic stage would initiate in larvae from 22 mm TL, when morphological alterations were observed in unfed larvae, indicating a total dependence on exogenous food.

Snout formation could be used as a diagnostic difference between alligator and tropical gar. Alligator gar larvae approached adult snout-length proportion at an earlier size and age than in tropical gar larvae (Fig.2). This suggests a faster metamorphosis in alligator gar and could contribute to earlier changes in alligator gar feeding habits and the onset of cannibalism, given that prey size is determinate by snout size and the lack of food can

promote cannibalism (Dabrowski & Bardega, 1984; Hecht & Pienaar, 1993; Busch, 1996). Similar changes concerning feeding habits for young gars were observed by Echelle & Rigs (1972) who reported a progressive increase in the frequency of fishes in the diet with the increasing size of gar, which starts at smaller size for longnose gar (17 – 36 mm TL) than for shortnose and spotted gars. In addition, Pearson et al. (1979) found that lognose gar began to consume fish larvae early in development (at 20 to 42 mm TL), although piscivory did not limit the intake of other, more readily available prey (i.e. cladocerans). These results indicate that larval gar are opportunistic feeders.
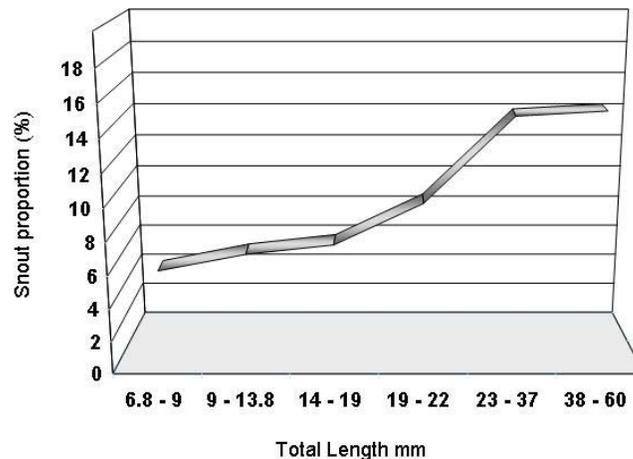

Fig. 2. Snout formation (Snout length/Total length) in alligator gar larvae

Results derived from a Stepwise discriminant analysis (SDA) of morphometric characteristics between intervals of total length indicated, on one hand, that the degree of development for alligator and tropical gar larvae could be mainly differentiated by snout length, suggesting that this characteristic may be used to evaluate growth of gar larva. On the other hand, the SDA results indicated that caudal peduncle depth, head width, and preanal depth were the morphometric characteristics that contribute most to differentiate between feed and starved gar larvae, and may be used to evaluate nutritional condition between gar larvae of similar developmental stages. In addition, a darker pigmentation observed in starving alligator and tropical gar larvae could be easily used to separate individuals in poor condition from large numbers of cultured larvae.

The growth rate observed in tropical gar larvae (1.0 mm/day) is similar to that reported for larvae of longnose gar (0.8 mm/day) and spotted gar (0.83 mm/day) (Pearson et al., 1979; Simon and Tyberghein, 1991). The fastest growth was that observed for alligator gar larvae during the first 10 DAH (1.55 mm/day) (Fig.3). Fastest growth rates were observed after 10 DAH (5.6 mm/day) exceeding juvenile growth rates reported for juvenile spotted gar (1.3 to 1.7 mm/day) and longnose gar (2.33 to 4.5 mm/day) (Echelle & Riggs, 1972; Netch &

Witt, 1962; Simon & Wallus, 1989). These data confirm that lepisosteids, and particularly alligator gar, are among fishes with the highest growth rate (Netch & Witt, 1962).
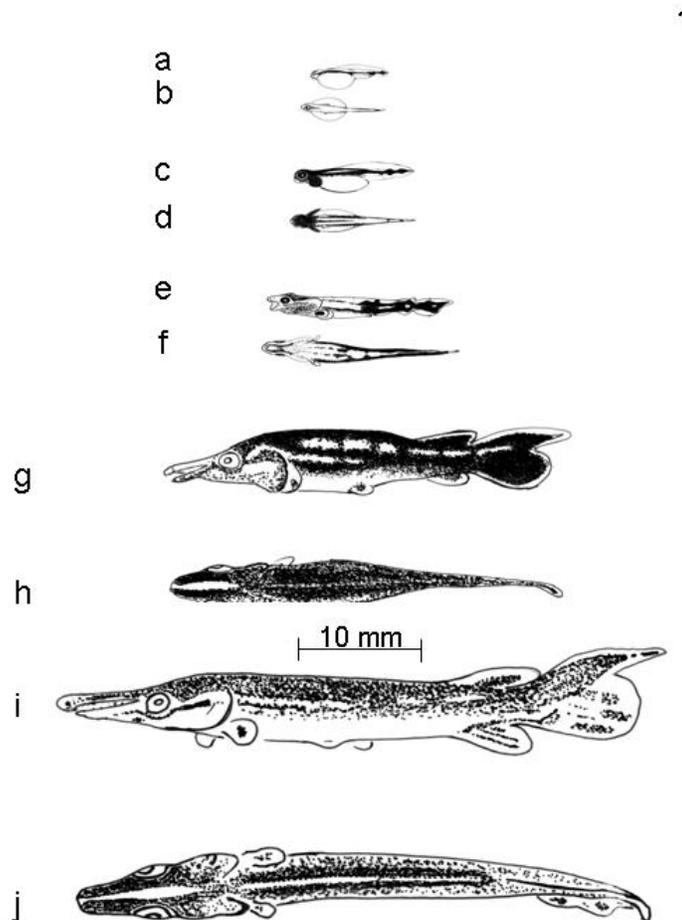

Fig.3.- Alligator gar, yolk-sac larva 7 mm TL, lateral (a) and dorsal view (b); 11 mm TL, lateral (c) and dorsal (d) view; larva 17 mm TL, lateral (e) and dorsal (f) view. Alligator gar larva 32 mm TL, lateral (g) and dorsal view (h); larva 50 mm TL, lateral (i) and dorsal (j) view.

It has been widely reported that larvae that go through short posthatch yolk-sac periods usually have difficulty in utilizing prepared diets until they complete metamorphosis. In contrast, some fishes such as salmonids that receive nutriments from the yolk sac for a long period after hatching can successfully utilize prepared diets from the time they first accept food (Baragi & Lovell, 1986; Gershanovich, 1991). Similarly to salmonids and sturgeons, eggs of lepisosteids are large and have a considerable quantity of yolk, which will nourish the larva for some days after hatching, even after the initiation of exogenous feeding. This mixed feeding period (5 to 8 DAH), allows a fast adaptation to available feed. During this period, the snout is not well developed yet, hindering larvae to ingest live prey of their size, therefore no cannibalism is present during this time. In fact, the release of larvae between 7

– 10 DAH, has been the common practice until now, because these can be cultured at high densities without mortalities related to cannibalism (Morales, 2000, personal communication), 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 reduce their piscivory behavior in further stages. This is supported by the fact that the feeding preferences of lepisosteids seem to depend on a variety of factors, among which food availability is the major factor affecting the kinds of food ingested by young gars. Thus, it has been suggested that their feeding habits are opportunistic rather than strictly piscivorous (Echelle & Riggs, 1972; Holloway, 1954; Resendez & Salvadores, 1983; Chavez-Lomelí *et al*., 1989). These feeding habits have been reported to appear early in the larval development (Pearson *et al.,*1979).

The high growth rate of alligator gar larvae in combination with rapid snout development suggests that they must be provided with a continuous food supply at the start of exogenous feeding, and that larvae should be capable to adapt to a variety of potential preys. This adaptation may be related to the structural and functional development of the digestive tract, necessary for an adequate and rapid digestion and absorption of food. Moreover, the fact that growth is expressed particularly in length is a consequence of the fast development of the snout, signifying that besides being a process involved in metamorphosis into the adult shape, it is an adaptation of larvae for capturing and ingesting larger preys. As a result, larvae will obtain a greater quantity of food by eating less, reducing in this way the energy expenditure for searching and capturing food. This fact is supported by the continuous development of the snout even in starved larvae, indicating that snout development is a metabolic priority for larval survival.

CONTROL OF LARVAL DEVELOPMENT

The nature of the growth promoting effect of thyroid hormones (TH) in larval fish metamorphosis may involve induction of morphological changes in the digestive tract and accessory organs, stimulation of renewal processes of the intestinal epithelium, increasing nutrient absorption capacity, and growth enhancement in general (Dabrowski & Culver, 1991). TH may have a great practical value in aquaculture because they do not only act as growth promoters, but also trigger and accelerate larval development (Lam, 1980). High concentrations of T4 (thyroxin) and T3 (3,3′,5-triiodo-l-thyronine) have often been observed in the yolk of unfertilized eggs, apparently originating from maternal plasma. Moreover, a rise in TH levels after yolk absorption has been reported, reflecting the production of these hormones by the developing thyroid gland of larvae. Therefore, larval development is continuously influenced by TH from maternal and endogenous sources (Brown *et al*., 1987). In addition, certain dietary factors may inhibit or stimulate TH activity (Inui *et al*., 1989).

In the case of alligator gar, T_3 levels during embryonic development are low, as shown by the concentrations determined in recently spawned eggs and eggs just before hatching (0.0117 and 0.00995 ng/egg, respectively). However, after hatching these levels increase,

reaching 2.22 ng/larva at 10 DAH, decreasing thereafter to 0.3 ng/larva at 13 DAH (Aguilera, 1999). This variation in T3 levels concur with the morphological changes previously described. These data, coupled with the morphological observations indicate that the metamorphic climax takes place around 10 DAH. This contention is supported by the occurrence of two main events before and after 10 DAH, the exhaustion of yolk reserves by the 8 DAH and the beginning of exponential growth at 11 DAH. For this reason, the conditioning to exogenous feeding from 5 to 8 DAH should be accomplished, allowing larvae in this way to have enough energetic reserves to undergo metamorphosis without detriment to their condition. Furthermore, these results indicate a precocious establishment of the thyroidian axis that would be functional at the onset of exogenous feeding (5 DAH). In contrast, several authors have suggest that metabolic and metamorphic processes in larvae are solely under the influence of the maternal source (Tagawa and Hiro, 1987; Kobuke *et al*., 1987; Sullivan *et al*., 1987; Brown *et al*., 1987; Tagawa *et al*., 1990).

Considering the use and importance of exogenous thyroid hormones and glucorticosteroids as potential regulators of larval gar development, we have determined the effects of the exposure of larvae to 3,3´,5-triiodo-l-thyronine (T3), hydrocortisone (HC) or thiourea (TU) (Mendoza *et al*., 2001, 2002a). Concentrations of T3 were three times higher in the T3 group, when compared to the other groups. This may have resulted from exposure of larvae to exogenous T3 added to the naturally high endogenous levels of T3 found in individuals of all groups. Abnormally high levels of T3 may be related to the high concentrations of cortisol determined in this group. Both hormones may have contributed to the lower survival and deformities observed in larvae treated with T3. Higher values of weight gain and total length were observed in the TU and control (C) groups. The lower weight attained by larvae exposed to T3 could be explained by energy invested in premature metamorphosis. Snout length was significantly reduced in the TU group, whereas in the T3 group snout development was accelerated.

These results showed the feasibility of altering snout development and present novel alternatives for the control of cannibalism under culture conditions, through the retardation of snout growth by exposure to anti-thyroid compounds (TU) without affecting growth and survival (Fig.4). Further investigations are needed to determine the optimal dose of T3.
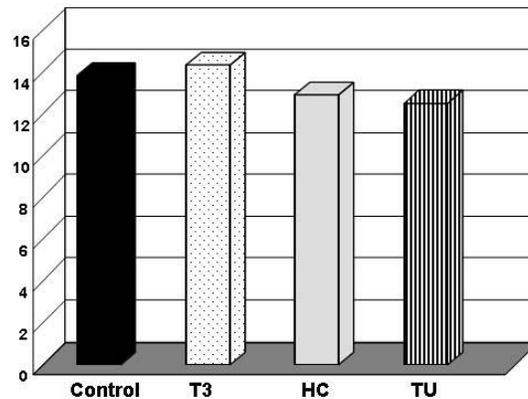
Fig. 4. Snout proportion (Snout length/Total length) of larvae treated with thyroid hormones (T3: triiodo-1-thyronine), glucocorticoids (HC: hydrocortisone) and goitrogens (TU: Thiourea). C: control.

HISTOLOGICAL ASSESSMENT OF THE DEVELOPMENT OF THE DIGESTIVE TRACT

Based on the morphological features of digestive system, teleost larvae may be roughly divided into two main groups. Newly hatched larvae of the first group, posses a digestive tract incipiently developed, this is observed commonly in larvae derived from pelagic eggs. While the second group is constituted by larvae originating from demersal eggs which present a variable degree of differentiation just after hatching (e.g. mouth already opened, liver and pancreas well differentiated) (Tanaka, 1969a).). In general, larvae have a straight intestine after hatching, formed by simple epithelia that remain unchanged until the yolk sac and lipid globule are absorbed. At this time, the intestine can be differentiated into foregut, midgut and hindgut (Grizzle & Curd, 1978; Govoni *et al*., 1986; Ferraris and de la Cruz, 1987; Verreth *et al*., 1992; Sarasquete *et al*., 1995). On the contrary, unlike other fish, but similar to amphibians, cleavage of acipenserid eggs is holoblastic resulting in the formation of a yolk endoderm which participates in the formation digestive system (Buddington & Christofferson, 1985; Buddington & Doroshov, 1986; Gawlicka *et al*., 1995; Gisbert *et al*., 1998).

At the start of exogenous feeding, telostean larvae may have a straight or convoluted digestive tract. Intestinal epithelium is composed exclusively by columnar absorptive cells and zymogene granules may be observed in the pancreatic tissue. From this moment onwards, several rapid morphological changes occur and larvae structurally resemble to adults of stomachless species (Tanaka, 1969b; Verreth *et al*., 1992; Segner *et al*., 1994; Kaji *et al*., 1996). After the start of feeding two conspicuous features appear in the epithelium of the intestine. One is the appearance of many vacuoles in the antero-median part of the intestine, and the other is acidophilic granules in the posterior part of the

intestine. The intestinal epithelium, particularly at the middle region, is highly vacuolated due to fat absorption, while acidophilic granules are a product of the accumulation of proteins incorporated by pinocytocis (Tanaka, 1971; Tanaka 1972; Sarasquete, *et al*., 1995; Kaji *et al*., 1996). These characteristics continue until the gastric glands of the stomach are differentiated (Watanabe, 1984; Walford & Lam, 1993). In the case of sturgeons, when larvae begin to feed they posses an anatomically complete and functional digestive tract with a high degree of morphological organization, which implies a well developed stomach and functional gastric glands (Buddington & Christofferson, 1985; Dabrowski, 1986; Buddington & Doroshov, 1986; Gawlicka *et al*., 1995; Gisbert *et al*., 1998). This particular development has made possible weaning sturgeon larvae onto artificial diets from the start of exogenous feeding, without the need to provide (Dabrowski *et al*., 1985).

Considering the previous statements, and the fact that lepisosteids have an embryonary development with intermediate characteristics between telosteans and chondrosteans (Dean, 1895), we have followed the appearance and development of different structures to determine the moment when the digestive tract of alligator gar was completely developed. Development of the digestive tract was assessed histologically (Aguilera 1999; Alvarez *et al*., 1999; Mendoza & Aguilera 2001; Mendoza *et al*., 2002a). Upon hatching, digestive tract develops from an undifferentiated epithelium located over the yolk sac. Differentiation takes place rapidly starting from the posterior end with the formation of the spiral valve in 3 DAH larvae. At the onset of exogenous feeding (5DAH) the stomach and pancreatic tissue can be observed, despite the presence of yolk reserves. This observations indicated that the digestive tract was completely formed (Fig.5). The degree of development of the digestive tract at the start of exogenous feeding, places gar among a reduced group of fishes, such as sturgeons and some salmonids, which have a differentiated stomach at this time. (Buddington & Christofferson, 1985; Dabrowski, 1986; Buddington & Doroshov, 1986; Gawlicka *et al*., 1995; Gisbert *et al*., 1998). Once gar larvae started feeding, some structural changes can be observed, such as the development of intestinal foldings, an enlargement of enterocytes size and number of organelles. According to Dabrowski (1986) this suggests a rapid maturation process during this period. In addition, through histological analysis we observed the existence of yolk reserves in the body cavity until 8 DAH. This confirms the periods proposed for the different nutritional phases in gar larvae: from 1 to 4 DAH lecitotrophic phase, from 5 to 8 DAH lecitoexotrophic phase, from 9 DAH onwards the exotrophic phase. Moreover, the existence of yolk reserves contribute to explain the absence of qualitative and quantitative differences (enterocytes height) between starved and fed larvae until the 8 DAH. Once these reserves are exhausted (9-10 DAH) conspicuous differences regarding mid-gut cell height of fed and starved larvae were observed (Alvarez *et al*., 1999). Thus, mid-gut cell height was shown to be a valuable tool for evaluating the suitability of artificial diets in gar larval culture, as it has been shown in other studies (Fig.6). (Theilacker & Watanabe, 1989; Oozeki *et al*., 1989; McFadzen *et al*., 1994; Theilacker & Porter, 1995). At 15 DAH, gastric glands of starved larvae were underdeveloped or degenerating when compared with those of fed larvae. Hepatic cells of starved larvae contained condensed cytoplasm with no intracellular spaces (areas of glycogen and lipid storage) and muscle fibers were atrophied. From these results, it was

possible to assume that the structural degree of development of the digestive tract of gar larvae would not hamper their weaning onto artificial diets.
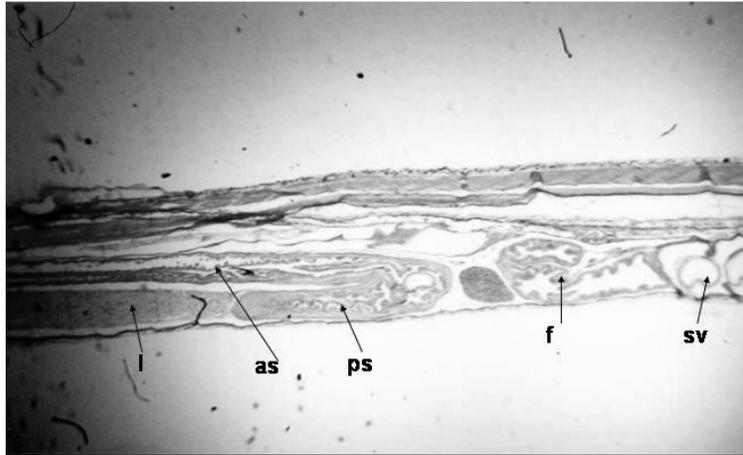

Fig. 5. Digestive tract of a 5 DAH larvae. Arrows in the picture show: (i) liver, (as) anterior stomach, (ps) posterior stomach, (f) foregut, (sv) spiral valve.
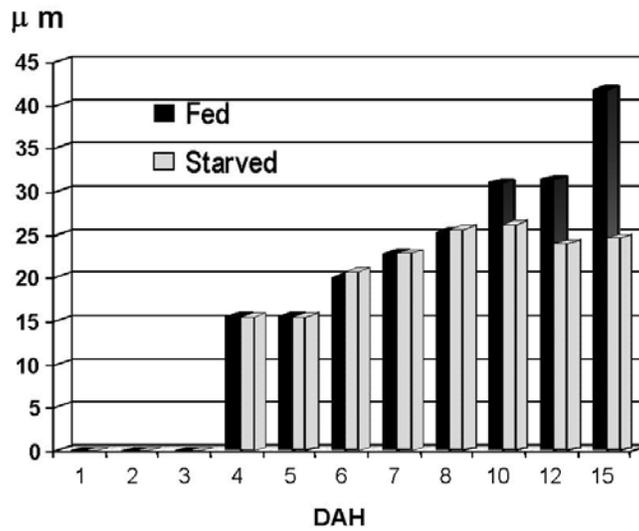

Fig. 6. Average midgut cell height (μm) of fed and starved larvae of alligator gar at different days after hatching (DAH).

ONTOGENY OF DIGESTIVE ENZYMES

Structural modifications occurring during the morphological development of the digestive tract are necessarily coupled with functional changes that are reflected in the production of different enzymes and their activity level. The poor performance of artificial diets as the sole food in the rearing of fish larvae has been attributed to the incomplete functioning of the digestive tract at the commencement of exogenous feeding. Moreover, the increase in enzymatic activity and functioning of the stomach has been related to the ability to digest artificial diets. (Alliot *et al*., 1977; Lauff& Hofer, 1984; Buddington, 1985; Ueberschär, 1988; Person-Le Ruyet, 1989; Dabrowski & Culver, 1991; Moyano *et al*., 1996).

In general, three stages have been defined to achieve the complete maturation of the digestive system in fish larvae. First, during endogenous nutrition, the alimentary canal and associated organs differentiate, but the enzymatic activity is still incipient. The second phase initiates with exogenous feeding; digestive tract differentiation is not complete yet and although some enzyme activity is present, the digestive capacity is low, but this aspect is compensated by macro-pinocytocis of proteins and their intracellular digestion as major mechanisms of nutrient acquisition. Finally, during the third phase the digestive tract is completely differentiated, with the formation of functional gastric glands, and is characterized by enzyme activity levels similar to those of juveniles (Budington, 1985), The latter stage is considered as the end of the larval period for most of the species (Watanabe, 1982; Person-Le Ruyet, 1989; Verreth & Van Tongeren, 1989; Verreth *et al*., 1992; Walford & Lam, 1993; Moyano *et al*., 1996). Nevertheless, this pattern varies in some species, for example in sturgeons, the gastric glands are already developed and proteolytic activity is similar to that of juveniles when exogenous feeding begins (Budington, 1985; Budington & Doroshov, 1986; Gawlicka *et al*. 1995). Maturation of the digestive tract has been considered the best indicator of the appropriate time to replace live food with artificial diets. (Alliot *et al*., 1977; Lauff & Haufer, 1984; Buddington, 1985; Ueberschär, 1988; Person-Le Ruyet, 1989; Dabrowski & Culver, 1991; Moyano *et al*., 1996).

The research pertaining to the ontogeny of digestive enzymes of alligator gar (Aguilera, 1999, Ruiz, 2000, Mendoza *et al*., 2000d; Mendoza *et al*., 2002a) can be summarized as follows: Pepsin-like acid proteolytic activity was detected from the beginning of exogenous feeding (5 DAH); before complete absorption of the yolk sac. Trypsin, chymiotrypsin and aminopeptidase-like activity gradually increased from 2-9 DAH. Enzymatic activity differed by digestive tract region. For example, trypsin and chymiotrypsin-like activity were highest in the fore-gut, whereas, aminopeptidase-like activity was highest in the hind-gut. Digestive enzyme activity, molecular weight, and number of enzymes within the two digestive tract regions, were confirmed and characterized using substrate-gel electrophoresis. Important lipase and acid phosphatase activities in 3-8 DAH larvae were related to yolk utilization. In contrast, increased activity of alkaline phosphatase at 8 DAH coincided with the maturation of enterocytes. Amylase activity was low throughout larval development and may be due to the carnivorous food habits of gar larvae. In contrast, fish with herbivorous or omnivorous diets have high levels of amylase activity (Vonk & Western, 1984, Munilla-Moran & Saborido, 1996). Similar to salmonids and sturgeons

(Buddington, 1985; Buddington & Dorshov, 1986; Gawlicka, *et al.*, 1995), alligator gar posses a well developed intestinal tract, characterized by a functional stomach and the secretion of pepsin-like enzyme from the beginning of exogenous feeding (Fig. 7). These characteristics are indicative of precocious digestive tract maturation.
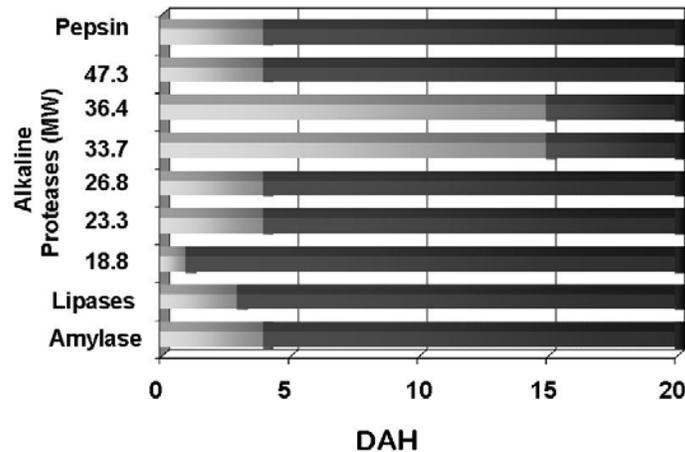

Fig. 7. Main digestive enzymes spectrum of alligator gar larvae during early developmental stages.

Taking into account the previous results, it can be concluded that the digestive system in alligator gar is highly developed at the start of exogenous feeding, particularly when compared with other species. This suggests the possibility of feeding the larvae artificial diets from first feeding.

Information gained from the isolation and purification of the main digestive enzymes may be used to develop an *in vitro* digestibility system to test different feed ingredients, resulting in the formulation of a cost-effective diet for cultured gar.

LARVAL METABOLISM

Growth Hormone

Alligator gar presents an amazing growth rate from early larval stages throughout its life cycle, allowing adults to reach up to 2 or even 3 meters. Unfortunately little is known regarding the physiology behind this amazing process. Fish Growth Hormone (GH) is a pituitary hormone responsible for lineal growth, however it can also be expressed in extrapituitary tissues of adults and in early embryos. GH is known to stimulate appetite (Pickford & Atz, 1957, Higgs *et al.*, 1975), food conversion efficiency (Markert *et al.*, 1977), lipid mobilization (Sheridan, 1986), nitrogen retention (Matty, 1962), amino acid

incorporation into tissues (Cheema & Maty, 1978; Fauconneau *et al*., 1996), stimulation of energy and protein metabolism at whole body level (Medale *et al*., 1988) and at tissue level (Foster *et al*., 1991). The growth promoting effect of exogenously administered GH in fish is well established. A number of studies have shown that the administration of mammalian GH enhances growth rate of fish (Pickford & Thompson, 1948; Komourdjian *et al*., 1976; Adelman, 1977), similarly a marked increase in growth rate, appetite and feed conversion efficiency has been reported by different authors using natural bovine growth hormone (Higgs *et al*., 1975, 1976; Markert *et al*., 1977). Cook *et al*. (1983) showed a highly significant increase in growth rate of goldfish after i.p. injection of bovine or carp GH and Wagner & McKeown (1981) reported an increase in body size and weight of juvenile rainbow trout injected with purified chum salmon GH. Recombinant GHs have similar effects in promoting growth in crussian carp (Xu *et al*., 1991). Agellon *et al*. (1988) have shown that administration of recombinant trout GH resulted in acceleration of growth rate of yearling rainbow trout, while recombinant bovine GH and recombinant chicken GH injections were found to enhance growth of juvenile Pacific salmon (Gill *et al*., 1985).

Within this context, we aimed our research at obtaining the coding sequence of the GH and studying its expression along larval development (Mendoza *et al.,* 2002d).

The cDNA coding for the GH was obtained by RT-PCR using total RNA extracted from alligator gar larvae heads. Two primer sets were designed on conserved regions, the first one allowed the theoretical amplification of all the coding sequence, while the second was located within the second and fifth exons. Although PCR conditions were standardized with both oligonucleotide sets, only the second set allowed the amplification of a 491-pb fragment. The PCR product was cloned using pBlueScript as a vector, and its nucleotide sequence was determined from three independent clones. The analysis of the fragment sequence confirmed that it corresponded to a GH gene, sharing 98 % of nucleotide similarity with the GH previously reported for *Lepisosteus osseus* (Rubin *et al*., 1996; GeneBank access number S82528). A single amino acid change (Val /Ala) in the fifth exon was observed between the two lepisosteidae. The few residue changes observed between the GHs of these two primitive fish that diverged 180 million years ago, sustain the fact of a very slow evolution of the hormone within the primitive fish, in contrast with the burst of changes observed in the euteleosts.

Using multiple hybridization (Multi-NPA[TM], Ambion), the GH transcripts were detected and quantified from eggs to late larval stages, using a ribosomal RNA probe as internal control. The mRNAs were detected in embryos and in several larval stages. The relative expression of GH along larval stages indicates the existence of substantial expression of this hormone in unfertilized eggs, declining thereafter until 3 DAH, then increasing around 5-7 DAH larvae, corresponding to the organogenesis. In 8 DAH larvae the expression diminishes again, this is consistent with the time when yolk reserves are depleted and with the onset of exogenous feeding. Lastly, the expression increases in 9-10 DAH larvae, probably corresponding to the formation of pituitary gland (Fig.8).
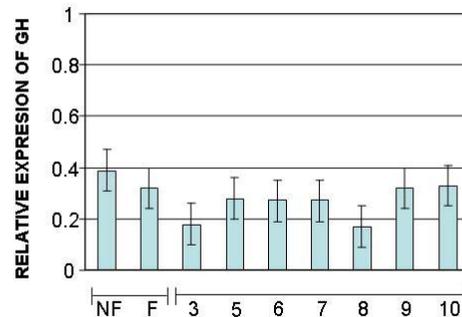
Fig. 8. Relative expression of Growth Hormone. NF: Non fertilized eggs, F:fertilized eggs.

These results corroborate previous reports on GH expression in early larval stages of an euteleost: the rainbow trout, and strongly suggest that the GH may play an important role during embryogenesis in fish. Until recently, the dogma was that GH has no role in embryo growth, but GH transcripts have been detected in mouse embryos at morula stage (Pantaleon *et al.*, 1997), rainbow trout embryos before pituitary organogenesis and even mature oocytes (Yang *et al.*, 1999). Additionally, functional GH receptors are expressed very early in mouse embryos (Pantaleon *et al.*, op. cit.), sheep blastocyst and fetal tissue (Lacroix *et al.*,1999). These data suggest a wider action of GH in controlling early development in vertebrates.

The cloning of GH and the elucidation of its role during larval stages will certainly contribute to the better understanding of alligator gar larval physiology and consequently could potentially facilitate the culture of larvae and juvenile gar to commercial size. With a view to applying GH in fish culture, the future direction of our research will be focused in synthesizing alligator gar GH by genetically engineered microorganisms in order to demonstrate its effect on the promotion of growth in tropical gar, a close related species whose slow growth rate, compared to alligator gar, delays its continuous production. Moreover, it is evident that fish respond with growth enhancement to treatment of most, if not all, kinds of GH. For this reason, GH genes or their cDNAs have been attracting attention for use in studies of fish transgenism, both as models and for the future application to aquaculture.

Nucleic Acids

Several approaches (i.e. morphological, histological, biochemical) have been performed to understand fish larval metabolism and nutritional condition that may reflect composite diets and natural feeding utilization (Ferron & Legget, 1994). However the need for sensitive and

reliable indicators of nutritional condition and growth has led to extensive research on molecular indicators, particularly nucleic acids that play a major role in growth and development. It has been shown that the ratio RNA/DNA is a useful indicator of nutritional condition. The amount of DNA is virtually constant in somatic tissues and tissue concentrations therefore reflect cell numbers. The amount of RNA in the cell is directly proportional to the amount of protein synthesis occurring. The relationship between RNA and DNA is an index of the cell's metabolic intensity and has been used to measure recent growth in fish (Clemmesen, 1996). RNA/DNA ratio relies on the fact that transcription dependent protein synthesis is positively correlated with ribosomal activity and increases in cellular RNA levels, while total DNA does not undergo such changes, furnishing a point of reference (Mommsen, 1998). This provides an excellent tool to assess the activity and growth of larvae and juveniles and has proven a useful indicator of the nutritional condition as shown in several larval fish studies (Westerman & Holt, 1988; Grant, 1996; Rooker, 1997; Buckley *et al*., 1999; Fukuda *et al*., 2001). The aim of using a molecular indicator to measure condition is to predict reliable survival probabilities under given food regimes.

By quantifying the total nucleic acids, according to the method of Westerman & Holt (1988), it was possible to observe that while alligator gar larvae fed live preys or artificial diets, increase in weight and size, the DNA and RNA concentrations increased too. This indicates cell proliferation and a parallel rise in the total quantity of RNA available for protein synthesis, that are channeled for growth, as previously reported by other authors (Mathers *et al*., 1994; Houlihan, 1993). At the same time, the RNA/DNA ratio increased until the 13 DAH (i.e. 9 day after feeding - DAF) and thereafter it remained constant, suggesting that the growth, as a product of protein accretion stabilizes at this stage and that larvae continue growing with the same metabolic intensity. This data agree with the morphological observations indicating the end of the metamorphosis, with the start of exponential growth, with the decrease of T3 levels and with the stabilization of the cell height of enterocytes. On the contrary, in starved larvae the concentrations of RNA, DNA and the ratio RNA/DNA was low and remained constant with a trend to decrease. These observations allowed to easily differentiate starved from fed larvae, and it was even possible to distinguish between larvae fed different diets. This was confirmed by means of other indices (Fig. 9) (Protein/Dry weight, Protein/DNA, RNA/Protein, DNA/Dry weight and RNA/Total length) (Mendoza *et al*., 2002b, 2002c).
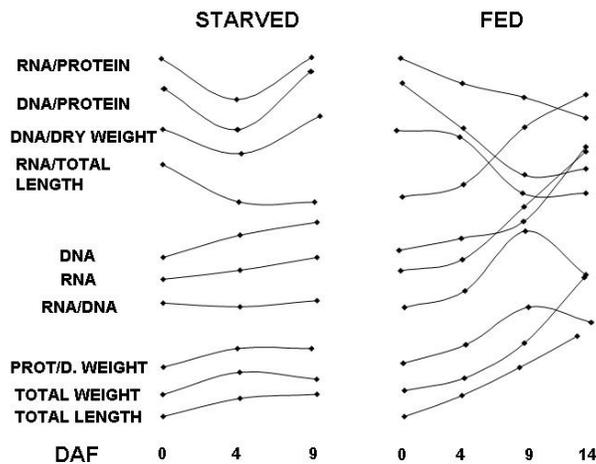
Fig. 9. Trends of different indices based on nucleic acids quantification observed in starved and fed larvae. DAF: days after feeding.

ACCEPTANCE AND CONSUMPTION OF ARTIFICIAL DIETS

Utilization of artificial diets in the culture of fish larvae bestows several advantages, among which the possibility to modify the dietary composition of prepared diets according to the nutritional requirements of the larvae is a major one (Bromley, 1978). Moreover, they represent an interesting alternative to avoid the extended use of live preys, (Duray & Bagariano, 1984). Artificial diets for the culture of marine and freshwater fish larvae has been extensively used (Duray & Bagariano, 1984). Three main strategies have been adopted for the conditioning of larvae to consume artificial diets and prevent or reduce the need of live preys. The first one is the so called direct conditioning, which refers to the supply of artificial diets since the start of the exogenous feeding. The second is the indirect conditioning, which consists in supplying artificial diets after feeding the larvae with live preys. The progressive conditioning is the third strategy, and implies the gradual replacement of live preys by artificial diets (Person-Le Rauyet, 1989; Lazo, 2000). Direct conditioning is usually achieved in the case of large larvae, like carps (Dabrowski *et al*., 1978), salmonids and sturgeons (Dabrowski *et al*., 1985) and soleids (*Solea solea*) (Appelbaum, 1985). However, several drawbacks have been associated to the use of this strategy. The occurrence of scoliosis, lordosis and erratic swimming behavior has been reported in coregonid larvae (*Coreogonus clupeaformis*) (Zitzow & Millard, 1988). Growth reduction and low survival have been observed in sparid larvae (*Sparus aurata)* (Tandler & Kolkovski, 1991). Problems reported with the use of this strategy have been attributed to the low quality of eggs and larvae (Dabrowski *et al*., 1985), to nutritional deficiencies of the diet (Zitzow & Millard, 1988), to a low affinity of proteolytic enzymes to artificial substrates offered in these diets that causes insufficient hydrolysis of dietary proteins and low food utilization (Dabrowski & Culver, 1991; Tandler & Kolkovski, 1991; Kolkovski *et al*., 1993) and to a low ingestion rate (Kolkovski *et al*., 1993). The rationale of the indirect conditioning strategy is that larvae at first feeding may sense and adapt to catching and ingesting live and mobile foods more readily than inert food, and once adapted, they also catch and accept inert feed. Indirect conditioning after feeding larvae with live preys during

the first days has been used in turbot larvae (*Scophthalmus maximus*), where it was observed that the use of limited amounts of live food was considered to be important for the successful weaning of very small larvae, although comparatively unimportant with large larvae (Bromley, 1978). An attempt to conditioning *Chanos chanos* larvae to artificial diets using an abrupt change was previously evaluated, but with a limited success. However, results were improved when a gradual conditioning was adopted (Duray & Bagariano, 1984). It has been observed that a longer feeding period with live preys postpones and increases the difficulty of weaning, while shorter periods of feeding live preys after which weaning to an inert diet is easier and the mortality rate during the weaning is lower but the growth rate is inferior (Appelbaum, 1985). However, the conditioning of small larvae is difficult only if it is performed directly wit artificial diets, but good results have been obtained with a mixed supply of live preys and artificial diets (Person-Le Ruyet, 1989). Using this strategy it has been possible to develop a gradual sequence from live food to artificial diets in *Lates calcarifer* larvae, allowing the reduction or prevention of cannibalism that occurs when live preys are replaced by artificial diets (Fuchs & Nedelec, 1990). Similarly, a gradual conditioning strategy has been successfully used with *Dicentrarchus labrax* larvae (Devresse *et al*., 1991).

The elaboration of an artificial diet implies taking into account the variety of nutritional requirements of different species and of their different developmental stages, as well as several physical and chemical characteristics of the diet (texture, color, size, density, attractability, stability, etc.). Several preliminary feeding experiments need to be performed to to determine the suitability of the diet.

Based on the results obtained through our multidisciplinary approach (morphological, structural, physiological and metabolic) it could be assumed that no difficulty for the conditioning of gar larvae onto artificial diets should exist. However, there was no previous successful report confirming this presumption for any of the evaluated lepisosteid species related with alligator gar (*i.e.* tropical gar or the Cuban gar). On the contrary, most attempts to culture larvae of this species failed, resulting in high mortality rates (León *et al*., 1978; García *et al*., 1997; Morales, 2000, personal communication; Marquez, 2001 personal communication). In addition, poor results were obtained when juveniles of these species were fed bait fish (Maldonado, 1991). Therefore, live preys were considered the only alternative for the successful of lepisosteid larvae, particularly for tropical gar (Rodriguez *et al.,* 1997; Hernandez *et al*., 1997; Hernandez, 1999). In the case of alligator gar and Cuban gar the culture trials to the juvenile stage were interrupted, and were only restricted to a stage where there were no problems of food acceptance or when cannibalism did not constitute a problem yet (Morales, 2000, personal communication). Our first attempts to culture gar larvae fed artificial diets between 1997 and 1999, were unsuccessful too. For these trials gar larvae were fed nauplii and adults of *Artemia salina*, but the culture ended at 15DAH, when larvae reached a maximum size of 50 mm, before the food supply could affect survival and growth. Although good growth and survival was not attained, we were able to identify some important characteristics of artificial diets, as well as the strategy for achieving their acceptance (Aguilera, 1999). The following year, the first successful results were obtained (Mendoza & Aguilera, 2000a; Mendoza *et al*., 2000c). What follows is a

summary of the experiments that led to the development of a successful strategy that allowed us to adequately culture larvae throughout the juvenile stage, exclusively fed on artificial diets.

To determine the acceptance of artificial diets by gar larvae, two feeding experiments were conducted at different times, each with their own control. Both feeding trials were performed with 3 DAH larvae and were evaluated at 15 DAH. In the first experiment, three different diets were tested: a) *Artemia nauplii*, as a positive control; b) a sinking pelleted diet of 0.5, 0.75 and 1.2 mm diameter; c) a combination of *Artemia nauplii* and the pelleted diet in equal proportions. In the second experiment the test diets were: a) *Artemia nauplii*, as a positive control; b) a sinking pelleted diet of 0.5, 0.75 and 1.2 mm diameter; c) an extruded 50% floating/50% slow-sinking micro-spheres diet of 0.5, 0.75 and 1.2 mm diameter. Diet particle size was increased through time (i.e., 0.5 mm pellets or micro-spheres 5 to 10 DAH; 0.75 mm pellets or micro-spheres 9 to 13 DAH; 1.2 mm pellets or micro-spheres 12 to 15 DAH), as snout length increased. Both artificial diets were donated by Purina S.A de C.V. Initial weight and total length of larvae were determined. The animals were blotted dry before being weighed to the nearest 0.01g. At the start of the feeding trials, larvae were selected according to the uniformity of their weight and size as determined by a one-way ANOVA. Larvae with a mean initial weight of 16.2 ± 1 mg and a mean initial length of 11.7 ± 0.5 mm were randomly assigned to twenty 45 L fiberglass tanks provided with freshwater in a recirculating system. The tanks were continuously aerated throughout the experiment by an air compressor. Each test diet was fed to 4-replicate tanks with 30 alligator gar larvae per tank. *Artemia nauplii* and artificial diets were fed *ad libitum* three times a day. At the end of the study, individual weight and total length were measured.

In the first experiment, larvae generally rejected the sinking pellet diet, despite the addition of a commercial betain-based feeding attractant. This resulted in 20% survival compared to 95% survival of larvae fed *Artemia nauplii*. A low ingestion rate of artificial diets may be the most likely cause of these results (Kolkovski *et al*., 1993). This problem has often been observed in species with evidently carnivorous habits (Lovshin & Rushing, 1989). Another plausible explanation is the feeding behavior of gar larvae, which feed mostly at the water surface. This 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 addition, this may suggests that the low floatability of artificial diets contributed to their low availability in the water column. It was not possible to maintain artificial feed in the water column even with the aid of supplemental aeration (Bromley, 1998). Moreover, air-lifts produced turbulence within the tanks and gar larvae, which are not active swimmers, tended to avoid such areas even though they may or may not have contained suspended food pellets. This behavior is possibly related with the habitats where gar adults reproduce. Spawning grounds are commonly flooded zones during the raining season (Perez-Sanchez, 1995). These suggestions were confirmed in the second experiment where gar larvae readily consumed the floating extruded microparticulated feed to which the same commercial attractant was added. In this case, in a similar experimental period,

survival increased to 80% and no significant differences were detected regarding growth and length when compared to the *Artemia nauplii* treatment group (Fig. 10a and 10b).
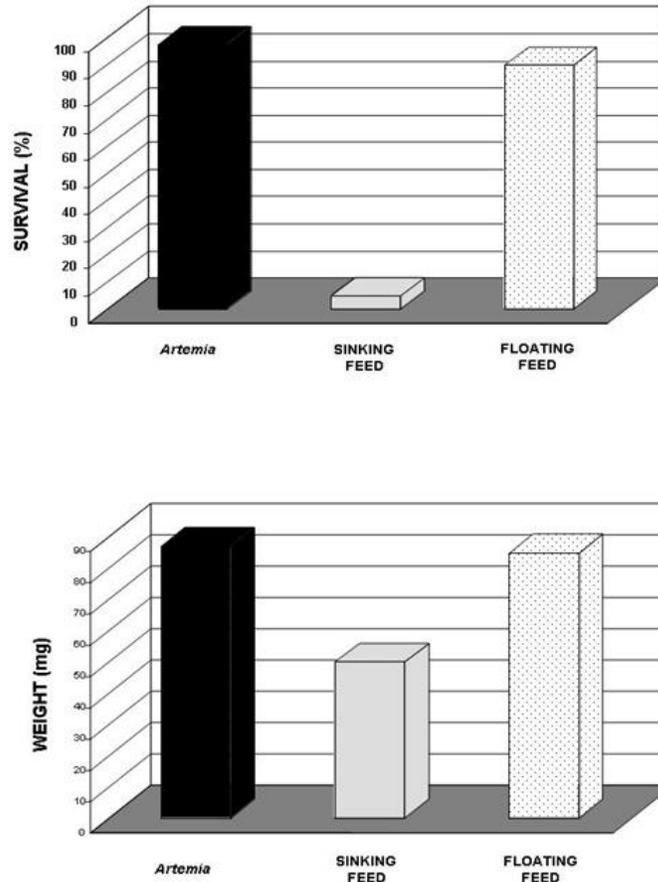

Fig. 10a. Survival of larvae fed natural preys and sinking or floating artificial diets. Fig. 10b. Weight gain of larvae fed natural preys and sinking or floating artificial diets. Different letters refer to significantly different means.

Improved attraction of the diet is paramount for an increased feed intake, and successful larval rearing. In the previous experiments we observed that once larvae approach the feed particles, they can stay next to these, and even have contact with them before their ingestion. This indicates that larvae probably use chemical perception to recognize their potential food. In this regard, the 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.

Thus, a third feeding bioassay was conducted to determine the acceptance of artificial diets by gar larvae (Mendoza *et al.*, 2002b). Four treatments were tested: a) Live feed (**LF**): consisted of *Artemia nauplii* and mosquito larvae, as a positive control; b) A 45%-protein

extruded micro-spheres diet (**45P**) of 0.5, 0.75 and 1.2 mm diameter with 50% floatability; c) A 52%-protein extruded crumbled diet (**52P**) of 0.3, 0.6 and 1.18 mm diameter with variable floatability; d) A control treatment (**C**) to which no feed was offered. Larvae with a mean initial weight of 27.9 ± 2 mg and a mean initial length of 15.7 ± 0.4 mm were randomly assigned to twelve 500 L fiber glass tanks provided with freshwater recirculated in a closed system. Each test diet was fed to 3-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 of different particle sizes was made at the 4th and 9th days of feeding (DF). The experiment was finished after 14 days of feeding Starved larvae (**C**) did not survive beyond the 9 DF, while in the rest of the treatments cannibalism was the main mortality cause of both preys and predators (Fig. 11a). The fact that cannibalism occurred only in those treatments where larvae were offered food could be explained by the rapid development of the snout coupled with an inadequate feeding supply. Higher growth was already evident at 4 DF for the LF and 45P treatments. At 9 DF, significant differences in growth were observed. Only larvae fed artificial diets continued to grow until the end of the experiment (Fig. 11b). The same trend was observed in morphometric indexes determined (*e.g.* weight gain, total length, snout length SGR, Fulton K index) and was confirmed by molecular indexes (*e.g.* RNA/DNA, protein/DNA; RNA/protein). Best performance resulted in those treatments where larvae were fed artificial diets, contrasting with most studies where artificial diets and live food have been compared (Person-Le Rauyet, 1989). The lower performance of larvae fed live food is explained by the size of live preys, which became rapidly inadequate considering the size of the snout of gar larvae (Fig. 12). This could be explained by the high energy expenditure of larvae in searching and capturing live preys, even if these were highly available, signifying that larvae would obtain a lower energetic reward than those larvae fed artificial diets, considering the increasing size of feed particles. Similarly Hunter (1981) reported that sea bream larvae selects food by size and that the size preference of sea bream increases faster than the increase in size of the food in the rearing tanks. Furthermore, the use of live food in alligator gar larvae culture implies the continuous and simultaneous production of high quantities of live preys of different size to satisfy the increasing demand of fast growing larvae. In practice, this is complicated and requires numerous facilities and extensive labor, resulting in a very costly strategy.
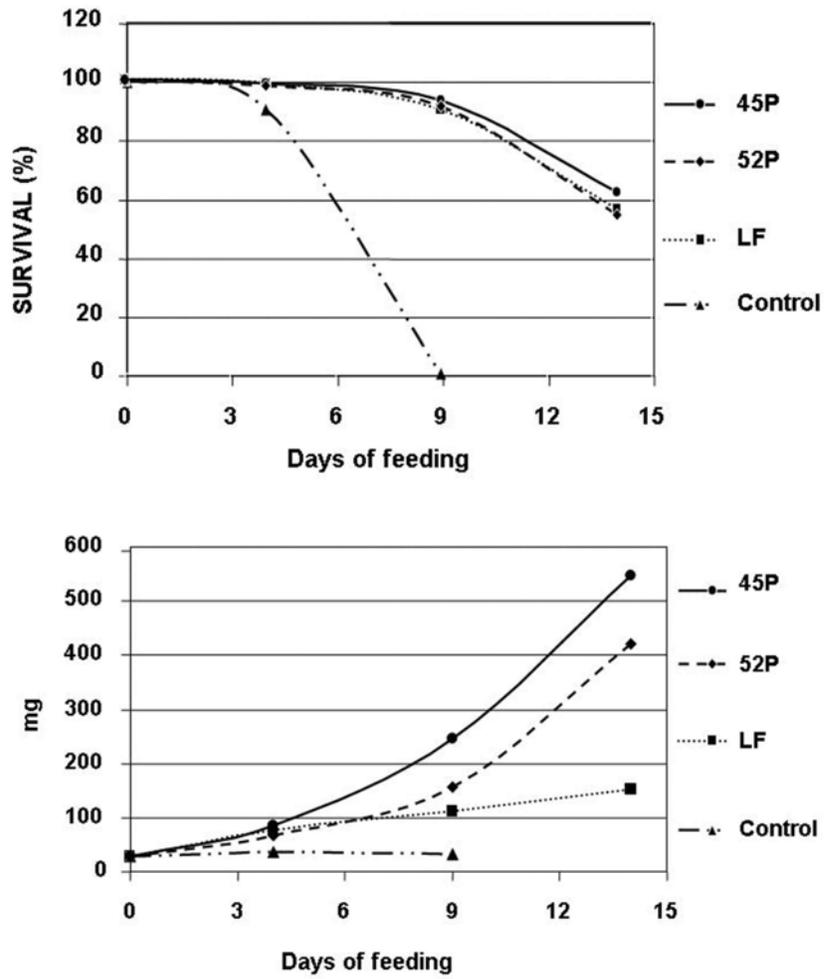
Fig. 11a. Survival of larvae fed natural preys (LF) and artificial diets (45P and 52P). C: Control. Fig. 11b. Weight gain of larvae fed natural preys.
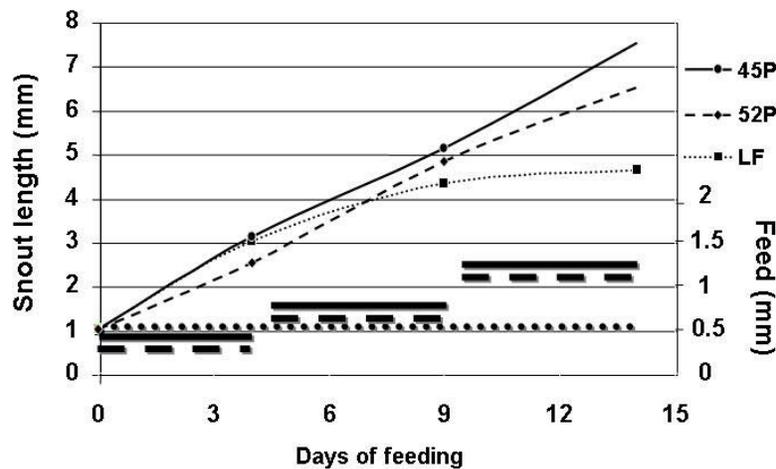
Fig. 12. Relationship between snout length and food size. LF: live food, 45P and 52P: artificial diets.

Differences in the performance of artificial diets 45P and 52P could be attributed to their manufacturing. It has been reported that during the process of manufacturing crumbled diets the individual particles tend to be of irregular shape and each particle end-up being a single ingredient which may result in selective feeding by fish and thus inadequate from a nutritional point of view (Marr, 1999). In addition, despite the higher floatability of crumbled diets (45 to 70 %), these were less available to gar larvae because they tend to agglomerate, losing their shape and remaining at the surface. In contrast, the micro-spheres had a lower floatability (30 to 60 %), but the particles did not lose their shape and could be individually ingested.

The results of these feeding trials made it possible to successfully culture larvae and juveniles using artificial diets. This feeding strategy produced 30 cm juveniles in four months. Our current research focuses on the determination of nutritional requirements of larval and juvenile alligator gar. Through our multidisciplinary approach, we determined that artificial diets could be used to replace live prey. This research provides a basis for the mass production of alligator gar larvae which ca now be used for repopulation experiments and for the culture of commercial-size alligator gar.

CONCLUSION

One of the major findings of the present research is that larvae of alligator gar can grow and develop to post-metamorphosis juveniles when fed solely on a formulated particulate diet. It was also demonstrated that the difficult and labor intensive period of Artemia feeding can be reduced considerably without any loss in terms of growth and survival. Because of the accelerated development of the digestive tract, gar larvae can be weaned on artificial diets

from their first week, provided that feed is of the appropriate nutritional quality, size and floatability. This has been demonstrated to be a requisite for particle consumption. Our current research focuses on the determination of nutritional requirements of larval and juvenile alligator gar

REFRENCES

Adelman, I. A., 1977. Effect of bovine growth hormone on the growth of carp (*Cyprinus carpio*) and the influences of temperature and photoperiod. J. Fish. Res. Bd. Can. 34,509-515.1977

Agellon, L. B., Emery, C. J., Jones, J. M., Davies, S. L., Dingle, A. D., Chen, T. T., 1988. Promotion of rapid growth of rainbow trout (*Salmo gairdnieri*) by a recombinant fish growth hormone. Can. J. Fish. Aquat. Sci., 45, 146-151.

Aguilera, C., 1999. Bases fisiológicas del desarrollo de larvas de catán (*Atractosteus spatula*) y perspectivas para su cultivo. Tesis Doctoral, Facultad de Ciencias Biológicas, U. A. N. L. Monterrey, N. L., pp.186.

Aguilera, C., Mendoza, R., Rodríguez, G., Márquez, G., 2002. Morphological description of alligator gar and tropical gar larvae, with an emphasis on growth indicators. Trans. Amer. Fish. Soc., 131, 899-909.

Alliot, E., Pastoureaud, A., Trellu, J., 1977. Evolution des activités enzymatiques dans le tube digestif au cours de la vie larvaire du bar (*Dicentrarchus labrax*). Variations des proteinogrammes et des zymogrammes. Actes de Colloques du C.N.E.X.O., 4, 85-91.

Alvarez, J., Sarmiento, M., Mendoza, R., 1999. Estudio histológico del desarrollo del tracto digestivo en larvas de catán *Atractosteus spatula* en condiciones de inanición. Resúmenes del XXIII Congreso Nacional de Histología, Monterrey, México, pp. 35.

Appelbaum, S., 1985. Rearing of the dover sole, *Solea solea* (L), through its larval stages using artificial diets. Aquaculture 49, 209-221.

AQUACOP, Calvas, J., 1990. State of the art of IFREMER in tropical aquaculture. In: Advances in tropical Aquaculture, Aquacop-IFREMER, Actes de Colloque 9, pp 625-642

Baragi, V., Lovell, R. T., 1986. Digestive enzyme activities in striped bass from first feeding through larval development. Trans. Am. Fish. Soc. 115, 478-484.

Beccaria, C., Diaz, J. P., Connes, R., Chatain, B., 1991. Organogenesis of the exocrine pancreas in the sea bass, *Dicentrarchus labrax* L., reared extensively and intensively. Aquaculture 99, 339-354.

Bromley, P. J., 1978. The weaning of hatchery reared turbot larvae (*Scophtalmus maximus* L.) on a dry diet. Aquaculture 13, 339-345.

Brown, C. L., Sullivan, C. V., Bern, H. A., Dickhoff, W. W., 1987, Occurrence of thyroid hormones in early developmental stages of teleost fish. American Fisheries Society Symposium, 2, 144-150.

Buckley, L., Caldarone, E., Ong, T.-L., 1999. RNA-DNA ratio and other nucleic acid-based indicators for growth and condition of marine fishes. Hydrobiologia. 401, 265-277.

Buddington, R. K., 1985. Digestive secretions of lake sturgeon Acipenser fulvescens during early development. J. Fish. Biol., 26, 715-724. 341

Buddington, R. K., Chrstofferson, P., 1985. Digestive and feeding characteristics of the chondrosteans. In: North american sturgeons: biology and aquaculture potential, (ed. by F.P. Binkowski and S.I. Doroshov), DR W. Junk Publishers, 31-41. Kluwer Academic Publishers Group, Boston.

Buddington, R. K., Dorshov, S. I., 1986. Development of digestive secretions in white sturgeon juveniles (*Acipenser transmontanus*) Comp. Biochem. Physiol., 83A,233-238. 344

Burns, T. A., Stalling, D. T., Goodger, W., 1981. Gar ichthyotoxin, its effect on crayfish, with notes on bluegill sunfish. The Southwestern Naturalist 25(4), 513-515.

Busch, A., 1996. Transition from endogenous to exogenous nutrition: larval size parameters determining the start of external feeding and size of prey ingested by ruegen spring herring *Clupea harengus*. Marine Ecology Progress Series 130, 39-46.

Chavez-Lomelí, M. O., 1989. Biología de los peces del río San Pedro en vista de determinar su potencial para piscicultura. Instituto de Investigaciones sobre Recursos Bióticos y la Fundación Universitaria para la cooperación, Jalapa, Ver. 90 pp.

Cheema, I. R., Maty, A. J., 1978. Increased uptake of L-leucine-^{14}C in the skeletal muscle of rainbow trout, *Salmo gairdnieri* after administration of growth hormone. Pak. J. Zool. 10,119-123.

Clemmesen, C., 1996. Importance and limits of RNA/DNA ratios as a measure of nutritional condition in fish larvae. In: Survival strategies in early life stages of marine resources (ed. by Watanabe, Y., Yamashita, Y. and Oozeki, Y.) Proceedings of an Internatíonal Workshop, Yokohama, Japan, 11-14 October 1994. AA. Balkema, Rotterdam, the Netherlands, pp. 67-82

Cook H., Cook, A. F., Peter, R. E., 1983. Ultrastructural immuno-chemistry of growth hormone cells in the goldfish pituitary gland. Gen. Comp. Endocrinol., 50,348-353.

Dabrowski, K. R., 1986. Ontogenical aspects of nutritional requerimients in fish. Comp. Biochem. Physiol., 85A(4),639-655.

Dabrowski, K., Bardega, R., 1984. Mouth size and predicted food size preferences of larvae of three cyprinid fish species. Aquaculture 40, 41-46.

Dabrowski, K., Culver, D., 1991. The physiology of larval fish, digestive tract and formulation of starter diets. Aquaculture Magazine March/April: 49-61.

Dabrowski, K., Dabrowska, H., Grudniewski, C., 1978. A study of the feeding of common carp larvae with artificial food. Aquaculture 13, 257-264.

Dabrowski, K., Kaushik, S. J., Fauconneau, B., 1985. Rearing of sturgeon (*Acipenser baeri* Brandt) larvae I. Feeding Trial. Aquaculture 47, 185-192.

Dean, B., 1895. The early development of gar-pike and sturgeon. Journal of Morphology 11(1), 1-55.

Dettlaff, T. A., Ginsburg, A. S., Schmalhausen, O. I., 1993. Development of prelarvae. In: Sturgeon Fishes, Developmental Biology and Aquaculture. Pp. 155-221 Springer-Verlag Ed., Berlin, Germany.

Devresse, B., Candreva, P., Sorgeloos, P., 1991. A new artificial diet for the early weaning of seabass (*Dicentrarchus labrax*) larvae. In: Larvi 91 - Fish and crustacean larviculture symposium, European Aquaculture Society, Special Publication, 15, 178-182.

Duray, M., Bagarinao, T., 1984. Weaning of hatchery-bred milkfish larvae from live food to artificial diets. Aquaculture 325-332.

Echelle, A. A., Riggs, C. D., 1972. Aspects of the early life history of gars (*Lepisosteus*) in Lake Taxoma. Transactions of the American Fisheries Society 101, 106-112.

FAO/PNUMA, 1984. Conservación de los recursos genéticos de los peces: Problemas y recomendaciones. Informe de la consulta de expertos sobre los recursos genéticos de los peces. FAO. Doc. Tec. Pesca, (217): 42p.

Ferraris, R. P., Tan, J. D., De La Cruz, M. C., 1987. Development of digestive tract of milkfish, *Chanos chanos* (Forsskal): hitology and histochemistry. Aquaculture 61, 241-257.

Ferron, A., Leggett, W. C., 1994. An appraisal of condition measures for marine fish larvae. Adv. Mar. Biol. 30, 217-303.

Fauconneau, B., Mady, M. P., LeBail, P. Y., 1996. Effect of growth hormone on muscle protein synthesis in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*).Fish Physiol. Biochem. 15(1), 49-56.

Foster, A. R., Houlihan, D. F., Gray, C., Medale, F., Fauconneau, B., Kaushik, S. J., LeBail, P. Y., 1991. The effects of ovine growth hormone on protein turnover in rainbow trout. Gen. Comp. Endocrinol. 81, 111-120.

Fuchs, J., Nedelec, G., 1990. Larvae rearing and weaning of seabass, *Lates calcarifer* (Block), on experimental compounded diets. Advances in Tropical Aquaculture, Actes de Colloque 9 pp. 677-697.

Fukuda, M., Sako, H., Shigeta, T., Shibata, R., 2001. Relationship between grow and biochemical indices in laboratory-reared juvenile Japanese flounder (*Paralichthys olivaceus*), and its application to wild fish, Mar. Biol., 138, 47-55.

García, J., Márquez, G., Páramo, S., 1997. Utilización de alimento fresco y artificial en la sustitución de alimento vivo para la cría de larvas y postlarvas del pejelagarto *Atractosteus tropicus*. In: Memorias de la Semana de Divulgación y Video Científico 1997, Secretaría de servicios académicos Universidad Juárez Autónoma de Tabasco, pp. 61-64.

Gatsoupe, F. J., Luquet, P., 1982. Weaning of the sole (*Solea solea*) before metamorphosis. Aquaculture, 26:359-368.

Gawlicka, A., Teh, S. J., Hung, S. S. O., Hinton, D. E., de la Noue, J., 1995. Histological and histochemical changes in the digestive tract of white sturgeon larvae during ontogeny. Fish Physiol. Biochem., 14, 357-371

Gershanovich, A. D., 1991. Lipid mobilization during early development of sturgeons. Ed. Acipenser, Cemagref Publ. pp. 41-51. P. Williot, Ed. Acipenser, Cemagref Pub., Moscow.

Gill, J. A., Sumpter, J. P., Donaldson, E. M., Dye, H. M., Souza, L., Berg, T., Wypych, J., Langley, K., 1985. Recombinant chicken and bovine growth hormones accelerate growth in aquacultured juvenile Pacific salmon, *Oncorhynchus kisutch*. Biotechnology 3, 643-646.

Gisbert, E., Rodríguez, A., Castelló-Orvay, F., Williot, P., 1998. A histological study of the development of the digestive tract of siberian sturgeon (*Acipenser baeri*) during early ontogeny. Aquaculture 167, 195-209.

Goodyear, C. P., 1967. Feeding Habits of Three Species of Gars, *Lepisosteus*, along the Mississippi Gulf Coast. Trans. Am. Fish. Soc. 96, 297-300

Govoni, J. J., George, W., Watanabe, Y., 1986. The physiology of digestion in fish larvae. Environmental Biology of Fishes, 16 (1-3), 59-77.

Grant, G. C., 1996. RNA-DNA ratios in white muscle tissue biopsies reflect recent growth rates of adult brown trout. J. Fish Biol. 48, 1223-1230.

Grizzle, J. M., Curd, M. R., 1978. Posthatching histological development of the digestive system and swim bladder of logperch, Percina caprodes. Copeia, 3, 448-455.

Hecht, T., Pienaar, A. G., 1993. A review of cannibalism and its implications in fish larviculture. Journal of the World Aquaculture Society 24(2), 246-261.

Hernández, U., 2002. Control reproductivo de *Atractosteus tropicus*: Evaluación de la inducción hormonal. Tesis de Maestría. Facultad de Ciencias Biológicas, U. A. N. L., Monterrey, N. L., 98 p

Hernandez, U., Marquez, G., Páramo, S., Félix, S., Hernández, S., 1997. Valor nutritivo de nauplios de *Artemia spp*. y uso en la larvicultura del pejelagarto *Atractosteus tropicus*. In: Memorias de la Semana de Investigación y Divulgación Científica de la Universidad Juárez Autónoma de Tabasco 1997. pp. 65-68.

Hernández, U., 1999. Punto crítico de no-retorno en larvas de pejelagarto *Atractosteus spatula* (Gill, 1863). Tesis de Licenciatura Inédita. División Académica de Ciencias Biológicas, Universidad Juárez Autónoma de Tabasco 1997. pp. 46

Higgs, D. A., Donaldson, E. M., Dye, H. M., McBride, JR., 1975. A preliminary investigation of the effect of bovine growth hormone on growth and muscle composition of coho salmon (*Oncorhynchus kisutch*). Gen Comp. Endocrinol., 27, 240-253.

Higgs, D. A., Donaldson, E. M., Dye, H. M., McBride, JR., 1976. Influence of bovine growth hormone and L-thyroxine on growth, muscle composition and histological structure of the gonads, thyroid, pancreas and pituitary of coho salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Ca., 33, 1585-1603.

Holloway, A., 1954. Notes on the life history and management of the shortnose and longnose gars in Florida waters. Journal of Wildlife Management 18(4), 438-449

Houlihan, D. F., Mathers, E. M., Foster, A., 1993. Biochemical correlates of growth rate in fish. In: Fish Ecophysiology (ed. by J. Cliff and B. Jensen) pp. 45-71. Chapman and Hall, London. pp.421

Hunter, J. R., 1981. Feeding Ecology and Predation of Marine Fish Larvae. In: Marine Fish Larvae (ed. by Lasker, R.), pp. 34-59. Washington Sea Grant Program Publisher.

Inui, Y., Tagawa, M., Miwa, S., Hirano, T., 1989. Effects of bovine TSH on the tissue thyroxine level and metamorphosis in prometamorphic flounder larvae. General and Comparative Endocrinology, 74, 406-410.

Kaji, T., Tanaka, M., Takahashi, Y., Oka, M., Ishibashi, N., 1996. Preliminary observations on development of pacific bluefin tuna *Thunnus thynnus* (Scombridae) larvae reared in the laboratory, with special reference to the digestive system. Mar. Freshwater Res., 4, 261-269.

Kobuke, L., Specker, J., Berni, H. A., 1987. Thyroxine content of eggs and larvae of coho salmon *Oncorhynchus kisutch*. J. Exp. Zool. 248, 168-176

Kolkovski, S., Tandler, A., Kissil, G. W., Gertler, A., 1993. The effect of dietary exogenus digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata*, *Sparidae*, *Linnaeus*) larvae. Fish Physiology and Biochemistry, 12(3), 203-209.

Komourdjian, M. P., Saunders, R. L, Fenwick, J. C., 1976. The effect of porcine somatotropin on growth and survival in seawater of Atlantic salmon (*Salmo salar*). Can. J. Zool. 54, 531-535.

Lacroix, M. C., Devinoy, E., Cassy, S., Servely, J. L., Vidaud, M., Kann, G., 1999. Expression of growth hormone and its receptor in the placental and feto-maternal environment during early pregnancy in sheep. Endocrinology, 140(12), 5587-97.

Lam, T. J., 1980. Tyroxine enhances larval development and survival in *Sarotherodon* (Tilapia) *mossambicus* Rupell. Aquaculture: 21, 287-291.

Lauf, M., Hoffer, R., 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. Aquaculture 37, 335-346

Lazo, J., 2000. Conocimiento actual y nuevas perspectivas en el desarrollo de dietas para larvas de peces marinos. In: Avances en nutrición acuícola V. Memorias del 5º Simposium Internacional de Nutrición acuícola (ed. by Cruz- Suárez, L. E., Ricque-Marie, O., Tapia-Salazar, M., Olvera-Novoa, M. A. y Civera-Cerecedo, R.), pp. 300-312. 19-22 Noviembre, 200. Mérida, Yuc., México

León, L., Aguiar, R., Hernández, 1978. Estudio sobre la biología y el cultivo artificial del manjuari (*Atractosteus tristoechus*) Bloch y Schmeider. Ministerio de la Industria Pesquera, Dirección Ramal de Acuicultura, Investigación No. 85, Cuba. Pp. 1-35.

Lovshin, L. L., Rushing, H. J., 1989. Acceptance by largemouth bass fingerlings of pelleted feeds with a gustatory additive. The Progressive Fish-Culturist, 51, 73-78.

Maldonado, E., 1991. Aprovechamiento de peces forrajeros en la alimentación de *Atractosteus tropicus* (Gill) en jaulas flotantes en el Estado de Tabasco, Mexico. Universidad y Ciencia, 8(15), 77-90.

Markert, J. R., Higgs, D. A., Dye, H. M., MacQuarrie, D. W., 1977. Influence of bovine growth hormone on growth rate, appetite, and food conversion of yearling coho salmon (*Oncorhynchus kisutch*) fed two diets of different composition. Can. J. Zool., 55, 74-83.

Márquez, G., 2000. Biología y Tecnología para el Cultivo del pejelagarto en el sureste de México. In: Redes nacionales de investigación en acuacultura, Memorias de la IV Reunión. (ed. by P. Álvarez, M. Guzmán, S. Contreras, y A. Silva), pp. 263-265. SEMARNAP, Instituto Nacional de la Pesca. México.

Marr, C., 1999. Improving efficiency in starter feeds. Aqua Feed, 4, 18-20.

Mathers, E. M., Houlihan, D. F., Burren, L. J., 1994. RNA, DNA and protein concentrations in fed and starved herring *Clupea harengus* larvae. Mar Ecol. Prog. Ser. 107, 223-231.

Matty, A. J., 1962. Effects of mammalian growth hormone on *Cottus scorpius* blood. Nature (London). 195, 506-507.

May, E. B., Echelle, A. A., 1968. Young-of-year alligator gar in Lake Taxoma, Oklahoma. Copeia. 3, 629-630.

McFadzen, I. R., Lowe, D. M., Coombs, S. H., 1994. Histological changes in starved turbot larvae (*Schophtalmus maximus*) quantified by digital image analysis. Journal of Fish Biology, 44, 255-262.

Medale, F., Fauconneau, B., Kaushick, S. J., 1988. Effect of GH administration on energy metabolism in rainbow trout. In: Energy Transformation in Cells and Organisms (ed. by W. Wieser and E. Gnaiger), pp. 240. Georg Thieme Verlag, Stuttgart.

Mendoza, R., 2000. Catán. In: Estado de salud de la acuacultura, (ed. by Alvarez-Torres), pp. 231-242. Instituto Nacional de la Pesca, Secretaria de Recursos Naturales y Pesca. Distrito Federal, Mexico.

Mendoza, R., Aguilera, C., 2000a. Morphophysiological studies on alligator gar *Atractosteus spatula* larval development as a basis of their weaning onto artificial diets. 24th Annual Larval Fish Conference. Alabama.

Mendoza, R., Aguilera, C., 2000b. Cultivo y pesquería de catán, In: Carta Nacional Pesquera pp. 56-57.. Diario Oficial de la Federación, Tomo DLXIII, No. 20, 28 de agosto de 2000.

Mendoza, R., Aguilera, C., Montemayor, J., Rodríguez, G., Márquez, G., 2000a. Biología de los lepisosteidos y estudios orientados hacia la recuperación de las poblaciones naturales del catán (*Atractosteus spatula*) (Lacepède, 1803). In: Redes nacionales de investigación en acuacultura, Memorias de la IV Reunión P. (ed. by Álvarez, Guzmán, M., Contreras, S. y Silva, A.), pp. 103-120. SEMARNAP, Instituto Nacional de la Pesca. Distrito Federal, México.

Mendoza, R., Aguilera, C., Montemayor, J., Rodríguez, G., Castro, R., 2000b. Aspectos reproductivos de los lepisosteidos, con énfasis en las perspectivas para el control de la reproducción del catán (*Atractosteus spatula*). In: Redes nacionales de investigación en acuacultura, Memorias de la IV

Reunión P. (ed. by Álvarez, Guzmán, M., Contreras, S. y Silva, A.), pp 281-290. Redes nacionales de investigación en acuacultura, Memorias de la IV Reunión. SEMARNAP, Instituto Nacional de la Pesca. Distrito Federal, México.

Mendoza, R., Aguilera, C., Rodríguez, G., Márquez, G., 2000c. Estrategias para la domesticación de especies en acuacultura: El catán (*Atractosteus spatula*). In: Redes Nacionales de Investigación en Acuacultura, Memorias de la V Reunion (ed. by Alvarez Torres, Ramírez-Flores, Torres-Rodriguez y Mora-Cervantes), pp. 95-102. Instituto Nacional de Pesca-SEMARNAP. Distrito Federal, México.

Mendoza, R., Aguilera, C., Ruiz, V., García-Carreño, F., Nolasco, H., Montemayor J., Rodríguez, G., 2000d. Enzyme ontogenetic larval development of the alligator gar Atractosteus spatula in México: Perspectives for its culture. World Aquaculture Society. New Orleans, USA. Feb, 2000. page 219.

Mendoza, R., Aguilera, C., 2001. Bases Fisiológicas del desarrollo de larvas de *Atractosteus spatula* y perspectivas para su cultivo. Ciencia-UANL. 4(2), 161-166

Mendoza, R., Aguilera, C., Montemayor, J., 2001. Effects of thyroid hormones and glucocorticoids on alligator gar Atractosteus spatula larval development. World Aquaculture Society. Orlando, Florida, USA. Page 434.

Mendoza, R., Aguilera, C., Rodriguez, G., González, M., Castro, R., 2002a. Morphophysiological studies on alligator gar (*Atractosteus spatula*) larval development as a basis for their culture and repopulation of their natural habitats. Reviews in Fish Biology and Fisheries. 00: 1-9, In press.

Mendoza, R., Aguilera, C., Carreón, L., Montemayor, J., González, M., 2002b. Early weaning of alligator gar *Atractosteus spatula* larvae. Aquaculture 2002, WAS. San Diego, California, Enero 27-30 2002. Page 214.

Mendoza, R., Aguilera, C., Carreón, L., 2002c. Acidos nucleicos para evaluar la condición de larvas de peces. Ciencia-UANL, 5(2), 211-217.

Mendoza, R., Garza-Rodriguez, M. L, Hernández-Montenegro, V., Aguilera, C., Barrera Saldaña, H., Revol, A., 2002d. Cloning of the growth hormone cDNA of alligator gar *Atractosteus spatula* and its expression along larvae stages. World Aquaculture Society. San Diego, California USA. January, 2002. Page 213.

Mommsen, T., 1998. Growth and Metabolism. In: The Physiology of Fishes. (ed. by D.H., Evans). CRC Press, Boca Raton, Florida. pp. 65-97.

Moore, G., Trautman, M., Curd, M., 1973. Description of postlarval gar (*Lepisosteus spatula Lacepede, Lepisosteidae*), with a list of associated species from the Red River, Choctaw County, Oklahoma. The Southwest Naturalist 18, 343-344.

Moyano, F. J., Diaz, M., Alarcon, F. J., Sarasquete, M. C., 1996. Characterization of digestive enzyme activity during larval development of gilthead sea bream (*Sparus aurata*). Fish Physiol. Biochem., 15, 121-130.

Munilla-Moran, R., Saborido, F., 1996. Digestive enzymes in marine species I. Proteinases activities in gut from red fish (*Sebastes mentella*), seabream (*Sparus aurata*) and turbot (*Scophtalmus maximus*). Comp. Biochem. Physiol. 113B, 395- 402. 384

Netch, N. F., Witt, A., 1962. Contributions to the life history of the longnose gar (*Lepisosteus osseus*) in Missouri. Transactions of the American Fisheries Society 91(3), 251-262.

Oozeki, Y., Ishii, T., Hirano, R., 1989. Histological study of the effects of starvation on reared and wild-caught larval stone flounder *Kareius bicoloratus*. Mar. Biol. 100, 269-275

Pantaleon, M., Whiteside, E. J, Harvey, M. B, Barnard, R. T, Waters, M. J., Kaye, P. L., 1997. Functional growth hormone (GH) receptors and GH are expressed by preimplantation mouse embryos: a role for GH in early embryogenesis?. Proc Natl Acad Sci U S A., 94(10), 5125-30.

Pearson, W. D., Thomas, G. A., Clark, A. L., 1979. Early piscivory and timing of the critical period in postlarval longnose gar at mile 571 of the Ohio River. Transactions of the Kentucky Academy of Science 40(3-4), 122-128.

Perez-Sanchez, E., 1995. Efecto de la gonadotropina coriónica humana (GCH) en la maduración gonádica del pejelagarto (*Atractosteus tropicus*) Gill 1823, en condiciones de laboratorio. Tesis de licenciatura Inédita, Universidad Juárez Autónoma de Tabasco, División Académica de Ciencias Biológicas, 28 pp.

Person-Le Rouyet, J., 1990. Early weaning of fish larvae onto microdiets: constraints and perspectives. In: Advances in tropical Aquaculture, Aquacop-IFREMER, Actes de Colloque 9, pp 625-642.

Pickford, G. E., Atz, J. W., 1957. The Physiology of the Pituitary Gland of Fishes, pp. 1-91. New York Zoological Society, New York.

Pickford, G. E., Thompson, E. F., 1948. The effects of purified mammalian growth hormone on the killfish Fundulus heteroclitus (Linn). J. Exp. Zool. 109, 367-383

Resendez, A., Salvadores, M. L., 1983. Contribución al conocimiento de la biología del pejelagarto *Lepisosteus tropicus* (Gill) y la tenguayaca *Petenia splendida* (Günther), del Estado de Tabasco. Biotica 8(4), 413-426.

Rodríguez, F., Marquez, G., Paramo, S., 1997. Evaluación del uso de cladóceros y nauplios de Artemia en la alimentación de larvas de pejelagarto *Atractosteus tropicus*. In: Memorias de la Semana de Investigación y Divulgación Científica . pp. 72-75. Universidad Juárez Autónoma de Tabasco 1997.

Rodríguez, G., Mendoza, R., Aguilera, C., Montemayor, J., 2000. Elaboración de una dieta artificial para larvas de catán (*Atractosteus spatula*) con miras a su repoblación y posible cultivo. In: Investigación para el Desarrollo Regional. pp. 160 -164Consejo Nacional de Ciencia y Tecnología. Ed., Distrito Federal, Mexico.

Rojas, P., Mendoza, R., 2000. El cultivo de especies natives en México. In: Estado de salud de la acuacultura (ed. by .. Alvarez-Torres, P.). pp. 431-476. Instituto Nacional de la Pesca, Secretaria de Recursos Naturales y Pesca. Distrito Federal, Mexico.

Rooker, J., 1997. Early life history of Red drum (*Sciaenops ocellatus*) in subtropical seagrass meadows: patterns of condition, growth, and mortality. Ph.D. Dissertation. Faculty of the Graduate School of the University of Texas at Austin. Pp. 191.

Rosas, M., 1976. Peces dulceacuícolas que se explotan en México y datos sobre su biología. Instituto Nacional de la Pesca/Subsecretaria de Pesca. México. Pp. 90.

Rosca, R., Appelbaum, S., 1985. Experiments on the suitability of dry food for larvae of Coregonus lavaretus L. Aquaculture, 48, 291- 302

Rubin, D. A., Youson, J. H., Marra, L. E., Dores, R. M., 1996. Cloning of a gar (*Lepisosteus osseus*) GH cDNA: trends in actinopterygian GH structure. J. Mol. Endocrinol. 16 (1), 73-80

Ruiz, V., 2000. Caracterización bioquímica de las principales enzimas digestivas del catán (*Atractosteus spatula*). Tesis de Licenciatura, Facultad de Ciencias Biológicas, U. A. N. L. Monterrey, N. L., 76 pp

Sarasquete, M. C., Polo, A., Yufera, M., 1995. Histology and histochemistry of the development of the digestive system of larval gilthead seabream, *Sparus aurata* L. Aquaculture, 130, 79-92.

Segner, H., Stroch, V., Reinecke, M., Kloas, W., Hanke, W., 1994. The development of functional digestive and metabolic organs in turbot, *Scophtalmus maximus*. Marine Biology, 119, 471-486.

SEPESCA-INP (Secretaria de Pesca - Instituto Nacional de la Pesca), 1994. Atlas Pesquero de México. SEPESCA. México. Ed. by M. Rodriguez, M. Palacios, R. Cruz and C. Díaz Pp. 234.

Sheridan, M. A., 1986. Effects of thryroxine, cortisol, growth hormone and prolactin on lipid metabolism of coho salmon Oncorhynchus kisutch, during smoltification. Gen. Comp. Endocrinol. 64, 220-238.

Simon, T. P., Tyberghein, E. J., 1991. Contributions to the early life history of the spotted gar, *Lepisosteus oculatus* Winchell, from Hatchet Creek, Alabama. Transactions of the Kentucky Academy of Science 52(3-4), 124-131.

Simon, T. P., Wallus, R., 1989. Contributions to the early life histories of gar (*Actinopterygii: Lepisosteidae*) in the Ohio and Tennessee River basins with emphasis on larval development. Transactions of the Kentucky Academy of Science 50(1-2), 59-74.

Sullivan, C. V., Iwamoto, R. N., Dickhoff, W. W., 1987. Thyroid hormones in blood plasma of developing salmon embryos. General and Comparative Endocrinology. 65, 337-345.

Suttkus, R. D., 1963. Order Lepisostei. In: Fishes of the Western North Atlantic. Part three, Soft-rayed fishes (ed. by . H. B. Bigelow) pp. 61-88. Yale University Memoir Sears Foundation for Marine Research, New Haven, Connecticut.

Tagawa, M., Hirano, T., 1987. Effects of thyroid deficiency in eggs on early development of the medaka, *Oryzias latipes*. The Journal of Experimental Zoology, 257, 36-366.

Tagawa, M., Tanaka, M., Matsumoto, S., Hirano, T., 1990. Thyroid hormones in eggs of various freshwater, marine and diadromus teleosts and their changes during egg development. Fish Physiology and Biochemistry. 8(6), 515-520.

Tanaka, M., 1969a. Studies on the structure and function of the digestive system in teleost larvae I. Development of the digestive system during prelarval stage. Japanese Journal of Ichthyology, 16(1), 1-8.

Tanaka, M., 1969b. Studies on the structure and function of the digestive system in teleost larvae II. Characteristic of the digestive system in larvae at the stage of first feeding. Japanese Journal of Ichthyology, 16(2), 41-49.

Tanaka, M., 1971. Studies on the structure and function of the digestive system in teleost larvae III. Development of the digestive system during postlarval stage. Japanese Journal of Ichthyology, 18(4), 164-173.

Tanaka, M., 1972. Studies on the structure and function of the digestive system in teleost larvae V. Epithelial changes in the posterior gut and protein ingestion. Japanese Journal of Ichthyology, 19(3), 172-180.

Tandler, A., Kolkovski, S., 1991. Rates of ingestion and digestibility as limiting factors in the successful use of microdiets in *Sparus aurata* larval rearing. European Aquaculture Society, Special Publication, 15, 169-171.

Theilacker, G. H., Porter, S. M., 1995. Condition of larval walleye pollock, *Theragra chalcogramma*, in the western Gulf of Alaska assessed with histological and shrinkage indices. Fish. Bull., 93, 333-344.

Theilacker, G. H., Watanabe, Y., 1989. Midgut cell height defines nutritional status of laboratory raised larval northern anchovy, *Engraulis mordax*. Fish. Bull. 87(3), 457-469

Ueberschär, B. F. R., 1988. Determination of the nutritional condition of individual marine fish larvae by analyzing g their proteolytic enzyme activities with a highly sensitive fluorescence technique. Meeresforsch. Rep. mar. Res. 32, 144-154.

Verreth, J., Van Tongeren, T., 1989. Weaning time in *Clarias gariepinus* (Burchell) larvae. Aquaculture, 83, 81-88

Verreth, J. A., Torreele, E., Spazier, E., Sluiszen, A. V., 1992. The development of a functional digestive system in the african catfish *Clarias garipinus* (Burchell). J. World Aquaculture Soc. 23(4), 286-298

Vonk, H. J., Western, J., 1984. Factors influencing enzymatic digestion. In: Comparative Biochemistry and Physiology of Enzymatic digestion. Academic Press, pp 448-491

Wagner, C. F., McKeown, B. A., 1981. The purification, partial characterization and bioassay of growth hormone from two species of Pacific salmon. In: B. Lofts and W.N. Holmes (editors), Current trends in Comparative Endocrinology. Proc. 9th Symp. Comp. Endocrinol. Hong Kong,p. 211.

Walford, J., Lam, T. J., 1993. Development of digestive tract and proteolytic enzyme activity in seabass (*Lates calcarifer*) larvae and juveniles. Aquaculture, 109, 187-205.

Watanabe, T., Kiron, V., 1994. Prospects in larval fish dietetics. Aquaculture, 124, 223-251.

Watanabe, Y., 1984. Morphological and functional changes in rectal epithelium cells of pond smelt during postembrionic development. Bulletin of the Japanese Society of Scientific Fisheries, 50(5), 805-814.

Westerman, M. E., Holt, G. J., 1988. The RNA-DNA ratios: measurement of nucleic acids in larval *Sciaenops ocellatus*. Contributions to Marine Sciences 30, 117-124.

Xu, K., Wei, Y., Guo, L., Zhu, Z., 1991. The effects of growth enhancement of human growth hormone gene transfer and human growth hormone administration on crussian carp (*Carasius auratus* gibelio Bloch). Acta Hydrobiol. Sin. 15, 31-34.

Yang, B., Green, M., Chen, T., 1999. Early Embryonic Expression of the growth hormone family Protein Genes in the Developing Rainbow Trout, *Onchorhynchus mykiss*. Mol Reprod. Dev. 53(2), 127-34.

Yeager, B. L., Bryant, R. T., l983. Larvae of the longnose gar, *Lepisosteus osseus*, from the Little River in Tennessee. Journal of the Tennessee Academy of Science 58(1-2), 20-22.

Zitzow, R. E., Millard, J. L., 1988. Survival and growth of lake whitefish (*Coregonus clupeaformis*) larvae fed only formulated diets. Aquaculture, 69, 105-113.