Development of Aquaculture of the Red Snapper *Lutjanus campechanus*: Research on Larval Nutrition

Desarrollo de la Acuicultura de Pargo Rojo *Lutjanus campechanus*: Investigacion sobre Nutricion de Larva

Développement de L'aquaculture du Vivaneau Campèche *Lutjanus campechanus*: Recherches en Nutrition Larvaire

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ABSTRACT

The red snapper *Lutjanus campechanus* is a reef fish of major economic importance in the southeastern United States. Red snapper aquaculture is being investigated both for stock enhancement, as a tool for rebuilding wild stocks, and for commercial production motivated by the high demand and high market value for this species. A major limitation to red snapper aquaculture is the difficulty associated with the rearing of early larval stages. Red snapper larvae require copepod nauplii as an initial essential feed for a significant portion of the larval rearing period although large scale production of copepods, as would be required to meet the needs of commercial aquaculture or stock enhancement, is not feasible. Reducing the dependency on copepods would thus greatly enhance red snapper aquaculture. The present research aims to understand the nutritional requirements and digestive capacity of red snapper larvae in order to support development of feeding protocols and, in particular, the identification of potential alternative feeds to copepods. The ontogeny of the digestive system is being described using histology and measurements of the activity of digestive enzymes performed on larvae sampled at different staged of development from hatching to the end of the larval period. Preliminary results indicate a slow growth of larvae during the first 12 days post hatch. Gastric glands, indicating acquisition of stomach functionality, were first observed in 18 day old larvae using histology. Further research in progress includes analysis of the proximate composition of red snapper eggs and larvae and that of live feeds.

KEY WORDS: Marine aquaculture, marine stock enhancement, larval nutrition, larval rearing

INTRODUCTION

The red snapper (*Lutjanus campechanus*) is a reef fish that frequents tropical and subtropical waters of the western Atlantic Ocean. The species is of major economic importance in the Southeast United States with landings for the period 2003 – 2007 averaging ~ 4.3 million lbs and ~ 4 million lbs for the commercial and recreational fisheries respectively (National Marine Fisheries Service, Fisheries Statistics Division, Personal communication). The abundance of red snapper in the northern Gulf of Mexico decreased by an estimated 90% between the 1970s and the 1990s (Goodyear and Phares 1990) due in large part to overfishing and bycatch mortality of juveniles taken by the shrimp-trawl fishery (Christman 1997, Gallaway et al. 1998). The stock was considered 'overfished and undergoing overfishing' until recently, and a rebuilding plan is currently in effect leading to highly restrictive regulation of fisheries since 2008 (South East Data Assessment and Review #7 Stock assessment of Gulf of Mexico red snapper). Similarly, results of recent assessments of the South Atlantic stock led to the complete closure of the fishery in that region Because of its high value both as a food fish and as a game fish, and its overfished status, red snapper is a primary candidate for the developing marine aquaculture industry in the United States. The potential benefits of red snapper aquaculture development are two fold, (i) the commercial supply of red snapper grown in aquaculture as a supplement to wild caught fish, and (ii) the production and propagation of hatchery reared juveniles to help rebuild wild populations in a stock enhancement program. Stock enhancement is currently being used in an increasing number of marine species in the United States and other countries (Lorenzen et al. 2010) as a management tool to overcome recruitment limitations. This approach seems worth exploring in red snapper because of the sedentary lifestyle of this species once juveniles settle on reefs; stocked juveniles would thus be expected to contribute to local fisheries post release.

STATUS OF RED SNAPPER AQUACULTURE

The first attempts to culture red snapper date back to work by Arnold et al. (1978) who reported spontaneous spawning of broodfish held in tanks and acclimated under controlled temperature and photoperiod conditions. Since these early results, spontaneous spawning of captive brooders held in tanks has been achieved in some studies (Papanikos et al. 2008, Bardon et al., Unpublished results) but success is still highly variable and unpredictable with some mating sets failing to

spawn during the entire spawning season or, when spawning occurs, a large proportion of spawns being unfertilized (Watanabe et al. 2004). For these reasons, egg production relies largely on hormonal induction of gamete maturation and strip-spawning of mature fish caught in the wild following the procedure described by Minton et al. (1983). Larval rearing is another major bottleneck to aquaculture production. Attempts to raise red snapper larvae to date have been made primarily using intensive culture approaches and led to very low survival rates during the larval period. High mortality rates are usually observed at or shortly after first feeding and during transition between different types of prey. A major difficulty with the rearing of early stages is that production of red snapper larvae has only been achieved when copepod nauplii were provided as an initial prey (Shields et al. 2005, Rhodes and Phelps 2008, Watanabe et al. 2004). An intensive culture method for the calanoid copepod *Acartia tonsa* was therefore developed at the Thad Cochran Marine Aquaculture Center of the University of Southern Mississippi that results in a mean production of 11 x 10^6 copepod nauplii per day (Lemus et al. 2008). Current implementation of the method involves feeding lab-cultured microalgae to a monospecific culture of *A. tonsa* (Lemus et al. 2008). This method supports the culture of red snapper larvae at an experimental scale but is labor intensive and cannot be scaled up to support production levels required for commercial purpose or stock enhancement. In consequence, a primary objective for red snapper aquaculture is to reduce dependency of larval rearing on copepods in particular to examine the potential use of alternative live prey for the rearing of larvae.

CHALLENGE TO FEEDING OF RED SNAPPER LARVAE AND RESEARCH OBJECTIVES

The culture of rotifers spp. is widely understood (Lee 2003) and inexpensive relative to that of copepods. In consequence, manipulation of rotifer enrichments has been the primary approach to develop efficient larval diets for marine fishes (Dhert et al. 2001). The origin of the inadequacy of rotifers as an initial prey for red snapper is not yet understood. Although the copepods produced in our laboratory (nauplius stage 1 – 114 µm x 61 µm) are smaller than the SS-rotifers (163 µm x 105 µm), the mouth gape of red snapper larvae at mouth opening (287 µm height x 198 µm width) would appear large enough to ingest rotifers (Lemus, Unpublished data). Also, rotifers are widely used to culture fish species with small larvae, therefore the inability of red snapper to capture rotifers seems not a likely cause for the lack of success of larval trials using rotifer preys. On the other hand, nutritional characteristics of rotifers are often unsuitable for marine fish larvae and enrichment procedures are usually required for the rotifers to meet nutritional requirements of the larvae (Dhert et al. 2001, Lee 2003). The lack of success of attempts to feed red snapper larvae with rotifers could thus be due to inadequate enrichment. Another potential issue is that red snapper larvae might not be able to digest rotifers efficiently until a certain developmental stage has been reached. An immediate priority for research in nutrition is therefore to understand the nutritional requirements of red snapper larvae and the ontogeny of their digestive system.

APPROACHES TO STUDY NUTRITIONAL REQUIREMENTS AND THE ONTOGENY OF THE DIGESTIVE FUNCTION IN FISH LARVAE

Implementation of experiments to study the nutritional requirements of fish larvae is challenging due to the fragility of this life stage and difficulties associated with accurately quantifying feeds ingested by the larvae. The change in the nutritional quality of enriched live preys through time is another challenge to implement and interpret such studies. In consequence determination of nutritional requirements has often been based on the determination of the proximate composition of fish eggs and larvae. Important information may also be gathered from the analysis of the composition of the natural prey for larvae of cultured species (Van der Meeren et al. 2008).

Studies on the nutritional requirements of marine fish larvae have largely focused on lipids (Coutteau et al. 1997, Lee 2003). The role of highly unsaturated (HUFA) lipids and in particular the central role of the balance between docosahexaenoic (DHA, 22:6n−3) and eicosapentaenoic acid (EPA, 20:5n−3) has been extensively documented in marine fishes (Rainuzzo et al. 1997). Requirements in arachidonic acid (ARA, 20:4n−6) initially received less attention, in part because of the relatively low representation of this fatty acid in marine fish tissues. However, recent studies have suggested the essential character of ARA for a number of marine fishes (Koven et al. 2001) including one lutjanid (Ogata et al. 2004). These findings highlight the need to investigate specific lipid and fatty acid requirements in red snapper larvae. Fewer studies have focused on larval requirements for protein and amino acids (Cahu and Zambonino Infante 2001). However, available data suggest that the content of amino acids and in particular the availability of appropriate amount and balance of Free Amino Acids (FAA) are essential to the successful development of marine fish larvae (Rønnestad et al. 1995, Finn et al. 1995, Rønnestad et al. 1999, Conceição et al. 2003, Brown et al. 2005). In addition, studies of nutrient content of natural preys of fish larvae (phyto- and zooplankton) have revealed high FAA levels (Fyhn et al. 1993, Helland 1995). Altogether, these results indicate that examination of lipid, protein and amino acid profiles is critical in order to define appropriate larval diets for red snapper.

Diets formulated for marine fish larvae also need to provide the necessary nutrients in a form that the larval digestive system is able to process and assimilate. Digestion of nutrients occurs in the gastrointestinal tract and is performed by digestive enzymes but also includes absorp-

tion and transport by intestinal cells (Zambonino Infante and Cahu 2001). In most teleost fishes, the digestive tract develops from an almost undifferentiated gut at hatching, to a complex and segmented digestive apparatus in juveniles and adults. In consequence, the digestive capacity of larvae usually differs from that of juveniles and rapidly evolves during larval development (Govoni et al. 1986, Péres et al. 1996, Zambonino Infante and Cahu 2001, Zouiten et al. 2008). Teleost larvae usually lack a functional stomach and their digestive capacity is limited mainly to pinocytosis and intracellular digestion and absorption (Govoni et al. 1986). Easy-to-digest prey such as rotifer is usually preferred during this phase (Chen et al. 2006). Intensive differentiation occurs as early as the endogenous feeding phase (Buddington 1985, Chen et al. 2006). Pancreas and liver show active differentiation shortly after the onset of exogenous feeding concomitant with elongation, thickening and undulations of the intestinal epithelium (Boulhic and Gabaudan 1992, Chen et al., 2006). Later events signal metamorphosis and transition to a juvenile digestive system and include formation of the gastric glands in the stomach and development of pyloric caeca (Chen et al. 2006). Digestive capacity and requirements of fish larvae are closely linked to the ontogeny of the activities of specific digestive enzymes that occurs concomitantly with the compartmentalization of the digestive tract (Zambonino Infante and Cahu 2001). Acidic protease activity is found mostly in the stomach. The exocrine pancreas produces several enzymes including glucosidases, lipases, alkaline proteases, amylase, trypsin and chymotrypsin. Enterocytes produce cytosolic enzymes (mostly peptidases) in cell cytoplasm, and enzymes found in the membranes of cells of the brush border (including peptidases and alkaline phosphatase). The kinetics of the ontogeny of the digestive system varies among taxa (Govoni et al. 1986), highlighting potential differences in digestive requirements at specific larval stages for different fish species. These results highlight the need to know the ontogeny of the digestive organs and associated enzyme activities in order to evaluate feeding sources and protocols.

COLLABORATIVE RESEARCH IN LARVAL NUTRITION OF THE RED SNAPPER

Research in progress at the Thad Cochran Marine Aquaculture Center aims to provide the basic information identified above in order to assist in the design of feeding sources and protocols for red snapper larvae. A first objective is to describe the kinetics of the morphological development of the digestive tract of red snapper and the ontogeny of the activity of digestive enzymes. Preliminary data on the morphological development of the digestive tract of cultured larval red snapper were obtained by Chiluiza (2003) using histology. However, the red snapper larvae used in that study were fed a mixed zooplankton assemblage (Ogle and Lotz 2006), which resulted in development rates that potentially differ from those observed in current rearing conditions. The kinetics is therefore currently being re-evaluated using a more thorough sampling (see below). A second objective is to investigate the nutritional requirements of red snapper larvae by studying the proximate composition of red snapper eggs and larvae at various stages of development along with that of copepod preys.

To address these research objectives, red snapper eggs and larvae cultured under current rearing protocol were collected at fertilization and at 1, 2, 3, 4, 9, 18, 24, and 31 days post hatch. Duplicate rearing tanks were sampled at each sampling date. Analyses in progress include histology of the digestive tract to describe the ontogeny of digestive organs, measurements of the activity of digestive enzymes and proximate composition of eggs, larvae and feeds.

Red snapper larvae show a slow growth during the first 10 to 12 days post hatch (Figure 1) with a general exponential pattern characterized by fast growth in length and height beginning 12 days post hatch. Initial observations of the digestive tract using histology revealed a rudimentary gut at hatch. Three gut compartments (foregut, midgut and hindgut), liver and pancreas were visible at 3 days post hatch and the mouth was open. The gastric glands were first detected in the largest 18 day old larvae. Additional observations are being conducted at each sampling date to refine the kinetics of the development of digestive organs and investigate the variability of development at a given age. Analysis of the activity of digestive enzymes is in progress. Preliminary results indicate an increase of lipase activity during the larval development period. Other enzymes evaluated include alkaline phosphatase, pepsin, amino-peptidase, chymotrypsin and acid phosphatase.

Study of the proximate composition of eggs, larvae and copepod and artemia preys is also in progress.

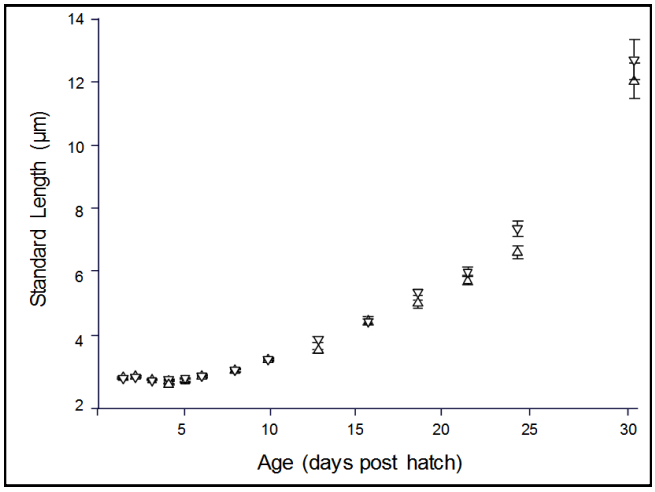

Figure 1. Growth represented as the change in Standard Length over time during larval development of *Lutjanus campechanus*. The two triangle symbols (upward and downward respectively) represent data from two replicate larval tanks surveyed at each sampling date.

FUTURE DEVELOPMENTS

The information obtained during the project described above will be precious to evaluate candidate alternative feed sources such as enriched rotifers for red snapper larvae, and to manage feed transitions. Additional research areas include studies of the effects of egg quality in particular egg nutrient content on larval fitness. The latter appears critical considering that most of the mortality is observed at first feeding time and may be related to larval fitness and the quality of the vitellin reserves. Larval survival and development is also highly dependent on feeding success and zootechnical approaches to improve first feeding and feeding transitions are also essential to improve performance of larval rearing. Additional potential approaches to study nutritional requirements and the ontogeny of the digestive system include the incorporation of radio isotopes or the analysis of stable isotopes in order to investigate questions such as nutrient source and metabolism post ingestion. The analysis of gene expression patterns may also provide useful information on the ontogeny of the activity of digestive enzymes. These new approaches can contribute to overcome the difficulties involved in studying larval nutrition and will be precious to improve the culture of sensitive life stages such as early larvae (Conceição et al. 2010).

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