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REPRODUCTIVE STRUCTURES AND EARLY LIFE HISTORY OF THE GULF TOADFISH, *OPSANUS BETA*, IN THE TECOLUTLA ESTUARY, VERACRUZ, MEXICO

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ABSTRACT Although the Gulf toadfish, *Opsanus beta*, is an abundant member of the nearshore Gulf of Mexico ichthyofaunal assemblage, little information exists regarding the ecology of the species, especially for southern Gulf of Mexico populations. We added to the existing knowledge of this species by describing the reproductive structures and examining the early life history of this species in the Tecolutla estuary, Mexico. Macro- and microscopic examination of 7 males showed spermatogenesis to be similar to other teleost species except for the occurrence of biflagellate spermatozoa. Histological examination of the male accessory gland showed 3 tissue layers, but their functions are still undetermined. We found asynchronous development of oocytes in the ovaries of 16 females, which may indicate multiple spawning over the long spawning season noted in this study. Batch fecundity estimates among females ranged from 79 to 518 mature ova with a mean ovum diameter of 3.5 mm. The above-mentioned factors along with large size at hatching, attached larval forms, and paternal care may account, in part, for the abundance of this species in highly dynamic systems.

RESUMEN Aunque el pez sapo del Golfo, *Opsanus beta*, es un miembro abundante del ensamble ictiofaunístico de las costas del Golfo de México, existe poca información acerca de la ecología de la especie, especialmente para las poblaciones del sureste del Golfo de México. Nosotros contribuimos al conocimiento existente de esta especie, describiendo las estructuras reproductoras y examinando las etapas tempranas del ciclo de vida de esta especie en el estuario de Tecolutla, México. El examen macro y microscópico de 7 individuos machos mostraron espermatogénesis similar al de otras especies de teleosteos, excepto por la presencia de espermatozoides biflagelados. El examen histológico de las glándulas accesorias del macho mostró una composición de tres capas de tejido, pero su función es aun indeterminada. Encontramos desarrollo asincrónico de los ovocitos de 16 hembras examinadas, que pueden indicar desoves múltiples a lo largo de la temporada de desove. La estimación de la fecundidad en las hembras examinadas presentó un intervalo de 79 a 518 huevos maduros con un diámetro de 3.5 mm. Los factores anteriores, junto con la talla grande al eclosionar, las formas larvales adheridas y el cuidado paterno, pueden responder a la abundancia de esta especie en sistemas muy dinámicos.

INTRODUCTION

The Batrachoididae is comprised of 69 species and 3 subfamilies, with the subfamily Batrachoidinae being most diverse in the western hemisphere (Nelson 1994). However, few directed biological studies exist regarding members of this family. Schultz and Reid (1937) described the taxonomic characters of *Opsanus* and gave a key for the Atlantic species, but this genus has received little attention in the ecological literature. Several studies have described the biology and ecology of the oyster toadfish, *O. tau*. For example, Gray and Winn (1961) studied its reproductive ecology and found sound production was related to reproductive season. Hoffman (1963a, 1963b) described the male gonads and seminal vesicles, including their respective ducts, noting seasonal variations in size and morphology. However, this information may not be applicable to congeneric species with a more tropical or subtropical distribution, such as the Gulf toadfish, *O. beta*. Most *O. beta* ecological studies to date have focused on

aspects of reproduction (Breder 1941), sound production (Walsh et al. 1987, 1989, Thorson and Fine 2002), or physiology (Walsh et al. 1990, Hopkins et al. 1997). *Opsanus beta* is a benthic species that commonly inhabits cavities, submerged tree trunks, rock crevices, or other objects. Although this species is highly abundant in subtropical seagrass environments (Serafy et al. 1997, Matheson et al. 1999), its use of 3-dimensionally structured habitats hinders organism collection with conventional gears (Kuhlman 1998, Shervette et al. 2001).

The objectives of this study were to describe macro- and microscopic aspects of the gonads and provide information on early embryonic and larval development of *O. beta*, an abundant species in the Tecolutla, Veracruz estuarine system.

MATERIALS AND METHODS

Field Procedures

Five collections were made in the Tecolutla estuary (20°30'N, 97°01'W) during March, September, and

November of 1996, and March and April of 1997. Fish were collected with a 50 m seine (12.7 mm mesh). In the field, all fish were sacrificed, measured for standard length (SL, to 1 mm), and weighed wet (WW, to 0.1 g). Individuals were fixed in 10% formalin and placed in plastic bags for transport to the laboratory where species identification was verified (Castro-Aguirre 1978). We also searched for submerged objects containing *O. beta* eggs specifically during collecting trips in April 1996, May 1997, and June 1997. All objects found with eggs were placed in dish pans and transported to a field laboratory where the eggs were aerated and kept alive for 8 d. Additional observations of *O. beta* nests and gravid females were made during April, October, and November 1998.

Laboratory procedures

The gonads were extracted and weighed (g, 0.001) on an analytical balance. The gonads were post-fixed in 10% formalin, rinsed in water for 1 h, and processed with standard histological techniques using amyl alcohol to clear the tissues. Tissues were sectioned at 5 and 8 μ m, stained with hematoxylin-eosin, ferric hematoxylin, aldehyde-fuchsin, or the PAS reaction, then examined and photographed with the use of a camera-equipped optical microscope. Histological descriptions of the oocyte stages were adapted from Forberg (1982) and Patino and Takashima (1995), with testicular descriptions following Brown-Peterson (2003).

Embryonic development was observed with a stereoscopic microscope, with photographs taken of each developmental stage. Embryo diameter, larvae length, and yolk

sac measurements were made with a caliper (to 0.1 mm). Descriptions of embryonic development follow the terminology of Martin and Drewry (1978).

RESULTS AND DISCUSSION

Twenty-three individuals of *O. beta* (16 females and 7 males) ranging from 44 to 220 mm SL (2.4 to 504.1 g WW) were used in the histological, macroscopic, and reproductive examinations. We consider *O. beta* to be a resident species within the Tecolutla estuary based on the presence of juveniles, adults, and nests containing eggs. This differs from the ecological category assigned by Castro-Aguirre (1978) who described *O. beta* as a euryhaline species with a marine component.

Male reproductive system

The testes of *O. beta* are elongate bodies with a white-yellow color and are situated on both sides of the spinal column and attached to the swim bladder by a delicate mesentery. The testes are covered with a thin and poorly vascularized tunica albuginea. The sperm ducts are thin and connected posteriorly to the urogenital complex. Histologically, the testes can be classified as a lobular type, with each lobule containing a series of spermatocysts. Spermatogenesis of *O. beta* is similar to other teleosts; spermatogonia, spermatocytes, spermatids, and spermatozoa were all observed in the testes. However, the occurrence of biflagellate spermatozoa (Figure 1A) observed in this species is unusual among teleosts (Lahnsteiner 2003).

Spermatogenesis appears to be a continuous process in *O. beta*. In contrast, Hoffman (1963a) reported that

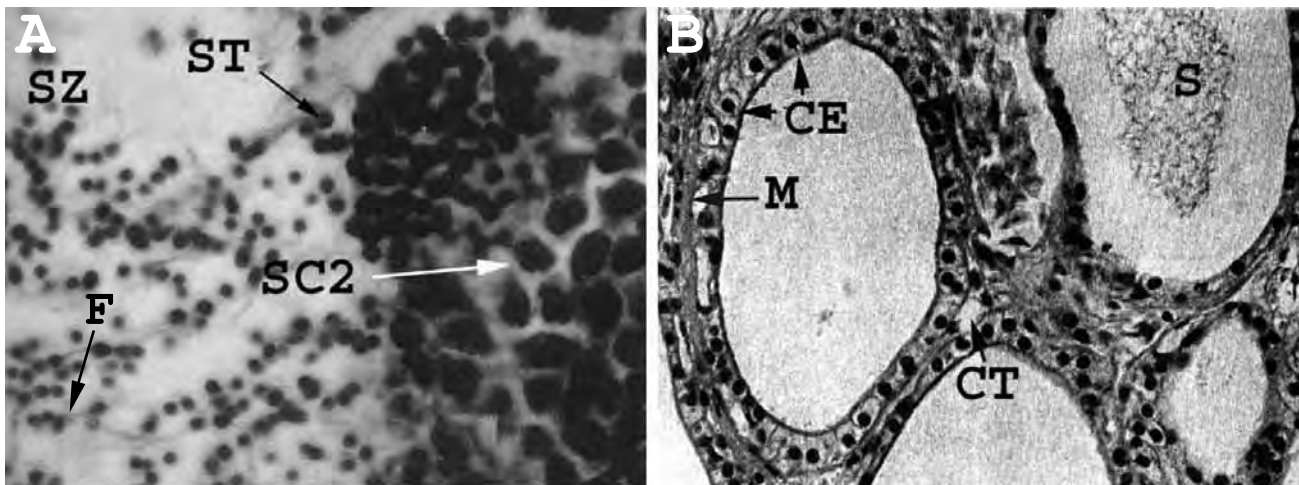


Figure 1. Histological sections from *Opsanus beta*. A) Testis, showing various stages of spermatogenesis, including spermatozoa (SZ), secondary (SC2) spermatocytes and spermatids (ST). Note the double flagella on some spermatozoa (F), 40x in phase contrast. B) Septae from part I of the accessory gland consisting of cubic epithelial tissues (CE). Note the secretion (S), connective tissue (CT) and muscular fiber (M), 20x.

spermatogenesis completely ceased in male *O. tau* that had a large amount of spermatozoa stored in the testis. We did not observe a total cessation of spermatogenesis in *O. beta*. Whereas few spermatocysts were present in individuals collected in September and November, the lobules in these fish contained large amounts of spermatozoa, corresponding to the regression maturational class of Brown-Peterson (2003).

The accessory glands of *O. beta*, present only in males, are large, fan-shaped paired organs with a spongy appearance. The glands usually have a hyaline, orange color with septa visible under transmitted light. They are situated in the posterior part of the abdominal cavity and are joined to the sperm ducts. The glands have 3 sections that differ microscopically. The section closest to the sperm duct is formed of very thin septa characterized by a layer of cubic or flat epithelial cells and a basal sheet of thin and compact connective tissue containing fibers of smooth muscle (Figure 1B). This section is not highly vascularized, but the blood vessels present are relatively large. The internal parts of the septa have secretions that are PAS and aldehyde-fuscia positive, indicating the presence of mucopolysaccharides and mucins (Gomori 1952). Although spermatozoa can be found in septa closest to the sperm ducts, this is not a common occurrence and indicates the accessory glands do not store mature sperm.

The second region of the gland is formed by larger septa, covered with a thick glandular epithelium consisting of 6–7 layers. This tissue is highly vascularized, and some secretions can be observed. The basal sheet is connective tissue with fibers of smooth muscle surrounding each one of the septa. The most distal region of the gland contains smaller septa and packets of 1–2 layers of highly convoluted cubical or columnar glandular epithelium, with nuclei undergoing active protein synthesis. In this region, there is reduced vascularization, but the blood vessels present are of greater diameter than in other regions. All septa have a basal cover of connective tissue with smooth muscle that is thinner than in the middle region. Finally, this region of the gland has nodules appearing to be formed by lymphoid tissue.

These accessory glandular structures have been called accessory glands, seminal glands, or seminal vesicles by various authors (Sundararaj 1958, Fishelson 1991, Lahnsteiner et al. 1990), and the accessory glands observed in *O. beta* are similar to those reported for *O. tau* (Hoffman 1963b). Similar accessory glands are found in male fishes within the Blenniidae, Gobiidae, Clariidae, Bagridae, Tripterygiidae (Fishelson 1991), Heteropneustidae (Sundararaj 1958), Haenopsidae (Patzner 1991) and Opistognathidae (Rasotto 1993). However, the accessory

glands present in the genus *Opsanus* are highly complex. Hoffman (1963b) reported that the glandular structure is divided into 4 different sections in *O. tau*, but we found only 3 sections in *O. beta*. It was not possible to establish the role of the accessory glands based on their position and development. However, some authors have proposed various functions for the glands and their secretions in other fishes. These include giving viability and motility to the spermatozoa (Sundararaj 1981 and references therein), increasing the seminal fluid viscosity and fulfilling phagocytic functions (Lahnsteiner et al. 1990), acting in nest formation (Fishelson 1991), serving as a place for sperm storage and maturation (Miller 1984), encouraging fertilization (Hoffman 1963b), and producing steroids that act like pheromones (De Jonge et al. 1989). It is possible that in *O. beta*, the glands fulfill one or more of these functions at the same time. The 3 sections of the glands could each produce a different type of secretion with different functions similar to that observed in *O. tau* (Hoffman 1963b). Detailed histochemical studies are necessary to reveal the exact composition of the glandular secretions and their possible functions in this species.

Oocyte maturation and spawning

The ovaries of immature females are a pair of thin, small, white sacks. When mature, the ovaries acquire an orange-yellow coloration, and the large oocytes are easily visible. Microscopically, the oocytes show asynchronous development. Pre-vitellogenic oocytes in the chromatin nucleolar, early perinucleolar, late perinucleolar, and cortical alveolar stages were observed in all but the smallest specimens. Vitellogenic oocytes in the primary, secondary, and tertiary stages were also observed. Asynchronous oocyte development, such as that observed in *O. beta*, is characteristic of fish that can spawn multiple times during the reproductive season (Brown-Peterson 2003). Oocytes in the tertiary vitellogenesis stage are fully mature, with a mean diameter of 3.28 mm. Ovulated oocytes had a mean diameter of 3.5 mm. Oocytes in the nuclear migratory and ovulatory stages were observed in *O. beta*, but oocytes undergoing germinal vesicle breakdown were not seen, likely due to the short duration of this stage.

Opsanus beta appears to have an extended reproductive season in the Tecolutla estuary. Females with mature oocytes were found from April through November, suggesting an 8 month reproductive season. In Pensacola, Florida, Allee (1998) noted spawning in May and September in *O. beta*. Nearly ripe males were found in March and June in Cedar Key, Florida, and a sexually mature female was taken in September (Reid 1954). This species also has relatively low batch fecundity: the largest

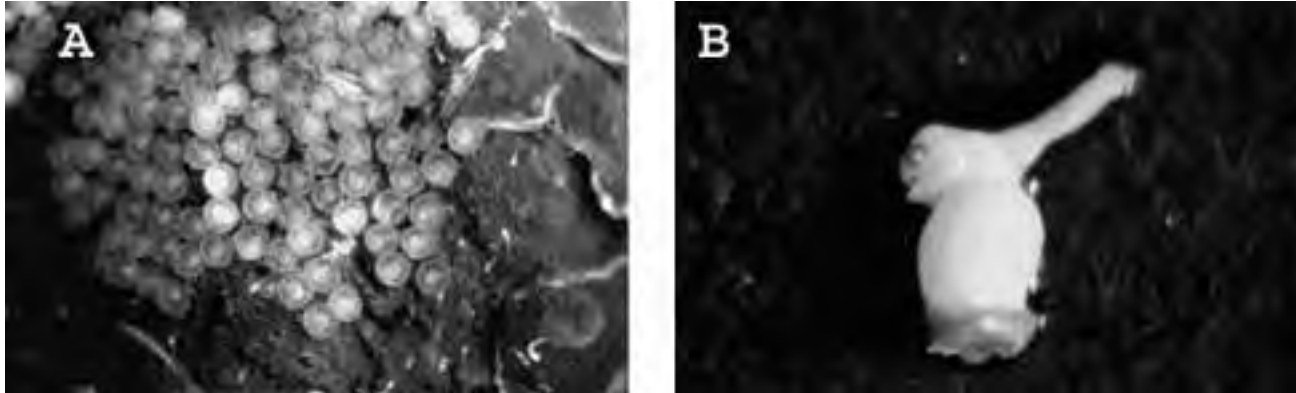


Figure 2. A) Nest of *Opsanus beta* eggs showing substrate typical of nests. Note the embryos are in different stages of development, suggesting at least two females spawned eggs in this nest. B) Larva of *Opsanus beta* with ocular pigment.

female captured (192 mm SL in April) contained 518 mature oocytes in the ovaries; a 141 mm SL female captured in October had 176 mature oocytes in the ovaries whereas the ovaries of the smallest mature female (111 mm SL, captured in November) had only 79 mature oocytes. The relatively low batch fecundity of *O. beta* is characteristic of this family (Breder 1941; Collette and Ruso 1981). However, low batch fecundity is offset by male parental care, which increases the survival probability of offspring. Additionally, the eggs are demersal and attached to submerged objects, which facilitates parental care. This reproductive strategy is typical for batrachoids (Breder and Rosen 1966) and was observed in the field.

Egg clutches of 2 *O. beta* were found in nests in April 1996 and May 1997 with a total of 350 and 412 eggs, respectively. One of these nests was in a cluster of oyster shells and another in a boot, suggesting *O. beta* will use any firm, relatively protected substrate as a nesting site. The eggs were spherical, orange colored, lacked oil droplets, and adhered to the substrate by an adhesive disc. Egg diameters varied between 2.1 and 4.6 mm, with a mean of 3.5 mm. The number of eggs found in the nests is greater than the batch fecundity of a single individual, suggesting that more than one female may spawn in the same nest. Indeed, a nest of eggs showed embryos in different developmental stages (Figure 2A), supporting this theory.

Embryonic and larval developmental

Various stages of early embryonic development, including the 8-, 16-, 32-, and the 64-cell stages, were observed among a recently spawned clutch of eggs. Unfortunately, these died before we could observe the morulation and gastrulation stages. However, a second clutch containing eggs in more advanced developmental stages was collected. In the late embryo stage, the yolk sac retained its spherical shape with the embryo located on the

surface of the yolk sac opposite the adhesive disc. Two stages of larval development were also observed in hatched eggs. The first stage was characterized by the yolk sac beginning to lose its round shape. The larvae were attached to the yolk sac and did not have ocular pigment. Larvae in this stage ranged in size from 4.1 to 7.0 mm TL, with the yolk sac measuring 2.9 to 3.7 mm wide and 2.9 to 6.0 mm long. In the second stage, the eye acquired pigmentation and the yolk sac began to shrink and acquire a cylindrical shape (Figure 2B). Larvae in this stage were 5.4 to 7.7 mm TL and the yolk sac was 2.8 to 7.1 mm long and 2.5 to 4.0 mm wide.

Breder (1941) established that *O. beta* spawn between February and March in Florida. In the Tecoluitla estuary, we observed spawning from March through May during the current study and have since seen toadfish nests in June (Martinez-Perez, personal observation). The development of large eggs and parental care in the toadfishes may be a response to environmental conditions. The estuaries in which these fish live have rapid fluctuations in temperature and salinity. Thus, resident fishes such as *O. beta* have developed strategies to increase reproductive success under these dynamic conditions.

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