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# GONADAL DEVELOPMENT AND SEXUAL DIMORPHISM OF *Gobiomorus dormitor* FROM THE ESTUARINE SYSTEM OF TECOLUTLA, VERACRUZ, MEXICO

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**ABSTRACT** The bigmouth sleeper, *Gobiomorus dormitor*, is a benthic, euryhaline species, and is very abundant in river mouths, coastal lagoons, and sites away from marine influence from south Florida to Dutch Guyana. There are few studies of its life history, ecology, and abundance, particularly within Mexican waters. Nine trips to Tecolutla estuary, Veracruz, Mexico, were taken between October 1995 and May 1998 to estimate the gonadal development and sexual dimorphism of *G. dormitor*. A total of 94 individuals ranging from 15–260 mm SL and 0.05–181 g were captured. Seventy-two specimens were adults (60 females, 12 males) and 22 were juveniles that did not show external sexual dimorphism. Both juvenile and adult stages of *G. dormitor* were captured year-round in seagrass beds and adjacent shallow, muddy or sandy areas. This study has shown that *G. dormitor* are resident and undergo sexual maturation in the Tecolutla estuary. Histological evidence suggests both males and females undergo gonadal recrudescence in the estuary and have an extended reproductive season from May through November. However, it is unclear if the species actually spawns in the estuary, since females in the final stages of oocyte maturation were not captured. Additional research on the reproductive biology and ecology of this under-studied species is necessary to determine its role in tropical estuaries in the southern Gulf of Mexico. Information learned from areas in the center of its distribution may aid in conserving the species at the periphery of its range in Florida, where it is considered threatened.

**RESUMEN** La guavena, *Gobiomorus dormitor*, es una especie bentónica eurihalina y es muy abundante en las desembocaduras de los ríos, lagunas costeras y lugares fuera de la influencia marina, desde el sur de la Florida hasta la Guyana Holandesa. Hay pocos estudios de su ciclo de vida, ecología y abundancia, particularmente dentro de aguas mexicanas. Se realizaron 9 muestreos en el estuario de Tecolutla, Veracruz, México, entre octubre de 1995 y mayo de 1998, para estimar el desarrollo gonádico y el dimorfismo sexual de *G. dormitor*. Fueron capturados un total de 94 organismos de entre 15–260 mm de longitud patron y 0.05–181 gramos. Sesenta organismos fueron hembras y doce fueron machos; 22 peces fueron juveniles y no mostraron dimorfismo sexual externo. Ambos estadios, juvenil y adulto, de *G. dormitor* fueron capturados todo el año en camas de pastos y áreas adyacentes poco profundas, lodosas o arenosas. Este estudio ha mostrado que *G. dormitor* es residente y sufre maduración sexual en el estuario de Tecolutla. La evidencia histológica sugiere que machos y hembras sufren una recrudescencia gonádica en el estuario y tiene una estación reproductiva que se extiende desde mayo hasta finales de noviembre. Sin embargo, no está claro si esta especie desova actualmente en el estuario, desde que hembras en estadio final de maduración del ovocito no fueron capturadas. En investigaciones adicionales de la biología reproductiva y ecología de la especie en estudio, es necesario determinar su papel en estuarios tropicales del sureste del Golfo de México. La información obtenida de áreas en el centro de su distribución, puede ayudar en la conservación de la especie en la periferia de su intervalo en Florida, donde son considerados como peces amenazados.

## INTRODUCTION

The eleotrid fishes (Family: Eleotridae) are one of the most widely distributed families world-wide and are commonly found in estuaries. In the Americas, the eleotrids are most abundant in Central America, with various species extending north to the United States and south to Brazil (Nordlie 1981). The bigmouth sleeper, *Gobiomorus dormitor* (Martinez and Sanabria 1993), occurs in the estuarine system of Tecolutla, Veracruz, and is known locally as “guavina.” *Gobiomorus dormitor* is a benthic, euryhaline species, very abundant in river mouths, coastal lagoons,

and sites away from marine influence (Darnell 1955, Castro-Aguirre 1978) from south Florida to Dutch Guyana, and is associated with muddy bottom, mangrove habitat and seagrass beds in Larios and Silvenia estuaries in Tecolutla, Veracruz (Torres 1992). This species is considered threatened in Florida (Gilmore 1992, Woods 1994).

There are few studies on the life history, ecology, and abundance of *G. dormitor*, particularly within Mexican waters. Darnell (1955) reported that *G. dormitor* exhibits nocturnal terrestrial habitats in headwaters of the Rio Tamesi in Mexico and is an opportunistic forager. McKaye et al. (1979a) reported that cichlids, poecilids, and

atherinids were the preferred prey of *G. dormitor* in Lake Jiloá, Nicaragua. The species inhabits stony areas and migrates to deeper water during the dry season, when spawning occurs (McKaye et al. 1979a). In contrast to other elotrids, *G. dormitor* appears to prefer open habitats adjacent to vegetation in freshwater streams (Gilmore and Hastings 1983, Winemiller and Ponwith 1998). Torres (1992) studied the larval stages of the families Gobiidae and Eleotridae in 6 estuarine systems in Veracruz state and reported that *G. dormitor* was the most abundant species in the 6 systems. *Gobiomorus dormitor* larvae were less abundant in the dry season and more abundant during rainy seasons in those estuarine systems. Adults are known to move downstream during the rainy season in estuarine areas of Mexico (Darnell 1962) and Florida (Gilmore and Hastings 1983). *Gobiomorus dormitor* was the second most abundant species in the Laguna Tortuguero, Costa Rica (Nordlie 1981), although Winemiller and Ponswith (1998) found the species to be the 4th most abundant species in the freshwater tributaries of the same system. *Gobiomorus dormitor* has been shown to be a successful invasive species when introduced into a crater lake in Nicaragua, displaying exponential growth and higher foraging success than a natural population in a nearby lake (Bedarf et al. 2001).

There is little information on the reproduction of *G. dormitor*. The breeding area for *G. dormitor* in Nicaraguan crater lakes ranges between 12–15 m. Nests are 6–11 cm wide, 60–120 cm long, and 40–90 cm deep and are located in deep, narrow rock fissures (Mc Kaye 1977, McKaye et al. 1979b). Both parents defend the nesting area, and females spawn about 4,000–6,000 eggs in one day (McKaye et al. 1979b). Winemiller and Ponwith (1998) and Bedarf et al. (2001) suggested year-round reproduction occurred in the Tortuguero, Costa Rica, estuarine system and in a crater lake in Nicaragua, based on gonadal observations and the occurrence of juveniles.

The reproductive life cycle of *G. dormitor* in Mexican waters is unknown. Thus, the present work contributes to the knowledge of some reproductive aspects of *G. dormitor*. The objectives of this study are 1) to determine the external morphological structures of sexual organs that permit differentiation between female and male *G. dormitor*, 2) to describe macro- and microscopically the gonadal morphology and sexual maturity classes of *G. dormitor*, and 3) to establish the reproductive season of *G. dormitor* in the Tecolutla estuary, Veracruz, Mexico.

### Study area

The Tecolutla estuary is located at 20°30'N and 97°01'W in the Gutierrez Zamora municipality in Veracruz

TABLE 1

Temperature and salinity data recorded during collections of *Gobiomorus dormitor* in Tecolutla River during this study.

Month	Temperature (°C)	Salinity (psu)
October	22	0
November	19	0
December	17	0
January	14	2
February	16	4
March	19	6
April	23	3
May	25	12

state, Mexico. Tecolutla estuary is oriented in a southwest to northwest direction, and the Tecolutla River is the principal inflow of fresh water. The estuary is divided into 2 principal sections before entering the Gulf of Mexico, the Larios estuary, and the El Negro estuary which has a smaller estuary to the east, the Silvenia estuary. The estuary has a warm, humid climate characterized by summer rains and an oscillation of annual temperature no more than 7 °C. The emergent vegetation in the estuary is principally composed of red mangrove (*Rhizophora mangle*), black mangrove (*Avicennia germinans*), and white mangrove (*Laguncularia racemosa*), with considerable seagrass, *Ruppia maritima*.

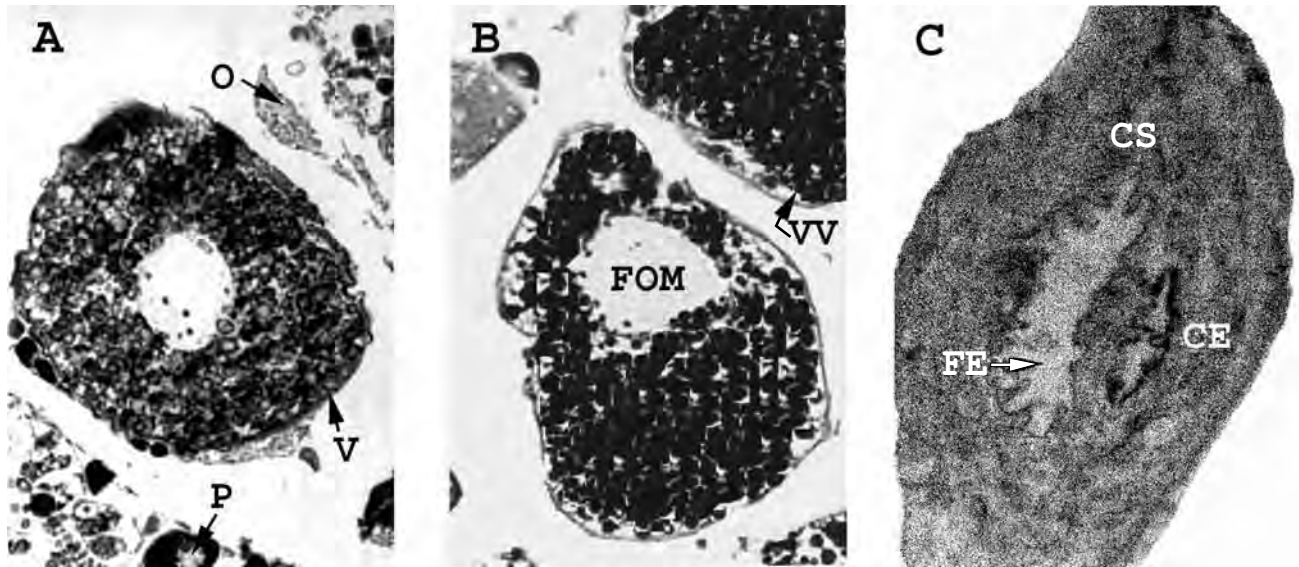
### MATERIAL AND METHODS

#### Field collections

Nine trips to Tecolutla estuary were taken between October 1995 and May 1998. Fishes were captured with a 50 m long beach seine constructed with 12.7 mm mesh net in *Ruppia maritima* seagrass beds. Fish were placed in 20 L buckets, sacrificed in ice water, and fixed in 10% formalin for transport to the laboratory. Water temperature and salinity was recorded at the time of sampling at each site.

#### Laboratory procedures

In the laboratory, fish were rinsed overnight in running water and identified (Alvarez 1970, Castro-Aguirre 1978). Meristic and morphometric measures were taken on the preserved fish with calipers, and all individuals were weighed to the nearest 0.01 g. The fish were dissected to extract the urogenital apparatus in both sexes, and the gonads were measured using vernier calipers and weighed to the nearest 0.01g on an analytical balance. A macroscopic description of the accessory glandule, gonad, urinary bladder, and genital papilla was made for both sexes.



**Figure 1.** Histological sections of the urogenital apparatus of female *Gobiomorus dormitor*. A) The ovary of a mature fish showing a nest of oogonia (O), perinucleolar stage oocytes (P), and an oocyte in secondary vitellogenesis (V), 200X. B) An oocyte in the migratory nucleus stage (FOM). Note the presence of an oocyte in the vitellogenin vesicle stage (VV), 200X. C) Transverse section of female papilla. Note the major duct with flat epithelial cells (FE), minor duct with cubic epithelium (CE) and connective stroma (CS), 40 X.

For microscopic descriptions, the urogenital apparatus was processed following standard histological techniques. Tissues were embedded in paraffin and sectioned at 5  $\mu\text{m}$ . For each organ different staining techniques were used: ovaries—hematoxylin-eosin (H-E), and Masson trichrome stains; testes—H-E, Masson trichrome and Hemotoxylin-Ferrous (H-F); seminal vesicle—H-F; urinary bladder and female papilla—H-E; male papilla—H-E and H-F. Oocyte stages were classified following Rodriguez (1992), while spermatogenic stages were classified following Hyder (1969). Oocyte diameter ( $\mu\text{m}$ ) was measured with an ocular micrometer.

## RESULTS

A total of 94 individuals ranging from 15–260 mm SL and 0.05–181 g, were captured during the course of this study. Seventy-two specimens were adults (60 females, 12 males) and 22 were juveniles that did not show external sexual dimorphism. Both juvenile and adult stages of *G. dormitor* were captured year-round in *R. maritima* grassbeds and adjacent shallow, muddy or sandy areas in Larios and Silvenia estuaries. Water temperature ranged between 14 and 25  $^{\circ}\text{C}$  and salinity between 0 and 12 psu (Table 1).

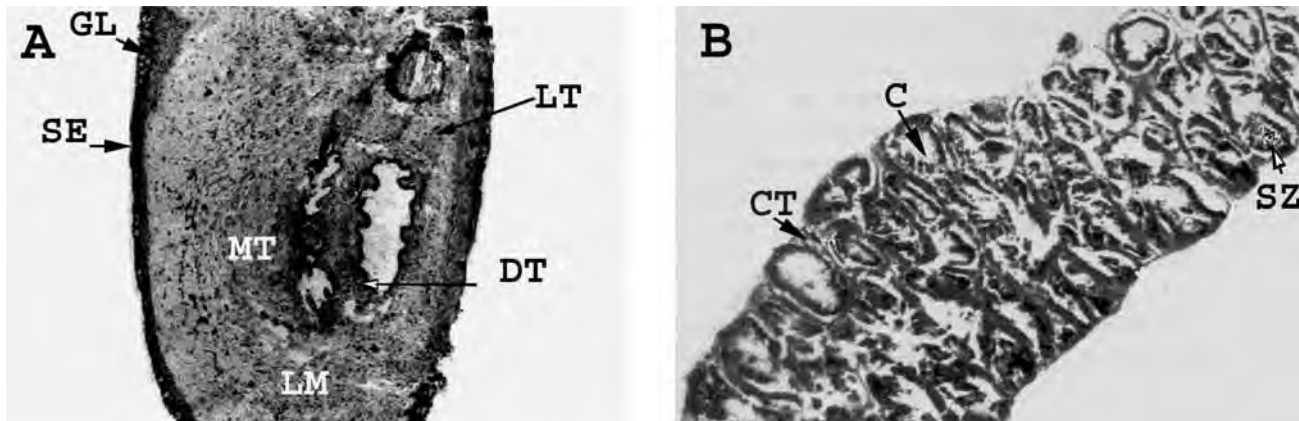
### Female reproductive system

The female urogenital apparatus is composed of 1 pair of ovaries, urinary bladder, and genital papilla. The ovaries have a ribbon form and a cream color, with a smooth

appearance in the immature and regressed classes. Oocytes become visible during vitellogenesis, and the ovary takes on a more lumpish appearance. There is a marked difference in size, with the right ovary smaller than the left. Both ovaries have a *tunica albuginea*, consisting of a thick layer of smooth muscle and dense, white connective tissue that forms septa in the interior of the ovary. In cross section, the ovarian lamellae are visible and contain oocytes in different stages of maturation, indicating asynchronous oocyte development.

Eight stages of oocyte development were identified in *G. dormitor*. Oogonia were small cells, with an acidophilic cytoplasm and a strongly basophilic nucleus. They occurred in nests in the *tunica albuginea* between the lamellae (Figure 1a). The chromatin nucleolar oocytes ranged in size from 12–22  $\mu\text{m}$  and were strongly basophilic with multiple nucleoli. Oocytes in the perinucleolar stage ranged in size from 23–79  $\mu\text{m}$ , were strongly basophilic, and were characterized by a large nucleus, nucleoli distributed in the periphery, and the appearance of the follicle (Figure 1a). The vitellogenin vesicle stage (80–100  $\mu\text{m}$ ; Figure 1b) is characterized by an oval shape and the appearance of small, light purple vesicles in the cytoplasm. In the primary vitellogenesis stage (101–129  $\mu\text{m}$ ), the oocyte loses its oval shape and becomes square or triangular. Vitellogenin globules begin to appear in the cytoplasm, and the oocyte becomes eosinophilic. In secondary vitellogenesis (130–163  $\mu\text{m}$ , Figure 1a), the oocyte, nucleus, and nucleoli return to an oval shape. There is a





**Figure 2.** Histological sections of accessory gonadal organs in male *Gobiomorus dormitor*. A) Transverse section of male genital papilla showing flat stratified epithelium (SE), layer of striated transverse muscle tissue (MT), striated longitudinal muscle tissue (LM), loose connective tissue (LT) and dense connective tissue (DT). Note the presence of small unicellular secretory glandules (GL), 40X. B) Seminal vesicle cyst (C) containing spermatozoa (SZ) and cyst wall formed of connective tissue (CT), 200 X.

large accumulation of vitellogenin globules in the ooplasm, and the oocyte rapidly increases in size. In the migratory nucleus stage (240–380  $\mu\text{m}$ , Figure 1b) the nucleus begins to migrate to the animal pole, the nucleoli are scarce, the globules of vitellogenin increase in size, and the *zona radiata* increases in thickness. Migratory nucleus oocytes are in the first stage of final oocyte maturation (FOM), indicating spawning is imminent.

The female urogenital papilla is an elongate structure with 2 central orifices, one below the other (Figure 1c). The lumen of the duct closer to the animal body has an irregular, contorted shape and a layer of flat epithelial cells. The second duct closer to the organism's skin is smaller, also of irregular shape and composed of cubic epithelium cells of glandular appearance. The glandular body is formed by conjunctive tissue with components of connective fibers, fibroblasts and blood vessels of different sizes. A stratified flat epithelium of 4 or 5 to 14 layers is present in the external portion.

### Male reproductive system

The male urogenital apparatus of *G. dormitor* consists of 1 pair of testes, seminal vesicles, and genital papilla. The *G. dormitor* testis is the unrestricted spermatogonial type (sensu Grier 1981), with spermatogenesis occurring within spermatocysts along the length of the lobules. All stages of spermatogenesis were identified in the testis of *G. dormitor*, including spermatogonia, spermatocytes, spermatids, and tailed spermatozoa.

The male urogenital papilla has a fusiform or extended shape with 2 central orifices, one under the other. The bigger and extended opening has contortions or projections in the lumen formed of cubic epithelium with basophilic basal nuclei. Under this layer there is connec-

tive tissue. The second opening also has an irregular form. Surrounding the openings are different layers of tissue; dense connective tissue, loose connective tissue, striated longitudinal muscle, striated transverse muscle, and the flat stratified epithelium of the skin (Figure 2a). Small unicellular glandules with PAS-positive stain were found in the epithelium, indicating a secretory function.

The seminal vesicles of *G. dormitor* are soft structures with a white or cream color that connect with the testes by means of a light fold or crease. Both vesicles are small extensions from the testes or sperm duct and are formed from a relatively few number of cells or tubules. A layer of connective tissue and blood vessels was observed histologically with H-E staining. The seminal vesicle consists of chambers, with glandular epithelial cells in the periphery. Histochemical staining suggests a secretory function. Spermatozoa were observed in the majority of the seminal vesicles analyzed (Figure 2b), suggesting the seminal vesicle is a spermatozoa reservoir.

### Reproductive seasonality

Females in the final stages of ovarian maturation were captured in May, September, and November from Tecolutla estuary, and females with vitellogenic oocytes in the ovary were found from April through November. However, females with vitellogenic oocytes were much more commonly taken from July through November (30–55% of females captured) than from April through June. This finding, in conjunction with the observation that the majority of males with spermatozoa in the sperm duct were captured from July through November, suggests the main reproductive season for this species in Tecolutla estuary is July through November.

## DISCUSSION

In the Tecolutla, Veracruz estuary, juvenile and adult stages of *G. dormitor* were captured in seagrass beds and adjacent shallow, muddy, and sandy areas. Our findings are in agreement with those reported by Darnell (1962) who captured *G. dormitor* in shallow water with modest currents, Nordlie (1981) who found the species in a shallow, vegetated lower estuary in Costa Rica, and Gilmore and Hastings (1983) who collected adults in silt/sand bottoms near vegetation in freshwater creeks in Florida. In contrast, juvenile *G. dormitor* were rarely captured in vegetation in freshwater streams in Costa Rica (Winemiller and Ponwith 1998), and both adults and juveniles were taken only in channel areas. Sexually mature individuals were taken year-round in Tecolutla, although large individuals have been previously suggested to be transitory (Nordlie 1981 and references therein) or absent (Gilmore and Hastings 1983) in estuaries.

In Tecolutla estuary, females with oocytes in the migratory nucleus stage were founded in May, September, and November, suggesting an extended spawning season. However, the percentages of vitellogenic females captured as well as males with spermatozoa in the sperm ducts suggest the major reproductive season in Tecolutla estuary is July through November. This is a later spawning season than previously reported. Nordlie (1981) reported mature *G. dormitor* were captured in May in Costa Rica, and McKaye (1977) found most breeding pairs of *G. dormitor* during May in Nicaraguan lakes, although observations were taken from February–September. However, competition for breeding sites with cichlids may have influenced the timing of the reproductive period in the Nicaraguan lakes (McKaye 1977). There is evidence that *G. dormitor* spawn year-round in Costa Rica and Nicaragua (Winemiller and Ponwith 1998; Bedarf et al. 2001), supporting evidence of the extended spawning season found in the Tecolutla estuary. The asynchronous oocyte development we observed suggests this species can spawn multiple times during the extended reproductive season, which coincides with reports by Nordlie (1981) and McKaye et al. (1979b). The final stages of FOM were not found in this study, perhaps because the female migrates to the coast to spawn (Darnell 1955, 1962), since spawning requires a hard substrate (McKay et al. 1979b) not present in Tecolutla estuary. However, the low sample size may have limited finding females in all stages of FOM.

The unique characteristic external morphology used to distinguish male from female *G. dormitor* is the presence of the genital papilla, which is observed in a few groups of fish such as the gobiids and eleotrids (Tavolga 1954; Miller 1986; Birdsong and Robins 1995). The function of the

genital papilla is still disputed. The presence of small unicellular secretory glandules in the apical part of the papilla was observed in male *G. dormitor*. These glandules may secrete a mucoid substance, which could have a lubricant or seminal function, to help in transport of spermatozoa to the exterior. Bianco et al. (1987), who observed that the genital papilla of male *Economidichthys pygmaeus* (Gobiidae) also had a secretory function, suggested it may be important in nest building or in adhesion to the substrate to keep newly spawned eggs from being displaced by currents. Hoffman (1963) mentioned that in male *Opsanus tau*, the apical portion of the papilla had blood vessels of different sizes, forming a vascular net with probable erectile function. The function of the female papilla is even less clear. The papilla of *G. dormitor* may have a contractile function, while the network of blood vessels forms a spongy structure which could aid in ovoposition. However, secretory glandules similar to those found in the male papilla were not seen in the female. Weisel (1949) reported the presence of the female urogenital papilla in *Gillichthys* and said that the function of the papilla could be in relation to the sexual cycle, an idea also mentioned by Hoffman (1963) who described this structure in *O. tau*.

The seminal vesicles are accessory sexual organs found in several families of teleost fish, such as Blenniidae and Gobiidae. The seminal vesicle of *G. dormitor* is type A, as described by Fishelson (1991). The presence of secretory glandular cells in the seminal vesicle together with spermatozoa suggests the structure has storage and nutritive functions for the spermatozoa, as described by De Jonge et al. (1989), Lahnsteiner et al. (1990), Seiwald and Patzner (1989) and Patzner (1991).

This study has shown that *G. dormitor* are resident and undergo sexual maturation in the Tecolutla estuary. Histological evidence suggests both males and females undergo gonadal recrudescence in the estuary and have an extended reproductive season from May through November. However, it is unclear if the species actually spawns in the estuary, since females in the final stages of FOM were not captured. Additional research on the reproductive biology and ecology of this under-studied species is necessary to determine its role in tropical estuaries in the southern Gulf of Mexico. Information learned from areas in the center of its distribution may aid in conserving the species at the periphery of its range in Florida, where it is considered threatened (Gilmore 1992, Music et al. 2001).

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