

Aspects of the reproductive biology of the toadfish, *Halobatrachus didactylus* (Schneider, 1801) (Pisces: Batrachoididae)*

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SUMMARY: The biology of *Halobatrachus didactylus* has received special attention during recent years because of its use as an experimental species in toxicology and cardiology experiments. The object of the present work is to supply basic information about the reproductive biology of this species. Samples were collected in Cádiz Bay between February 1999 and March 2000. A total of 901 specimens (440 females and 461 males) were examined. Sex ratio did not differ from the theoretical 1:1, except in May 1999, and February 2000. Minimum size at maturity was 191 mm total length for females and 160 mm total length for males. Approximately 50 % of the population matured at 262 mm for females and 302 mm for males. The spawning season extends from March to August, with a peak in May-June. Fecundity ranged from 227 to 1233 eggs/female, and was related to body length and weight. Length-weight relationship for the sexes differed, and showed positive allometry. The condition factor showed a minimum in the spawning season, which is interpreted as the result of mobilization of somatic energy reserves needed for reproductive development, and/or the use of reserves stored in the body as a source of energy in spawned fish, influenced by reduced feeding during this period.

Key words: reproduction, toadfish, *Halobatrachus didactylus*, Batrachoididae, Bay of Cádiz.

INTRODUCTION

The toadfishes (Family Batrachoididae) include 12 genera and 40 species of shallow water bottom-dwelling fishes that live in soft sand, rocky bottoms and reefs (Cervigón, 1980). Some batrachoid species have traditionally been used as laboratory animals in the field of physiology (Hopkins *et al.*, 1997; Gilmour *et al.*, 1998; Perry *et al.*, 1998; Paert *et al.*, 1999), toxicology (Gutiérrez *et al.*, 1978; Sinovcic *et al.*, 1980; Sarasquete *et al.*, 1982), ethol-

ogy (Ament *et al.*, 1997; Bass, 1998), neurobiology (Rabbitt *et al.*, 1995; Fine *et al.*, 1996; Hirsch *et al.*, 1998), cardiology (Benítez *et al.*, 1994a,b; Coucelo *et al.*, 1996), and endocrinology (Fine *et al.*, 1996; Knapp *et al.*, 1999). Owing to this, their biology has been extensively studied in most of species. Along the Iberian Peninsula, the Batrachoidids are represented by only one species, *Halobatrachus didactylus*. Its distribution extends from the Bay of Biscay to Ghana and the west Mediterranean (Roux, 1986). In the Bay of Cádiz, it is abundant and represents an important component of the fish communities (Arias, 1976).

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H. didactylus is generally found in shallow water –tidal channels and creeks, inlets, beaches– down to about 50 m. It is a sedentary and solitary species that lives partly buried in soft sand and mud, hidden between weeds and sea grasses or concealed in rock crevices. It is an euryhaline species, capable of colonizing diverse biotopes, from brackish waters of river estuaries such as those of the Guadalquivir (Spain), Mira, Sado, and Tagus (Portugal) to hypersaline waters of the salt ponds (Arias and Drake, 1990; López de Cunha, 1994; Costa *et al.*, 1995; Fernández-Delgado *et al.*, 2000).

The biology of *H. didactylus* has received special attention in recent years because of its use as a laboratory animal in toxicological and cardiological experiments (Benítez *et al.*, 1994 a,b; Coucelo *et al.*, 1996). Cárdenas (1977), and Costa *et al.* (1995) examined its feeding habits; and Arias and Drake (1990) studied juvenile distribution in Cádiz Bay. Abundance and distribution in the Mira, Sado and Tagus Rivers estuaries was studied by Costa (1993), Costa *et al.* (1995) and López de Cunha (1994), who considered this species as one of the most important and abundant fish in those habitats; biochemical and histochemical features of different organs were assessed by Gutiérrez *et al.* (1987), Establier *et al.* (1986), and González de Canales *et al.* (1989, 1990); and haematology has been studied by Gutiérrez and Sarasquete (1985), and Sarasquete (1983, 1984). Published information on its reproductive biology is scarce. There only exist reports on the histological, histochemical, and biochemical structure of the gonads (Blanco, 1991; González de Canales *et al.*, 1992; Rosety *et al.*, 1992).

The objective of this study was to investigate some reproductive traits (sex ratio, size at sexual maturity, spawning period, fecundity, length-weight relationship and condition factor) of *H. didactylus*.

MATERIALS AND METHODS

Samples of fish were obtained monthly from artisanal fishermen's catches in Cádiz Bay (South-Western Spain) from February 1999 to March 2000.

All specimens were measured (total length, L_T), and weighed (body weight, W_T ; eviscerated weight, W_E ; gonad weight, W_G ; and liver weight, W_L). Sex and maturity (I, undeveloped; II, ripening or recovering; III ripe, gamete running; and IV, spent) were also recorded.

Sex was established by macroscopic examination of the gonads. A Chi-square test (Steel and Torrie,

1985) with Yates correction for continuity was used to compare sex ratios in each month, and at each 20-mm length interval with a hypothetical 1:1 ratio.

To estimate the mean total length at maturity (L_{50}) for males and females, the fraction of mature fish in each 10 mm interval was fitted to a logistic function (Gaertner and Laloé, 1986) by using the Marquardt method (Draper and Smith, 1966).

The spawning season was inferred from the study of monthly variation in gonadosomatic index and hepatosomatic index and the relative frequencies of gonad maturity stages through the study period. Gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as: $GSI = 100(W_G \cdot W_E^{-1})$; $HSI = 100(W_L \cdot W_E^{-1})$.

Fecundity was estimated using 73 ripe ovaries preserved in Gilson's fluid. Relative fecundity (RFL_T : fecundity related to total length; RFW_T : fecundity related to total weight; RFW_E : fecundity related to eviscerated weight; RFW_G : fecundity related to gonad weight) for each fish was calculated as: $RFL_T = F \cdot L_T^{-1}$, $RFW_T = F \cdot W_T^{-1}$, $RFW_E = F \cdot W_E^{-1}$ and $RFW_G = F \cdot W_G^{-1}$. Fecundity was regressed on body length, on body weight and ovary weight by linear regression on logarithms using model II regression as suggested by Ricker (1973). The oocyte diameter (mm) was estimated from 140 randomly chosen ripe oocytes, measured by the ocular micrometer of a binocular microscope.

Length-weight relationship was modeled by linear model II regression analysis on the log-transformed variables (Ricker, 1973). Relative condition factor (Kr) for adult specimens was calculated as the percentage ratio of the observed weight of a fish to the weight expected from the calculated length-weight relationship (Le Cren, 1951).

Monthly changes in relative condition factor, gonadosomatic index and hepatosomatic index were assessed by using a one-way analysis of variance (ANOVA). Significant ANOVAs ($p \leq 0.05$) were followed by Duncan's multiple range test (Steel and Torrie, 1985). GSI data were \log_{10} transformed to satisfy the assumptions of the analysis.

RESULTS

In total, 901 specimens measuring 122-470 mm L_T were examined. Overall, 440 (48.8 %) specimens were females (122-366 mm L_T ; 30-1075 g W_T) and 461 (51.2 %) were males (137-470 mm L_T ; 50-2105 g W_T).

TABLE 1. – Number of male and female *H. didactylus* by month and results of the Chi-square test for a 1:1 ratio.

Month	Females	Males	Total	χ^2
February/99	10	14	24	0.38 ^{ns}
March	4	11	15	2.40 ^{ns}
April	38	58	96	3.76 ^{ns}
May	84	36	120	18.41*
June	20	25	45	0.36 ^{ns}
July	19	29	48	1.69 ^{ns}
August	28	41	69	2.09 ^{ns}
September	43	40	83	0.05 ^{ns}
October	45	55	100	0.81 ^{ns}
November	31	31	62	0 ^{ns}
December	45	28	73	3.51 ^{ns}
January/00	22	20	42	0.02 ^{ns}
February	22	47	69	8.35*
March	29	26	55	0.07 ^{ns}
Total	440	461	901	

ns= p> 0.05; *= p ≤ 0.05

Males were found to be larger (t = 7.94; p < 0.01) and heavier (t = 7.52; p < 0.01) than females.

Sex ratio

Sex ratios did not differ (p > 0.05) from 1:1 except in May 1999 when females were favored significantly, and February 2000 when males predominated (Table 1).

The number of males and females was equal at lengths <220 mm L_T , and between 260 and 300 mm L_T , but females outnumbered males at 220-260 mm L_T . Males dominated in specimens >300 mm and all individuals with lengths over 380 mm were males (Table 2).

Size at sexual maturity

The smallest mature fish were a 191 mm L_T female and a 160 mm L_T male. Fifty percent maturity occurred at 262 mm L_T for females and at 302 mm L_T for males (Fig. 1).

According to these results, adults were defined as those individuals at/or exceeding the minimum size at sexual maturity and at least stage II.

Spawning season

Gonadosomatic Index (GSI)

GSI increased with stage of development. GSI for stage I and II females remained below 19.13. For mature females, GSI was between 3.98 and 40.12. Immature males had a GSI of 0.10-4.07, while for

TABLE 2. – Number of male and female *H. didactylus* by length class and results of the Chi-square test for a 1:1 ratio

Length class	Male	Female	Total	χ^2
120 – 140	1	2	3	—
140 – 160	6	3	9	0.44 ^{ns}
160 – 180	11	12	23	0 ^{ns}
180 – 200	56	62	118	0.21 ^{ns}
200 – 220	65	78	143	1.01 ^{ns}
220 – 240	51	87	138	8.88*
240 – 260	42	69	111	6.09*
260 – 280	58	60	118	0.01 ^{ns}
280 – 300	47	39	86	0.57 ^{ns}
300 – 320	41	18	59	8.20*
320 – 340	24	7	31	8.26*
340 – 360	12	2	14	5.79*
360 – 380	20	1	21	15.43*
380 – 400	13	0	13	11.08*
400 – 420	6	0	6	—
420 – 440	5	0	5	—
440 – 460	2	0	2	—
460 – 480	1	0	1	—
Total	461	440	901	

ns= p> 0.05; *= p ≤ 0.05

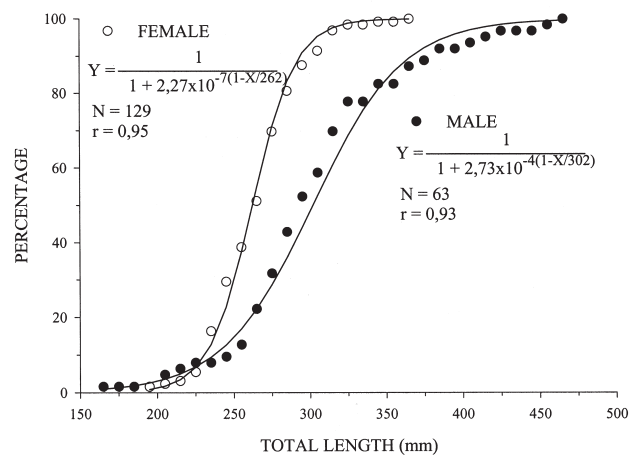


FIG. 1. – Sexual maturity of male and female toadfish, *H. didactylus* and corresponding size-at-maturity, L_{50} .

mature fish it ranged from 0.59 to 11.37. GSI was significantly higher in females and showed a high variability throughout the year in both sexes.

GSI of adult females ranged between 0.33 and 40.12, and varied between months (F = 71.82; p < 0.01). Higher GSI values occurred in March-May 1999 and declined sharply thereafter, indicating that oocytes were released. Low values occurred in July-November. In December, the gonad cycle started again (Fig. 2a).

Spawning periods for males were not as marked as for females. However, male GSI values decreased in June-July coincident with those of females. GSI values varied from 0.01 to 11.37, and showed significant monthly differences (F = 15.00; p < 0.01). Mean GSI

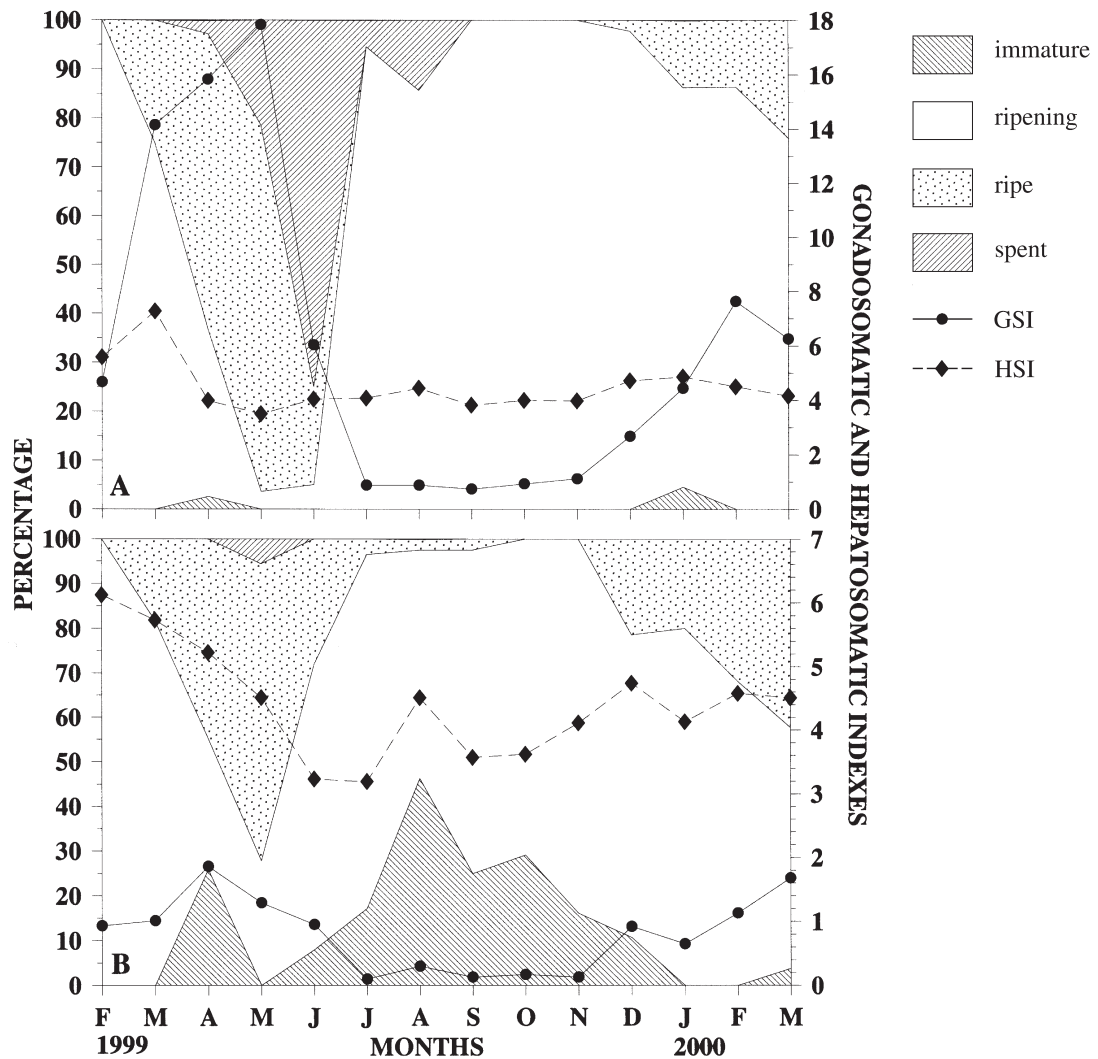


FIG. 2. – Monthly changes in GSI, HSI and in the frequency of occurrence of the various maturity stages of gonads for female (A) and male (B) *H. didactylus*.

for males remained high and variable in February-May, and low in July-November (Fig. 2b).

Hepatosomatic index (HSI)

HSI showed strong variations during the sampling period for both sexes (Fig. 2a,b). Differences between months were statistically significant ($F = 8.04$; $p < 0.01$) in females. Mean HSI values were significantly higher in December-March, and were minimum in April-November. Males also showed monthly differences in HSI ($F = 6.01$; $p < 0.01$). Highest values were found in February-April 1999, and lowest ones in June-July 1999.

The seasonal cycle of the HSI for females is not related to that of GSI ($r = -0.03$; $p > 0.05$). That of males showed a slight correlation to GSI ($r = -0.12$; $p < 0.05$).

Monthly variation in the proportion of different maturity stages

Both ripe males and females were collected between March and September 1999 and December 1999-March 2000 (Fig. 2).

The percentage of mature females was higher in April (61%), and May (75%). The rest of the year, almost all fishes were ripening or recovering. Spent females were observed in low percentages between April and August. The highest proportion (75%) occurred in June 1999 (Fig. 2a).

The greatest proportions of ripe males were found in April (45%), and May (64%). Maturing males was found in high proportions throughout the year, except in April and May when ripe individuals dominated. Spent males were collected in May (Fig. 2b).

TABLE 3. – Absolute and relative fecundity statistics in the toadfish *H. didactylus*

Characteristic	Mean	Standard error	Range
Fecundity	490	20	227-1233
RF-L _T	1.8	0.06	0.91-3.56
RF-W _T	1.44	0.03	0.88-2.10
RF-W _E	2.00	0.05	1.16-2.91
RF-W _G	9.05	0.32	5.86-19.37

RF-L_T: fecundity related to total length, RFW_T: fecundity related to weight, RFW_E: fecundity related to eviscerated weight, RFW_G: fecundity related to gonad weight, N = 73.

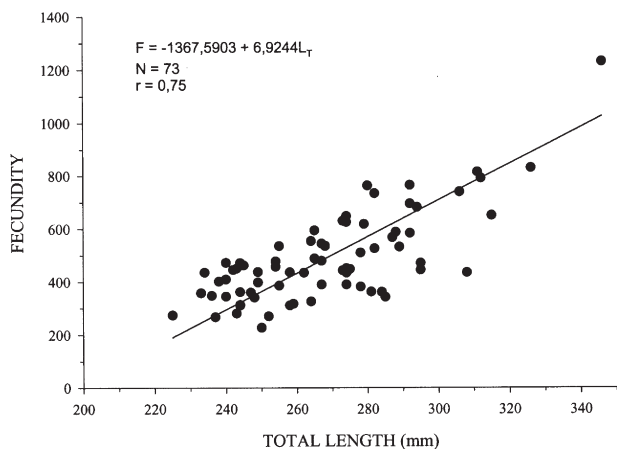


FIG. 3. – Relationship between fecundity and body length in *H. didactylus*.

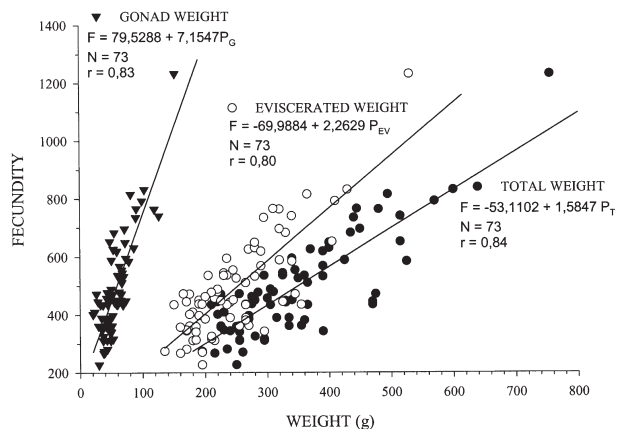


FIG. 4. – Relationship between fecundity and body weight, eviscerated weight and gonad weight in *H. didactylus*.

Peaks in GSI, along with the changes in percent frequency of ripe and spent individuals, indicates that *H. didactylus* spawns in March-August and has a reproductive peak in May-June.

Fecundity

Estimates of fecundity (F) are shown in Table 3. Fecundity increased linearly with body length, total

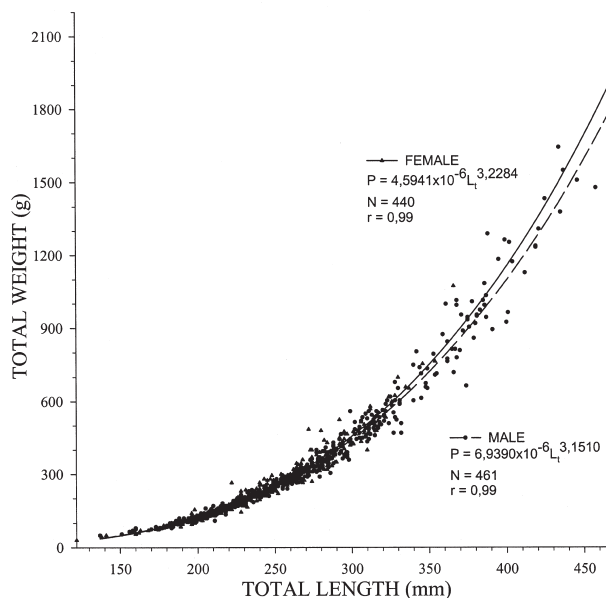


FIG. 5. – Length-weight relationship of male and female *H. didactylus*.

weight, eviscerated weight and ovary weight (Figs. 3 and 4). The number of eggs was higher in the left portion of the ovaries ($t = 7.19$; $p < 0.01$). This portion of the gonad was also larger ($t = 13.57$; $p < 0.01$) and heavier ($t = 7.75$; $p < 0.01$). Fresh mature eggs were 4.70-7.88 mm ($\bar{x} = 6.38 \pm 0.05$) in diameter and weighed 0.06-0.14 g ($\bar{x} = 0.10 \pm 0.001$).

Length-weight relationship

Length-weight relationships were derived from 901 specimens ranging from 122 to 470 mm and 30 to 2105 g.

Equations for the sexes differed ($t = -2.70$; $p < 0.01$), with relationships represented in Figure 5. The exponents of the two regressions showed a positive allometry ($t = 9.39$, $p < 0.01$ for males and $t = 9.53$; $p < 0.01$ for females)

Condition Factor (Kr)

Mean Kr for males and females showed a similar seasonal pattern (Fig. 6). In adult males, Kr showed monthly differences ($F = 5.40$; $p < 0.01$). Kr was higher in February-April 1999, and decreased sharply thereafter. Kr for adult females also varied significantly between samples ($F = 4.32$, $p < 0.01$). Highest condition was attained in March-April. The lowest values occurred in June.

Kr was correlated significantly with GSI in either males ($r = 0.18$; $p < 0.01$) or females ($r = 0.42$; $p < 0.01$).



FIG. 6. – Mean monthly variation in Condition Factor of male and female *H. didactylus*.

DISCUSSION

In fish, sex ratio varies considerably from species to species but in the majority of species it is close to 1:1. It differs from one population to another of the same species and may vary from year to year in the same population (Nikolsky, 1963). Monthly variations in sex ratio of *H. didactylus* samples apart from those of May 1999, and February 2000 showed no significant differences from that of the expected 1:1 ratio. The greater number of females compared to males in May, coincident with the reproductive peak, could be attributed to the fact that, as in other batrachoid species such as *Opsanus beta*, *O. tau*, and *Amphichthys cryptocentrus*, males actively guard the eggs, and maintain residence in the nests until young attain the free-swimming stage (Breder, 1941; Gray and Winn, 1961; Hoffman and Robertson, 1983), making them less exposed to capture.

Costa *et al.* (1995) obtained an overall 1:1 sex ratio for *H. didactylus* in the Mira estuary, but these authors did not test the presence of a monthly or length related pattern. Granado and González (1988) also reported a 1:1 sex ratio for *A. cryptocentrus*, in tropical waters, during most of the year.

Size related differences in sex ratios were observed. Significant deviations from parity usually occurred among the medium and largest sizes, with females predominating the medium while the largest sizes were disproportionately male. The differences observed in the size-specific sex ratio of the toadfish have been reported for other fish species (Turner *et al.*, 1983; Stergiou *et al.*, 1996), and have been related to sexual differences in growth, mortality and energetic cost of reproduction.

Males and females of *H. didactylus* mature at different lengths. Males began maturing earlier than females, a fact that has been attributed to gains in fecundity with size (Stearns, 1992). L_{50} for males was greater than for females, which may indicate that after first maturation females reduce growth and allocate energy mainly for the production of gametes while males, with a smaller reproductive effort, continue growing.

No previous estimates of size at maturity are available for the species investigated here. In *A. cryptocentrus*, a species that attains similar sizes to *H. didactylus*, males also mature at a larger size than females. Sizes at sexual maturity for both sexes of this species (males, 270 mm, and females, 310 mm) (Granado and González, 1988) are very similar to those of *H. didactylus*.

Our estimate of the spawning season in *H. didactylus* in Cádiz Bay is similar to that of Blanco (1991) who stated that this species spawns during spring. However, his finding that the maturation-spawning occurs in March-April in females and earlier (February-March) in males differs from the peak observed in the present study (May-June). This may indicate that in *H. didactylus*, as in other teleosts, the spawning season is affected by year to year climatic changes (i.e. in water temperature), that delay or advance maximum spawning. Gray and Winn (1961) indicated that local differences in shallow water and deep-water populations and yearly climatic differences might be expected, particularly if temperature is causative.

The spawning season of *H. didactylus* is similar to that reported by Gudger (1910) and Schwartz (1974) for *O. tau* in North Carolina and Chesapeake Bay. Gray and Winn (1961) observed that *O. tau* spawning in South Carolina begins in May and extends to early August. In the tropics, *A. cryptocentrus*, can reproduce throughout the year (Granado and González, 1988).

Although no significant correlation was observed between gonadosomatic and hepatic indices for females, the decrease in HSI in females during April-May, may indicate the use of liver reserves during gonad growth and maturation. It is known that in non-mammalian vertebrates, the principal events responsible for the enormous growth of oocytes involve the storing and packaging of a hepatically derived plasma precursor, vitellogenin, into yolk protein (Wallace and Selman, 1981). This suggests that liver reserves may

accumulate prior to reproduction, being then transferred through the blood stream to the ovaries during oogenesis. Bustamante (1983) observed an increase in weight of the liver in fishes prior to reproduction, and pointed out that this was due to an increase in the synthesis of lipids and proteins necessary for gonad development.

As for other batrachoid species, fecundity in *H. didactylus* is very low. This must be compensated for by a high survival of eggs and juvenile fish that results from males guarding the nests until young are free-swimming. The high amount of yolk, and the great egg sizes may also be mechanisms to assure a high survival rate in embryos and larvae such as has been reported by Yáñez-Arancibia *et al.* (1976) and Palazón *et al.* (1994) for the Ariidae, *Galeichthys caerulescens*, and *Bagre marinus*, respectively. The fecundity estimates of *H. didactylus* and other Batrachoids show that these species have a reproductive strategy common to other fish families with parental care, i.e. they produce a low number of large eggs.

Even though the size of eggs in *H. didactylus* is similar to that reported for other Batrachoids, the fecundity of *H. didactylus* is higher than for *S. barbatus* (245-499; Hoffman and Robertson, 1983), and *A. cryptocentrus* (45-467 eggs; Granado and González, 1988). Relative fecundity in this later species was estimated as 0.39-1.01 eggs/g, which is considerably smaller than the values observed in *H. didactylus*.

The increase in fecundity with body length and weight is consistent with the observations made in other fish species (Granado and González, 1988; Gartner, 1993; Barbin and McCleave, 1997).

Hepatosomatic index is a good indicator of recent feeding activity (Tyler and Dunns, 1976); the decrease in HSI during the spawning period suggests that *H. didactylus* may decrease its feeding activity during reproduction. The condition factor also showed a minimum value in the spawning season, which as stated by Maddock and Burton (1999) can be the result of mobilization of somatic energy reserves needed for reproductive development, and/or the use of reserves stored in the body as a source of energy in spawned fish, influenced by reduced feeding during this period. In a previous study, Cardenas (1977) observed that 4-5 % of stomachs were empty in summer-autumn, with the value increasing to 10-17 % in winter-spring, the higher values being registered in spring.

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