

Reproductive biology of dolphinfish, Coryphaena hippurus (Actinopterygii: Coryphaenidae), in Saint Peter and Saint Paul Archipelago, Brazil

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Summary: In order to elucidate the main aspects related to dolphinfish reproduction around Saint Peter and Saint Paul Archipelago, 862 individuals caught in that area by commercial handline fishing were examined between 2007 and 2011. From those specimens, it was possible to assess the sex in 782 specimens (271 males and 511 females) and the levels of gonadal maturation in 536 of them (175 males and 361 females). The fork length (FL) of the examined specimens varied between 27 and 150 cm. Sex ratio found was 1.0 male: 1.9 females, but the difference was not statistically significant in April (χ^2 =0.08), August (χ^2 =0.82) and October (χ^2 =3.63). However, by size interval, sex ratio was 1.0 male: 1.0 female for FL between 120 and 130 cm. The gonadal index showed the highest values in February for males and in April for females. Most spawning-capable specimens were found in February (males 36.4%) and in May (females 42.2%). The length of size-at-50%-maturity (L₅₀) was estimated at 70.66 cm FL for males and 68.60 cm FL for females. The results suggest that the period of highest reproductive activity occurs between April and June.

Keywords: reproduction; sexual proportion; GI; L₅₀; spawning season; maturation.

Biología reproductiva del dorado, Coryphaena hippurus (Actinopterygii: Coryphaenidae), en el Archipiélago de San Pedro y San Pablo, Brasil

Resumen: Con el objetivo de estudiar la reproducción del dorado capturado con línea de mano por la flota pesquera en los alrededores del Archipiélago de San Pedro y San Pablo, entre 2007 y 2011 se examinaron un total de 862 ejemplares de esta especie. Fue posible identificar el sexo de 782 de estos ejemplares (271 machos y 511 hembras), así como los estados de madurez gonadal de 536. La longitud furcal (LF) de los ejemplares examinados varió entre 27 y 150 cm. La proporción de sexos fue de 1 macho por 1.9 hembras, aunque en abril (χ^2 =0.08), agosto (χ^2 =0.82) y octubre (χ^2 =3.63) la proporción de sexos valores en febrero para machos y en abril para hembras. El mayor porcentaje de individuos maduros se observó en febrero y mayo. La talla de primera madurez sexual (L₅₀) se estimó en 70.66 cm LF para machos y 68.60 cm LF para hembras. Los resultados sugieren que el periodo de mayor intensidad reproductiva se sitúa entre abril y junio.

Palabras clave: reproducción; proporción de sexos; IG; L₅₀; época de puesta; madurez sexual.

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INTRODUCTION

Because of its strategic geographical position between the northern and southern hemispheres and between the American and African continents, the Saint Peter and Saint Paul Archipelago (SPSPA) (Fig. 1) exerts a strong influence on the life cycle of various migratory species, for which it has great importance as



Fig. 1. - Location of the Saint Peter and Saint Paul Archipelago (Bezerra et al. 2013).

a reproduction and feeding site. In relation to meteorological condition, the climate at SPSPA is directly influenced by the Intertropical Convergence Zone, which explains why the area has one of the highest precipitation indexes in the Atlantic Ocean, particularly between December and April, and consequently a rather low sea surface salinity. In addition, the vertical temperature profile of the SPSPA is relatively stable throughout the year, with a shallow thermocline, around 25 m, and surface temperatures ranging between 27.13°C and 27.92°C (Campos et al. 2009, Vaske-Jr 2010).

The dolphinfish, *Coryphaena hippurus* (Linnaeus, 1758), one of the species commonly caught in the archipelago, has a circumtropical distribution in the Atlantic, Pacific and Indian oceans (Beardsley 1967, Rose and Hassler 1968, Carpenter 2002). It is a migratory, pelagic and fast-swimming fish which is intensively caught by commercial and sport fisheries around the world (Erdman 1956, Zaneveld 1961, Sacchi et al. 1981). In the southeast Caribbean, it constitutes the largest portion of the large pelagic fish landed by commercial fisheries in both weight and revenue (Mahon et al. 1981).

Although the reproductive biology of the dolphinfish has already been studied in different places around the world (Potoschi et al. 1999, Alejo-Plata et al. 2011, Furukawa et al. 2012), in Brazil studies of the reproductive biology of the species are scarce (Souza 1998). Furthermore, the available studies show great heterogeneity in the area and the geographic and environmental conditions in which the data were obtained. It is therefore difficult to extrapolate from one region to another so studies must be carried out in geographically specific areas.

According to FAO statistics (FAO 2010), in 2008, 54339 t of dolphinfish were caught worldwide, of which about 25% (13491 t) were caught by Ecuador, about 17% (9307 t) by Taiwan and about 16% (8870 t) by Brazil.

Because the dolphinfish is a migratory species, with stocks shared and exploited by various nations, it is crucial to generate information about its biology, not only to allow a suitable assessment of their stocks but also to ensure the sustainability of its exploitation and conservation. Based on this scenario, the goal of this work was to elucidate the main aspects related to the reproduction of the dolphinfish near the SPSPA.

MATERIALS AND METHODS

The examined specimens were caught by commercial tuna fishing boats operating near the SPSPA (00°55'02"N, 29°20'42"W) (Fig. 1) between 2007 and 2011, using hand lines. Samples from the four years were pooled (Table 1) to construct a "year type cycle", as shown in the figures.

All specimens were identified and measured at the time of boarding, with their total length (TL) and fork length (FL) being recorded. Subsequently, the gonads were collected and preserved in formaldehyde 10% for later analysis.

In the laboratory, the gonads were weighed and measured, and the sex and stage of gonadal development were then identified by macroscopic analysis. The gonads were fixed in a solution of 10% formalin for 48 hours, suffered the first cleavage after 24 hours, were returned to the fixative solution, and then preserved in 70% alcohol. For histological analysis, the methodology described by Behmer et al. (1976) was applied. Six maturity phases were established for females: I, immature; II, developing; III, spawning-capable; IV, actively spawning; V, regressing; and VI, recovering. Five phases, adapted from Hunter et al. (2011), were identified for males: I, immature; II, developing; III, spawning-capable; IV, regressing; and V, recovering.

The sex ratio was estimated from the total number of females and males caught throughout the sampling period and by size interval. Statistically significant differences in the sex ratio were tested by the chi-square test (χ^2) (p<0.05) (Snedecor and Cochran 1989).

Table 1. – Numb	er of	dolphinfish,	Coryphae	ena	hippurus,	sampled
ł	y mc	onth between	2007 and	201	1.	

Month	Samples (n)	
January	88	
February	115	
March	67	
April	28	
May	101	
June	75	
July	109	
August	102	
September	35	
October	66	
November	37	
December	39	
Total	862	

The gonadal index (GI) was obtained by the model proposed by Schaeffer and Orange (1956), using the following relation between the weight of the ovaries and size of the individuals: $GI=(GW10^5)/FL^3$, where FL is the fork length and GW is the gonad weight. Immature specimens were excluded from the analysis because they had not yet begun their reproductive cycle. A statistical comparison of the GI between months was done using the Kruskal-Wallis test (p<0.05) (Kruskal and Wallis 1952). In this study, the GI was used in place of the gonadosomatic index owing to the absence of weight scales on the boats. Nevertheless, through the determination of the months with the highest reproductive activity, the GI allows us to identify the possible spawning period. Thus, the spawning season was determined by the monthly distribution of female mean GI and monthly frequency of different maturational stages.

The average length of size-at-50%-maturity (L_{50}) was obtained from the following logistic curve for both sexes, suited using the maximum likelihood approach with Statistica 7 software (Zar 2010): Y=1/[1+exp(a+bFL)], where Y is the fraction of individuals who are able to reproduce (Beverton and Holt 1956), a and b is coefficient of linear regression and FL is the fork length each length class.

RESULTS

Of the 862 specimens studied, 511 (59.2%) were females, 271 (31.5%) were males and 80 (9.3%) could not have the sex identified. The FL of sampled specimens ranged between 27.0 and 150.0 cm, with females ranging from 27.0 to 124.5 cm, and males from 39.0 to 150.0 cm. The highest frequency for both females and males was observed between 80 and 90 cm FL (Fig. 2).

The overall sex ratio was 1.0 male: 1.9 females. Females were significantly more frequent in all months, except for April (χ^2 =0.08), August (χ^2 =0.82) and October (χ^2 =3.63), when females were still predominant, but the difference was not statistically significant ($\chi^2_{calculated}$ = 73.66> $\chi^2_{tabulated}$ =19.68) (Table 2). Regarding the size classes, there were no statistically significant differences ($\chi^2_{calculated}$ =73.66> $\chi^2_{tabulated}$ =19.68) (Table 3), except between 70 and 110 cm FL. Between 120 and 130 cm FL the sex ratio was equal to 1 male:1 female. Between 20 and 120 cm FL females were more abundant and between 130 and 150 cm FL no females were sampled.



Fig. 2. – Frequency distribution of fork length of specimens of dolphinfish, *Coryphaena hippurus*, caught in the vicinity of the Saint Peter and Saint Paul Archipelago (n total=862, n females=511, n males=271) between 2007 and 2011.

Table 2. – Sex ratio of males and females of dolphinfish, *Coryphaena hippurus*, and results of the chi-square (χ^2) .* Statistical difference level of significance 5%

	Males (n)	Females (n)	Total	χ^2
Jan	32	53	85	5.2*
Feb	42	69	111	6.6*
Mar	12	29	41	7.05*
Apr	6	7	13	0.08
May	27	68	95	17.7*
Jun	19	54	73	16.8*
Jul	41	62	103	4.3*
Aug	45	54	99	0.82
Sep	7	28	35	12.6*
Oct	20	34	54	3.63
Nov	10	24	34	5.8*
Dec	10	29	39	9.26*
Total	271	511	782	73.66*

Table 3. – Sex ratio by size interval of males and females of dolphinfish, *Coryphaena hippurus*, and results of the chi-square (χ^2).* Statistical difference level of significance 5%

Size interval (cm FL)	Males (n)	Females (n)	Total	χ^2
20-30	0	1	1	1
30-40	2	3	5	0.2
40-50	0	2	2	2
50-60	3	7	15	1.6
60-70	20	24	47	0.4
70-80	43	75	128	8.7*
80-90	97	183	295	26.4*
90-100	57	131	201	29.1*
100-110	34	63	131	8.7*
110-120	11	20	38	2.6
120-130	2	2	4	0
130-140	1	0	2	1
140-150	1	0	1	1
Total	271	511	870	73.66*

According to the histological analysis of the testes, of the 175 males examined, 16 were immature (39.0 to 71.0 cm FL), 56 were developing (72.0 to 124.0 cm FL), 11 were spawning- capable (81.0 to 150.0 cm FL), 8 were regressing (91.0 to 108.0 cm FL) and 84 were recovering (70.0 to 108.0 cm FL). The frequency distribution of monthly stages of gonadal maturation indicates a higher frequency of immature males in July (43.7%), developing in January (21.4%), spawning-capable in February (36.4%) and recovering in July (27.4%) (Fig. 3).

Of the 361 females examined, according to the histological analyses, 42 were immature (27.0 to 72.0 cm FL), 140 were developing (43.0 to 115.0 cm FL),







Fig. 4. – Relative frequency of maturity stages of female dolphinfish, *Coryphaena hippurus* (n=361), caught in the vicinity of the Saint Peter and Saint Paul Archipelago between 2007 and 2011 (the bars represent the standard deviation).

45 were spawning-capable (64.0 to 124.5 cm), 8 were actively spawning (75.0 to 117.0 cm), 33 were regressing (72.0 to 108.0 cm) and 93 were recovering (63.0 to 106.0 cm FL). There was a higher frequency of immature females in July (26.2%), developing in January (14.2%), July and August (13.5%), spawning-capable in May (42.2%), actively spawning also in May (37.5%), regressing in February and June (21.2%), and recovering in June (16.13%) and July (25.8%) (Fig. 4). The gonad maturation phases for both males and females are described in Tables 4 and 5, respectively.

The weight of male gonads ranged from 0.49 to 90.00 g, with the monthly mean GI ranging from 0.58 to 3.73, with a peak in February (Fig. 5). For females, the gonad weight ranged from 0.77 to 597.20 g, with the monthly mean GI ranging from 3.24 to 34.90, with a peak in April, suggesting that spawning occurs between April and June (Fig. 6). The monthly mean GI for both males and females showed statistically signifi-



Fig. 5. – Monthly mean gonadal index (GI) of male dolphinfish, *Coryphaena hippurus* (n=175), caught in the vicinity of the Saint Peter and Saint Paul Archipelago between 2007 and 2011 (the bars represent the standard deviation).

Table 4. – Macroscopic and microscopic characterization of the maturity phases of the testes of *Coryphaena hippurus* in the Saint Peter and Saint Paul Archipelago, adapted from Hunter et al. (1985), Murua et al. (2003) and Brown-Peterson et al. (2011).

Stage	Macroscopic characteristics	Microscopic characteristics
I. Immature	Small and threadlike testes often clear (translucent) with a light yellow colour. Difficult for sex distinction.	Compact cell form. Displays only primary spermatogonia; the lumen is not divided into lobes.
II. Developing	Testes with yellow-white colour (translucent to opaque with a small width and reduced thickness, but measurable); due to its reduced thickness, there is an inaccuracy in the observation of the ventral streaks and the cross section median. At the end of this phase, a few sperm may be present in the sperm channel.	Spermatocytes present throughout the lobules. May show secondary spermatogonia, primary and secondary spermatocytes, spermatids and spermatozoa. No sperm is present in the lumen of the lobules or sperm ducts. Germinal epithelium is continuous throughout the testes.
III. Spawning- capable	Large and firm testes, white to pink coloration, sometimes with reddish spots with increased vascularization; tubular form; ventral streaks usually present (in the form of lobes); outer membrane is easily ruptured when handled, ripping by its own weight. Abundant semen throughout the testis.	All spermatogenesis stages may be present. Spermatocysts are present throughout the testis, active spermatogenesis. Germinal epithelium may be continuous or discontinuous.
IV. Regressing	Small and flaccid testicles, no semen released with pressure. Has residual spermatozoa in the lumen of the lobules and the spermatic duct.	Spermatocysts are widely scattered near the periphery containing secondary spermatocytes, spermatids and spermatozoa. Little or no active spermatogenesis. Spermatogonial proliferation and regeneration of common germinal epithelium at the periphery of the testes (cell disorganization present).
V. Recovering	Small testes, often threadlike. No spermatocytes are present. The lumen is not divided into lobes.	Proliferation of spermatogonia throughout testis. Continuous germinal epithelium throughout testis, different from the immature testis due to the testicle wall thickness and evident spacing, indicating previous reproductions.

Table 5. – Macroscopic and microscopic characterization of the maturity phases of the ovaries of *Coryphaena hippurus* in the Saint Peter and Saint Paul Archipelago, adapted from Hunter et al. (1985), Murua et al. (2003) and Brown-Peterson et al. (2011).

Stage	Macroscopic Characteristics	Microscopic Characteristics
I. Immature	Small and threadlike ovaries, often translucent, yellowish colour, without opaque oocytes. Ovary has a thin wall on which blood vessels are indistinct, making sex distinction difficult.	Only oogonia and oocytes in primary growth are present. Atresia is not present. Thin ovarian wall and small space between oocytes, oocytes demonstrate a cluster conformation with visible cellular organization.
II. Developing	Medium sized ovaries, orange colour, still with no noticeable opaque oocytes, and blood vessels are becoming more distinct.	Beginning of vitellogenesis; oocytes are in primary growth, cortical alveoli and vitellogenesis 1 and 2. No advanced stage of vitellogenesis is present.
III. Spawning-capable	Large ovaries, bright yellow colouration, prominent blood vessels. Individual oocytes are macroscopically visible.	Oocytes present in vitellogenesis 3. A few hydrated oocytes may be present. Early stages of oocyte maturation are present. In some oocytes, the yolk appears dense with multiple transparent lipid bodies around the nucleus.
IV. Actively spawning	Translucent oocytes, which may or may not flow when pressure is applied. Hydrated oocytes are larger than the opaque oocytes. The gonad becomes delicate to the touch and may break.	Presence of oocytes with germinal vesicle migration. Hydrated oocytes (translucent) in large quantities and with few post-ovulatory follicles (POFs).
V. Regressing	Very flaccid ovary, appearing haemorrhagic with orange colour and purple spots. Occasionally with a few remaining translucent oocytes.	Atresia (at any stage) and POFs are present. Presence of cortical oocyte and/or vitellogenesis 1 and 2. Cellular disorganization is noted.
VI. Recovering	Small ovaries, orange-dark brown coloration, opaque oocytes are not present; contains a thicker membrane than the immature fish. Displays reduced blood vessels.	Only oogonia and primary growth oocytes are present. Muscle bundles, dilated blood vessels, thick ovary wall, has degenerating POFs and previous atresia (gamma/ delta).



Fig. 6. – Monthly mean gonadal index (GI) of female dolphinfish, *Coryphaena hippurus* (n=361), caught in the vicinity of the Saint Peter and Saint Paul Archipelago between 2007 and 2011 (the bars represent the standard deviation).

cant differences (Kruskal-Wallis, p_{males} =0.0008072, $p_{females}$ =1.496e-11).

The size-at-50%-maturity (L_{50}) estimated for males and females (Fig. 7) was equal to 70.66 and 68.60 cm FL, respectively. Of the 271 males examined only 26 (9.6%) had an FL smaller than the size at first maturity, whereas of the 511 females only 29 (5.7%) exhibited an FL lower than that. These results indicate a predominance of adults in the sampled population.

DISCUSSION

The sex ratio found in this study (1.0 male:1.9 females) was very close to that found by Potoschi et al. (1999) in the western and Central Mediterranean, equal to 1 male:2 females for juvenile samples and 1 male:1 female for adult specimens.

Castro et al. (1999) obtained in the Canary Islands a sex ratio equal to 1.0 male:1.4 females for dolphinfish, with females predominating in size intervals lower than 85 cm FL, while in size intervals larger than 90 cm FL the males predominated. However, in a similar study conducted for *Coryphaena equiselis* the authors



Fig. 7. – Length at first sexual maturity L_{50} for males (n=175) and females (n=361) of *Coryphaena hippurus* caught in the vicinity of the Saint Peter and Saint Paul Archipelago between 2007 and 2011.

concluded that, although the sex ratio was also 1.0 male:1.4 females, for both the adults and the period in which the highest values of GSI were shown, the sex ratio found was 1 male:1 female. The sample size (150 *Coryphaena equiselis* and 36 *Coryphaena hippurus*), however, probably influenced these results.

On the other hand, Lasso and Zapata (1999) found a sex ratio very close to 1:1 (1.0 male:0.96 females) on the coast of Colombia and Panama; the males were predominant from June to December and females from January to May. A fairly equal amount of males and females was found in January.

Similarly, Massuttí and Morales-Nin (1997) found a sex ratio equal to 1 male:1 female at the island of Majorca, with no significant difference. The distribution of sexes by size interval, however, showed a significant predominance of females in specimens smaller than 25 cm FL and a high proportion of males for individuals ≥150 cm FL, though this interval was not significantly

Table 6. –	 Size at first sexual maturity 	(L_{50}) for males and females	of dolphinfish,	Coryphaena hippurus	reported in the literature.
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Author	Year	Place	L ₅₀ Males	L ₅₀ Females
Campos et al.	1993	Costa Rica	130 cm TL	130 cm TL
Massuttí and Morales-Nin	1997	Western Mediterranean	61.8 cm FL	54.5 cm FL
Wu et al.	2001	Taiwan	51 cm FL	51 cm FL
Schwenke and Buckel	2008	North Carolina	47.6 cm FL	45.8 cm FL
Alejo-Plata et al.	2011	Gulf of Tehuantepec, Mexico	50.57 cm FL	48.38 cm FL
Zúñiga-Flores et al.	2011	Gulf of California, Mexico	80 cm FL	80 cm FL

different from 1:1 because of the small number of specimens sampled (5 males and 2 females).

Zúñiga-Flores et al. (2011) found a sex ratio equal to 1 male:1 female in the Gulf of California, with no significant difference. The proportion of females, however, was greater for FL below 90.0 cm, while the opposite was observed for larger sizes.

Campos et al. (1993) reported a sex ratio off Costa Rica equal to 2 males:1 female, which, according to them, provided evidence of a separation between adult and young males, with the latter staying nearer the coast.

Potoschi et al. (1999) obtained juvenile samples (between 20 and 60 cm FL) from a commercial fleet using surrounding-nets and adults samples as bycatch from a commercial fleet using longline, drifting-net and harpoon. Massuttí and Morales-Nin (1997) obtained the samples from commercial catches of two different fisheries: juveniles by small-scale fishery using surrounding net and adults by Xiphias gladius longline fishery. Zúñiga-Flores et al. (2011) obtained their samples from a sportfishing fleet and an artisanal fishery, using trolling surface baits and artificial lures and small longlines, respectively. Lasso and Zapata (1999) obtained their samples from an industrial fleet targeting sharks by surface nets. Although various fishing methods were used to obtain the specimens of the cited authors, all of our specimens were captured using a single method (hand line). The results found in this study suggest that the sex ratio might be influenced by both size and sexual stage, as proposed by Potoschi et al. (1999), because for individuals between 120 and 130 cm FL the sex ratio was 1 male:1 female in the SPSPA.

Regarding the gonad maturation phases, the microscopic descriptions shown in this study are similar to the ones found by Alejo-Plata et al. (2011) and Zúñiga-Flores et al. (2011) for females, in stages III (spawningcapable), IV (actively spawning) and V n(regressing). Likewise, Zúñiga-Flores et al. (2011) showed a similar microscopic description for males in stages II (developing) and III (spawning-capable).

The highest monthly mean values of GI coincided with those of other species in the SPSPA, such as wahoo (Viana et al. 2008), rainbow runner (Pinheiro et al. 2010) and blackfin tuna (Bezerra et al. 2013), which also exhibit a period of increased reproductive activity in the first half of the year, coinciding with the presence of flying fish at the study site.

Castro et al. (1999) reported values of gonadosomatic index (GSI) with a peak in June for both sexes in the Canary Islands, coinciding with the summer in that region. Schwenke and Buckel (2008) reported that the highest values of GSI occurred in May for males

and females in North Carolina. Similarly, Furukawa et al. (2012) found the highest values of GSI in May for males and in July for females in the northern East China Sea. Campos et al. (1993) observed peaks of GSI in April, August and September in Costa Rica. Potoschi et al. (1999), working with individuals from the Mediterranean Sea, found the highest values of GSI between June and September, when all the individuals sampled were mature, with a peak in July. Wu et al. (2001) found peaks of GSI for males and females in February and March in Taiwan, but suggested that the dolphinfish has a long breeding season, during which it spawns almost continuously. Similarly, Alejo-Plata et al. (2011) observed a long spawning season for the dolphinfish off Mexico, with several peaks of GSI and the top two happening in September-November and between February and April. Massuttí and Morales-Nin (1995), based on the GSI, reported a spawning season from June to August for females, and from June to September for males at the island of Majorca. Zúñiga-Flores et al. (2011) concluded that the period of greatest reproductive activity in the Gulf of California coincided with the highest abundance, which, in turn, coincided with the increase in sea surface temperature. Most of these results therefore indicate that, as in the SPSPA, the greatest reproductive activity for the dolphinfish occurs in the warmest months of the summer in various regions of the world.

The spawning season for dolphinfish were defined and identified by a combination of several techniques, including sex ratio, GI and frequency distribution of monthly stages of gonadal maturation. The period of greatest reproductive activity of the dolphinfish in the SPSPA occurs just after the peak of the spawning of flying fish (between December and March), one of its preferred prey, when it becomes particularly abundant at the site, probably providing the energy the dolphinfish requires to grow and mature its gonads. This event seems to influence the migratory cycle and reproduction not only of dolphinfish but also of other species that feed on the flying fish (Hazin 2009). This would explain the decrease in reproductive activity of the species in the second half of the year, coinciding with a lower food availability and consequently greater difficulty for the maturation of the gonads. If no abrupt changes occur in the local weather throughout the year, the reproduction in the SPSPA is probably conditioned by the trophic factor.

The length size-at-50%-maturity (L_{50}) found for both males and females (70.66 and 68.60 cm FL, respectively) is within the range reported in the literature (Table 6). The fact that only 7% of males and females sampled were below the L_{50} is a positive aspect for the sustainability of the fishery conducted in this region.

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