Impact of seasonal variation on the spermatozoa reproductive cycle of Hemantura walgo (Müller & Henle, 1841) from Iraqi Marine waters.

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Impact of Seasonal Variation on The Spermatozoa Reproductive Cycle of *Hemantura Walgo* (Müller & Henle, 1841) From Iraqi Marine Waters

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Abstract
The impact of seasonal variation on the spermatozoa reproductive cycle of *Hemantura walgo* in Western North of Arabian Gulf from January 2016 to December 2017 was studied. A total 225 individuals males of *H. walga* were collected using beach net. Gonads Somatic index (GSI) was high during May (10.1) and September (9.98) and the lowest values were recorded during on February, July and October with their values were 0.85, 1.21 and 1.43 respectively. Mature males were noticed during May and September which indicates there are two reproduction periods for *H. walga* during the year. The relationship between weight and width desk of *H. walga* was also investigated and generally demonstrated positive allometric growth (b > 3) for this species Thus, this study supplies to a better understand of seasonal variation on the spermatozoa reproductive cycle of *H. walgo*. The findings of this study also are essential to the estimation, protection and management of *H. walgo* , in addition to providing plan for the improvement of conservation strategies

Keywords: Seasonal variation,. Spermatozo , *Hemantura walgo* , Iraqi Marine waters.

دراسة التاثيرات الموسمية على الدورة التكاثرية لنضج الحيوانات المنوية لذكورقوبع *Himantura walga*

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الخلاصة
تم دراسة التأثيرات الموسمية على الدورة التكاثرية لنضج الحيوانات المنوية لذكورقوبع *Himantura walga* في المياه البحرية العراقية شمال غرب الخليج العربي للفترة من كانون الثاني 2016 لغاية كانون الاول 2016، إذ جمع 225 فرد من الذكور بوساطة شباك الجر (الكرفة) . كانت اعلى دالة مناسل في شهري مايس (10.1) وايلول(9.98) وانخفضت في اشهر شباط ، تموز و تشرين الاول (0.85، 1.21 و 1.43بالتتابع) ، حيث ظهرت الذكور الناضجة في شهري مايس وشهر ايلول مما يدل على وجود فترتي تكاثر للقوبع المدروس. تضمنت الدراسة علاقة وزن الجسم وعرض قرص القوبع المدروس و اظهرت الدراسة عن نمو ايجابي (b > 3) لهذا النوع. و هكذا فان هذة الدراسة توفر فهم افضل للتغيرات الموسمبة على الدورة التكاثرية لنضج الحيوانات المنوية لذكورقوبع *H. walga* . كما أن نتائج هذه الدراسة ضرورية للتقدير والحماية والإدارة لذكورقوبع *H.*walga . بالإضافة إلى توفير خطة لتحسين استراتيجيات للمحافظة عليها.

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Introduction

The marine fisheries classify into coastal and deep sea fisheries. cartilaginous fishes has a significant role in each marine environment and arranged at the top of the predators list in marine water. These fishes comprise about 60 families, 189 genera and about 1200 species [1] They include sharks, batoids and chimaeroid fishes [2]. Later, Ghotbeddin et al. [3] described that Batoidea , in general, recognized as rays in Oman sea. Infact, rays and the sharks form the subclass Elasmobranchii. However, rays are the major group of cartilaginous fishes which covered about 26 families and 600 species. Rays are characterized by their flattened bodies, pectoral fins which are compound to the trunk, and gill slits which are found on their ventral surfaces.

The production of batoids occurs by using Internal fertilization [4]. This helps batoids to protect sperm and does not leave the eggs to be lost or consumed by predators and also maintains the essential energy for reproduction instead to be vanished to the environment [5, 6]. However, all skates and several rays are oviparous laying their eggs in leathery egg cases while other rays are ovoviviparous which they give birth to young one which grows in a womb except with no involvement of a placenta [7-9]. In spite they are classified as unwanted fishes, rays are vulnerable to extreme fishing, therefore an attempt is required to preserve and to protect these fishes in the environment. *Himantura walga* is the one frequent species collected by the fisher men in big amounts as by-catch of bottom trawl and trammel net fisheries in Arabian Gulf. It made about 21.8% of the total fish catch in Oman sea [3] and frequently caught by fishermen in Iraqi water and it used commercially as a food fish. Males of *H. walga* attain sexual maturity earlier and have smaller body than females. Therefore, it is suggested for the fishermen to employ selective fishing gears which are merely capable to mature ones [10]. The study reproduction biology of *H. Walga* will help in their conservation which cover width size, growth pattern, sex ratio, sex maturity level and width size of males and females [10] . Novariani, et al. [10] stated that the ratio for male and female were well balanced, the width-weight correlation between male and female was allometric positive. Males sexual maturity were at fully calcified stage (FC), concurrently the majority females were at immature stage (TKG 1), and finally the males reach sexual maturity earlier and have lesser body than females. From these results, it might be recommended for fishermen to use selective fishing gears which are merely capable to collect the adult rays.

In spite of the unique gonadal organization and reproductive strategies known for *H. walgo* from Iraqi extremely small is reported concerning the reproductive biology of local species. The purpose of the investigation was to show the structure of the male test is and also to describe the seasonal difference on the spermatozo reproductive cycle of *H.walgo* from Iraqi Marine waters. This study will be also very useful for planning, development and successful management policy to management organizations.

Materials and methods

Samples were collected monthly by beam trawl in western of Arabian Gulf From January 2016 to December 2017. Fish specimens were identified [2]. Disc width (DW, mm) , total fish weight (TW, gm) and total length of claspers (TL, cm) were measured [11]

TW- DW relationship was estimated as:

W = a Lb, where W is total weight and L is the Disc width (DW)

The fish sex assessed based on the claspers situated on the inside edge of the pelvic fin of the males. The claspers length-Disc width relationship was also calculated . The testes were removed and fixed in Bouin's fluid for about 15 min. Then, both sides of testes were removed. The testes were cut out into small pieces and put in the same fixative for 18– 24h. They were processed by a normal paraffin technique, cut at 5–6μm thick, stained with hematoxylin and eosin, periodic acid-Schiff (PAS)-hematoxylin, and Masson'strichrome for light microscopy examination [12- 14].

The gonadosomatic index(GSI) was calculated following [15,16].

Results

The total of 225 specimens of *H. walga* were studied at Iraqi Marine water from January 2016 to December 2017. The light microscopic structure of the testis of the *H. walga* was studied. The testes of the *H. walga* are paired, elongated organs which are situated in the peritoneal cavity somewhere they are separated from the dorsal wall with the mesorchia (plate 1). The test is formed of different sizes of lobes with color ranged from red to orange, containing many spermatocytes in a dorsoventral zonated arrangement (plate 2). The germinal papilla at the mid dorsal surface of the testicular lobe is the basis site of spermatocytes development, where mesenchymal-like cells are mainly found (plate 3) The newly formed spermatocytes at the dorsal end of the germinal zone replace the older ones, which are sequentially moved to the ventral side and are termed spermatogonia, spermatocyte, spermatid, spermatozoa, and degenerate zones . The mature and immature cells are shown in plates 3 and 4 respectively.

Seasonal reproductive activity was obvious when the gonadosomatic index (GSI) achieved a maximum in May (10.10) and September (9.97) when the temperature is moderate and a minimum in February (0.45). Ripe running males were observed in large number in May and September respectively (Figure-1).

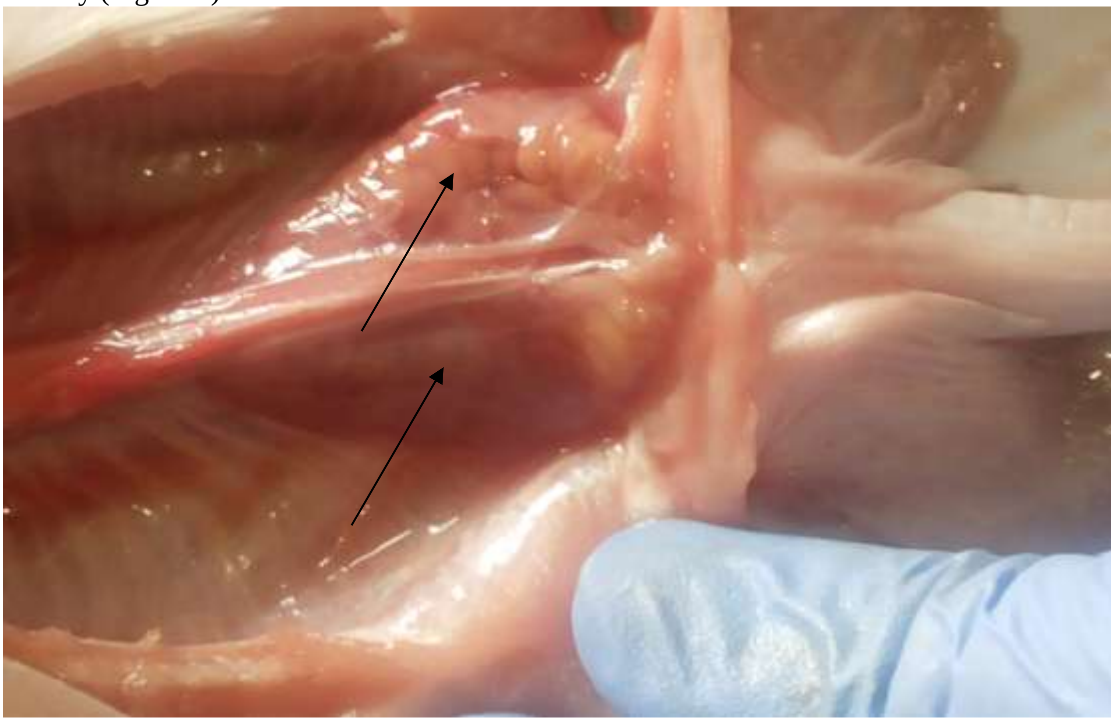

Plate 1-Location of testicles in the dorsal side of *Hemantura walgo* collected from Iraqi Marine water.

Plate 2-Mature testes of *Hemantura walgo* collected from Iraqi Marine water showing different sizes of lobes.

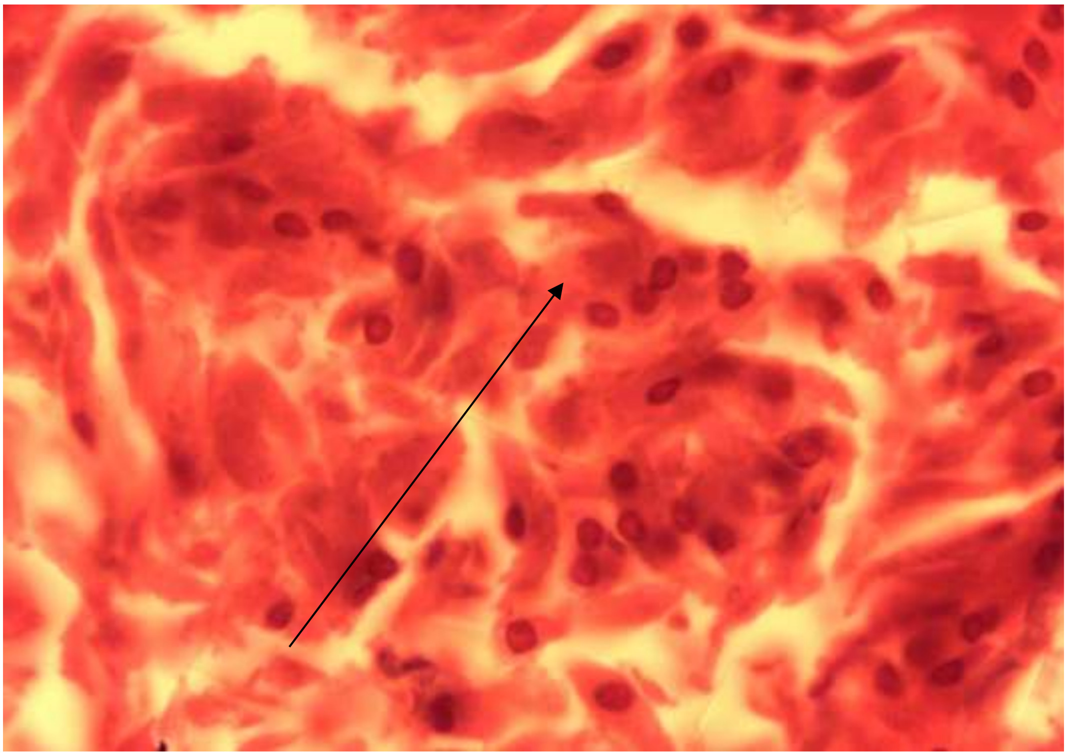

Plate 3-Mature testis of *Hemantura walgo* showing mature Spermatozoa are clearly the dominating cell type and they are released in the lobule lumen.

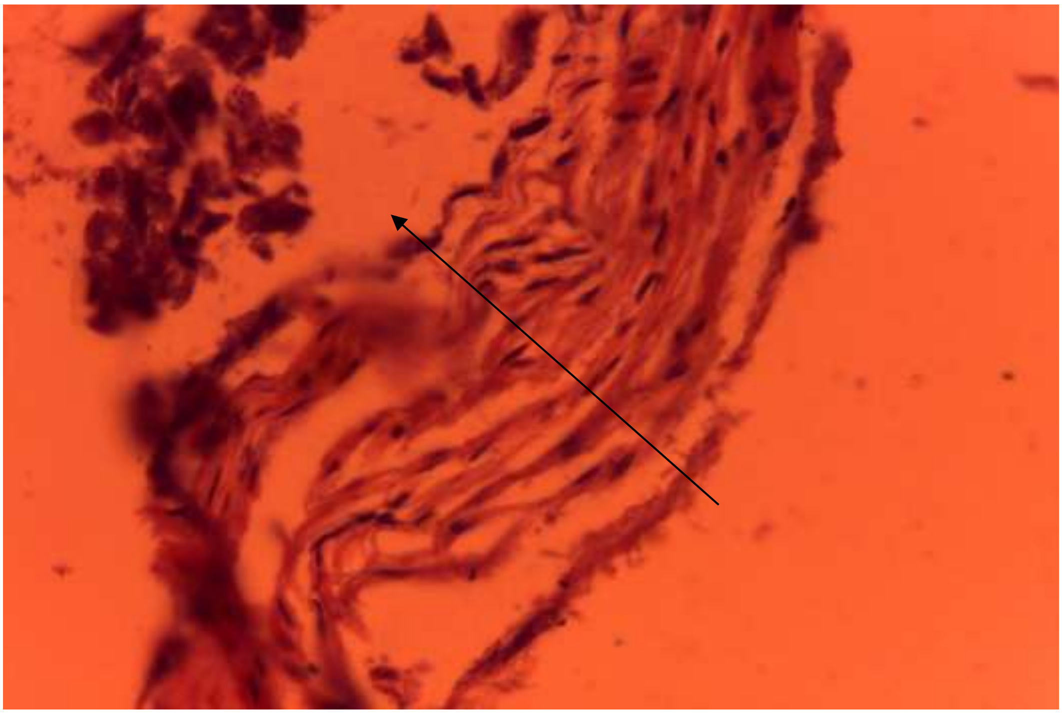

Plate 4-Mature testis of *Hemantura walgo* showing Immature Spermatozoa

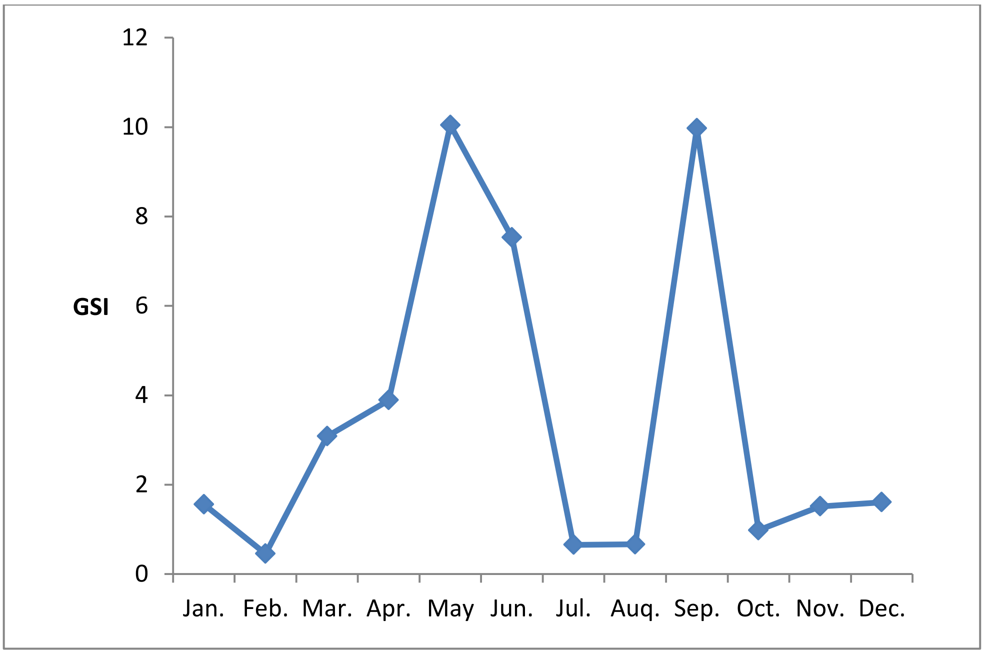

Figure 1-Variation of Gonadosomatic Index (GSI) between January 2016 and December 2016 in males of *Hemantura walgo.*

As shown from Figure 2, TW significantly related to DW of male *H. walga* and presented in the following equation:
W=0.0003 x $(DW)^{2.595}$ (n= 225, r= 0.71, $p < 0.05$)

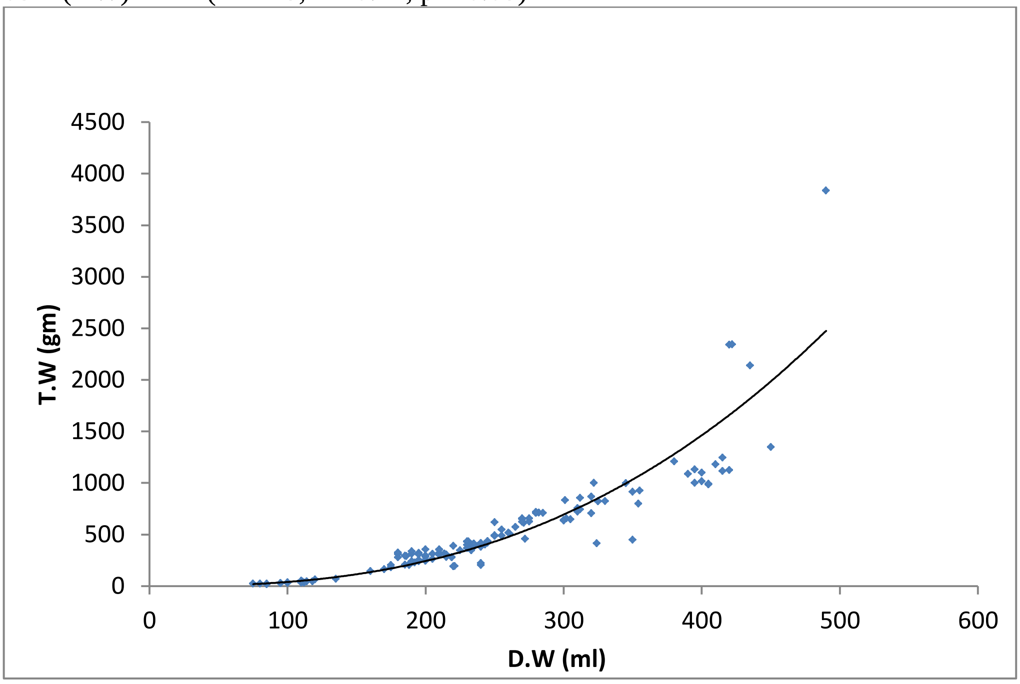

Figure 2-The relationship between total weight (T.W) in gm and Disc width (D.W) in ml) of *Hemantura walgo* for the period From January 2016 to December 2016.

H. walga showed a significant difference in their TL- DW relationship as following (Figure 3):
TL= 92.4133 + 0.2233 DW (n= 225, r= r= 0.71 , $p < 0.05$).

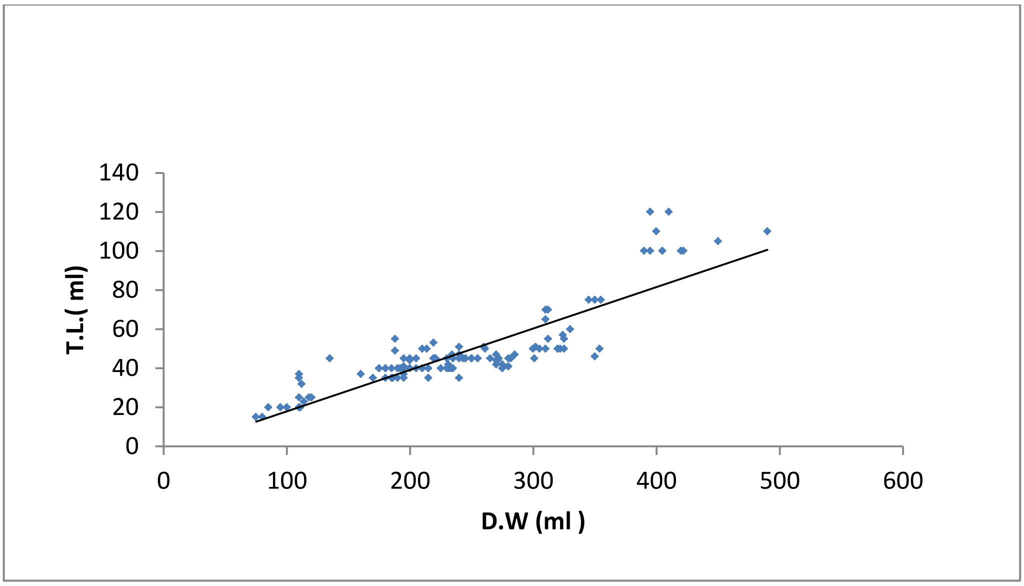

Figure 3-The relationship between claspers length (T.L.) in mm and Disc width (D.w) in mm of *Hemantura walgo* for the period From January 2016 to December 2016.

Discussion

The evolutionary improvement of the cartilaginous fishes reproductive system has been brought widely attention of many scientists. Furthermore, the way of genital tract improvement and sex demarcation in the cartilaginous fishes are close comparable to amniotes than to teleosts [17] . The zonated arrangement of the spermatocytes and the close relationship of the testis with hemopoietic epigonal organ give distinctive characteristic for cartilaginous fishes which related to testicular structure and spermatogenesis of the cartilaginous fishes [18]. In the degenerate zone, the spermatocytes decline after releasing the spermatozoa into the intratesticular duct, where they are additional transported throughout the extratesticular duct system and lastly stored at the seminal vesicle. The epithelial lining of the genital duct is a pseudostratified ciliated columnar with no muscular layer underneath; consequently, sperm are got across throughout ciliary activity [14].

The length-weight relationship based on disc length and width normally demonstrates positive allometric growth ($b > 3$) for *Himantura walga* in Malaysian waters [4] . Lim et al. [4] reported that DW shows to be the greatest variable (as standard length) for defining the length-weight relationship of the stingray species. Furthermore, this relation changes during the year depending on several factors for example temperature , season, , salinity, sex , gonadal development, spawning, fish health , size composition and food abundance and [19-22]. However, Knowledge of size/disc width and size/ weight at sexual maturity and seasonal reproductive activity in the *H. walga* may demonstrate critical to ensure effective management and conservation strategies for this species.

Males tend to attain sexual maturity in advance and have lesser body than females. Therefore, it is recommended for the fishermen to apply selective fishing gears that are merely capable to catch mature rays [14]. Therefore, this current investigation is quite important and will represent a better understanding of the reproductive morphology vulnerable stingray and this biological information should consider as a good details for useful technique of rearing, reproduction, conservation, and guard of the threatened stingray. On the other hand, deeper knowledge about this endemic species is required. Further biological and ecological issues have to be considering as demographic and stock estimation in conjunction to environmental situation are essential in order to protect this species in the Arabian Gulf

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