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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Macroscopic Examination of Cownose Rays (*Rhinoptera bonasus*)

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Abstract: The cownose ray, *Rhinoptera bonasus*, is currently listed as vulnerable and threatened species by the IUCN Red List due to overfishing and bycatch in worldwide resulted declines of the steep population declines of 30-49% in only 43 years. We aimed to present external and internal inspections for a necropsy of cownose ray with a large, rhomboid-shaped wing-like pectoral disc and compressed dorsoventrally using a specific approach to observe external and internal anatomy. The fish were obtained from a public aquarium after natural death and were dissected with four incision area to open chondrocranium, gills, pericardial cavity and pleuroperitoneal cavity. The dorsal surface of the body is completely naked and smooth, greenish-brown colored, in contrast the ventral surface of the body is white. The liver fills most of the pleuroperitoneal cavity and covers the esophagus, stomach, duodenum, valvular intestine, spleen, kidney and uterus. The valvular intestine or spiral valve is one of the largest organs of the digestive system. As a conclusion, the external and internal examination of cownose rays shed light on to the approach to organs that enable to detect abnormalities in the organs.

Keywords: Anatomy, dissection, ray, rhinopteridae.

İnek Burunlu Vatoz (*Rhinoptera bonasus*) Balığının Makroskobik Olarak İncelenmesi

Öz: İnek burunlu vatoz, *Rhinoptera bonasus*, balığı popülasyonu son 43 yıl içerisinde aşırı ve bilinçsiz avcılık nedeniyle %30-49 oranında azalmış ve IUCN Kırmızı Listesi tarafından nesli tükenme tehlikesi altındaki balıklar listesine dahil edilmiştir. Bu çalışma dorsoventral basık bir vücut şekline sahip olan inek burunlu vatoz balıklarının eksternal ve internal anatomik yapılarının incelenmesi amacıyla yapılmıştır. Balıklar, doğal ölüm sonrası bir şehir akvaryumundan laboratuvara taşınmış ve chondrocranium, solungaçlar, perikardiyal boşluk ve pleuroperitoneal boşluğun açılması amacıyla dört ana kesi alanı ile diseke edilmiştir. Balığın dorsal yüzeyi pürüzsüz, yeşilimsi-kahverengi renkli, ventral karın yüzeyi ise beyazdır. Karaciğer pleuroperitoneal boşluğun büyük kısmını doldurmakta ve özefagus, mide, duodenum, valvüler bağırsak, dalak, böbrek ve uterusu örtmektedir. Valvüler bağırsak veya spiral valf, sindirim sisteminin en büyük organlarından birini oluşturmaktadır. Sonuç olarak, inek burunlu vatoz balıklarında eksternal ve internal incelemeler sonucu elde ettiğimiz bulguların bundan sonraki süreçte organ ve dokularda bulunan anormalliklerin incelenmesine yardımcı olacağı kanısındayız.

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Anahtar kelimeler: Anatomi, diseksiyon, ray, rhinopteridae.

INTRODUCTION

The cownose ray, *Rhinoptera bonasus* (Mitchill, 1815) is a species of *Batoidea* belonging to the *Rhinopteridae* family, mainly found in the Atlantic Ocean and throughout a large part of the western Atlantic and Caribbean, from New England, the United States to southern Brazil. The cownose ray is a semi-pelagic species which found in tropical and temperate seas and estuaries (Neer & Thompson, 2005), and its population index is not known, but groups of thousands of individuals may be seen during migration (Baldassin et al., 2008). About 47 species of 13 families of sharks, and 31 species of nine families of rays, were born in captivity (Gonzalez, 2004).

The characteristics of cownose rays are sizeable maximum size, high maximum age, slow maturity, and low fecundity, similar to other elasmobranchs, which make this species vulnerable to overexploitation (Hoenig & Gruber 1990; Musick, 1999). The cownose rays do not become fully mature until reaching ~70% of their maximum size, which corresponds to six to seven years for males (>85 cm disc width) and seven to eight years for females (85-88 cm disc width) (Fisher et al., 2013; Smith & Merriner, 1987). According to the estimations, the oldest individual female observed was 21 years old, and the male was 18 years old (Fisher et al., 2013). Females typically have one generation per year, pupping in late June- early July after an 11-12 month gestation period (Fisher et al., 2013; Smith & Merriner, 1986), yet there have been documented cases of twins (Fisher et al., 2014). Because of the slow population growth and bycatch, most ray species are nearly threatened with extinction or decreasing population status (Carlson et al., 2020).

More than 50 public aquariums or zoos were established worldwide to interact between this fish species and humans in "touch tanks" specialized for cownose rays alone or with other fish. Well-designed simulations of the natural habitat of animals, naming public aquaria, are a valuable means of raising awareness on the saving of threatened species and providing animal rescue, care, rehabilitation and conservation services through education and observation (da Silva et al., 2019; Reid et al., 2013; Tlusty et al., 2013; Wolfensohn et al., 2018). Keeping these animals in captivity requires an understanding of their anatomy so that staff veterinarians can care for them or, at times, perform a necropsy. The anatomical differences of fish need different approaches to dissection techniques and necropsy. Determining and examining the gross anatomy of cownose rays would be beneficial for understanding their morphology and estimating the impact of a possible fishery upon the species and the role within the ecosystem as upper-level predators.

This study presented the external and internal anatomy of cownose rays and dissection to reach and observe the internal organs. The recommended approach will shed light on researchers, aquatic professionals, and veterinarians in diagnosing cownose ray diseases by using an appropriate dissection method.

MATERIAL AND METHOD

Specimen Collection and Measurements: *Rhinoptera bonasus* (n=6) were obtained from a public aquarium after natural death and were transferred to our laboratory under cold-chain conditions for necropsy.

Specimens were photographed with a camera (Canon, Model: 600D, Tokyo, Japan). Images were transferred to a computer, and Solidworks R17 3D CAD software (Dassault Systèmes, Waltham, MA; USA) was used to measure the specimens' external dimensions and the internal organs' size.

Dissection;

Brain: First, a circular incision was made on the skin between the eyes on the dorsal aspect of the head region to open the brain. The chondrocranium was removed by cutting with the help of scissors and a scalpel, and the brain tissue was exposed (Figure 1A, 1B).

Gills: The gill arches were exposed by removing the skin overlying the gill slits through two triangular incisions. (Figure 1C, 1D).

Pericardial Cavity and Heart: The heart was examined after opening the pericardial cavity by making a triangular incision. The pericardial cavity was opened due to an incision made along a border by palpating the cartilage cage forming the margins of the cavity (Figure 1C, 1E).

Pleuroperitoneal cavity and internal organs

An incision was made on the coracoid bar using scissors. Then, with the help of forceps, an incision was made by scalpel along the process of the scapula up to the pelvic entrance. Thus, the pleuroperitoneal cavity was opened, and the organs in this cavity were observed (Figure 1C, 1F)

RESULTS AND DISCUSSION

The present study revealed our results from external and internal examinations.

External examination: In this study, cownose ray had triangle-like wing-shaped pectoral fins began at the anterior end of the snout and ended just caudal to the pelvic fin. The head, trunk and pectoral fins were originated form the disc. Pelvic fins were located caudal to the disc in a single lobe with rounded ends. There was a single and prominent dorsal fin on the midline at the base of the tail. There were no tail fins in our specimens (Figure 1A-1F). The shape and position of the fins differ between fish species. Taskin et al., (2022) reported that two dorsal tail fins existed in guitarfish, which had the features of sharks on the posterior half of their body. Although the cownose rays and the guitarfish belong to the same subclass (Euselachii), these structures were not found in the cownose ray in this study.

The dorsal surface of the cownose ray body was greenish-brown colored, with faint dark stripes, and completely naked and smooth (Figure 1A), while McEachran et al., (2002) and Taskin et al., (2022) reported that the surface had fine denticles in guitarfish and some stingrays. The body was broad, compressed dorsoventrally.

We observed that cownose ray appeared to have a large, rhomboid-shaped wing-like pectoral disc. The disc had a mean width and length of 653.59 mm and 442.73 mm, respectively. The disc width length ratio was 1.47. However, Poulakis, (2013) stated that the average disc width on cownose rays was 712 mm. Fisher, (2010) observed the disc width ranging between 300 and 1105 mm. Also, Compagno and Last, (1999) reported that the disc width was between 610 and 2100 mm, and the disc width/length ratio was between 1.6-1.8 in adult batoids. Jones et al., (2017) also stated that the disc width varies between 346-965 mm, and the DW/DL ratio is between 0.47 and 0.66. Along with our findings, the researches we mentioned have suggested that cownose rays may have a wide range of disc width and length.

The head of the cownose ray was narrower than the body. The snout was short and consists of a pair of round lobes separated by a notch in the midline. The eyes were located lateral to the head and anteromedial to the spiracles. Spiracles were small holes behind each eyes, which were directly connected to the respiratory system. The position of these spiracles was the same as reported in the literatures by McEachran et al., (2002) and Taskin et al., (2022).

It was observed that the tail protruded separately from the body and has a prominent spiky needle on the dorsal surface of the tail just behind the pelvic fins. The tail base was cylindrical, and starting thick and gradually tapering, and its length was close to the disc's length. Tail length/disc length ratio was 1.06. Consistent with our findings, Compagno and Last, (1999) reported that the tail was slender and whip-like, and 1 to 3 or more times disc length.

In our specimens, the ventral surface of the body of the cownose ray was white. Thompson and Springer, (1965) reported that all skates and rays had five gill slits on each side of the lower surface. Similar to this literature, in the present study, on the anterior half of the lower surface, caudal to the mouth, five small slit-shaped gill openings were observed on each side (Figure 1C). At the free ends of the gill slits, small saw tooth-like projections were seen.

The mouth was in the form of a narrow transverse slit close to the anterior margin of the lower surface (Figure 1C). There were fringes on the upper edge of the mouth opening and vertical grooves on the lower edge. The nostrils were located cranial to the mouth. The nostrils stood apart at a distance of mouth opening from each other, and there were naso-oral grooves between each nostril and mouth angle. Paired cephalic lobes were observed under the head. Fisher, (2010) stated that those cephalic lobes were anterior protrusions of the pectoral fins in cownose rays.

In the macroscopic examination of the lower surface of the disc, numerous black dots were seen spread from the tip of the snout to the area between the gills. According to Murray, (1960, 1962, 1965), these dots were sensory pores that guide the cownose ray to navigate the ground while swimming and help to locate prey or creatures below the seafloor. Fisher, (2010) also mentioned these sensory pores were spread out over the head region and were intensely situated around the mouth. The mean measurements of the body components and organs were presented in Table 1.

Table 1. Mean measurements of the body parts and organs of the cownose ray.

Body part	Measurement	Body part	Measurement
Disc length	442.73 mm	Valvular Intestine length	102.12 mm
Disc width	653.59 mm	Colon length	34.72 mm
Tail length	473.04 mm	Rectal gland length	34.15 mm
Conus arteriosus length	16.11 mm	Spleen length	60.14 mm
Conus arteriosus width	8.96 mm	Spleen width	21.01 mm
Atrium length	10.74 mm	Right kidney length	70.38 mm
Atrium width	21.66 mm	Right kidney width	17.80 mm
Ventricle length	17.24 mm	Left kidney length	68.57 mm
Ventricle width	27.38 mm	Left kidney width	16.44 mm
Liver length	137.60 mm	Ovary length	29.52 mm
Liver width	162.14 mm	Ovary width	15.37 mm
Mouth opening width	84.07 mm	Oviduct length	133.47 mm
Esophagus length	55.34 mm	Uterus length	70.65 mm
Stomach length	88.47 mm	Right Epigonal Organ length	40.31 mm
Duodenum length	26.61 mm	Left Epigonal Organ length	44.62 mm

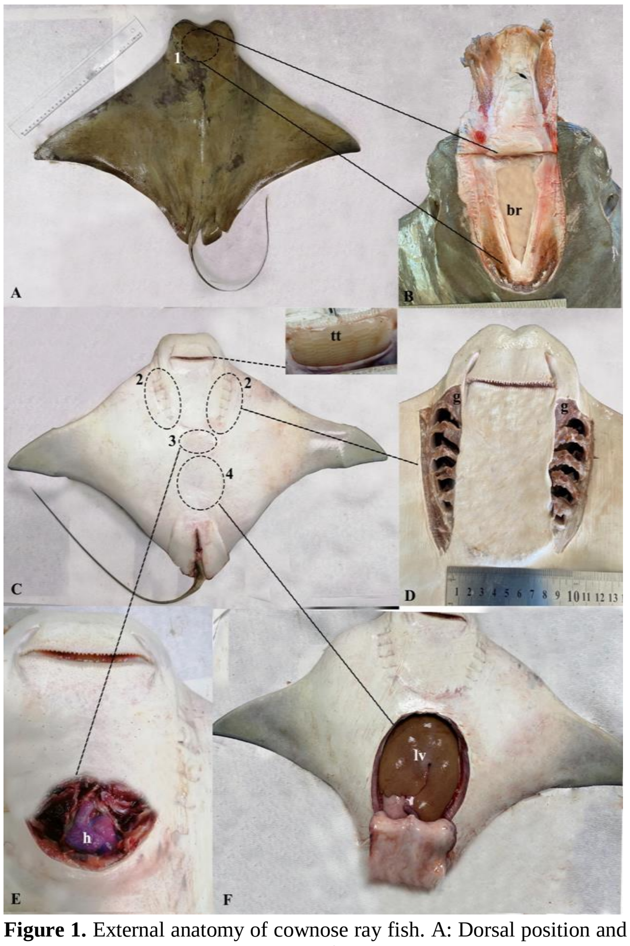

Figure 1. External anatomy of cownose ray fish. A: Dorsal position and brain location (area 1), B: Opening of the brain tissue, br: Brain; C, D: Ventral position of fish, gills location (area 2), heart location (area 3), h: Heart and pleuroperitoneal cavity (area 4), tt: Teeth, g: Gills (D); E: Heart location; F: Pleuroperitoneal cavity, lv: Liver

Internal Examination;

Brain: The brain (br) was located in the cerebral cavity between the eyes. Under the skin, the brain was covered by a cartilaginous case, chondrocranium. When the chondrocranium was opened, the brain was seen that it was covered with thin membranes and had a yellowish-white color with a soft consistency. The brain consisted of two hemispheres, with folds and grooves (gyri and sulci) on the surface (Figure 1B).

Gills: Gills (g) were located on the ventral side of the body, on either side of the head, just behind the mouth. Gills was observed on either side of the pericardial cavity, arranged in transversal leaves (Figure 1D). Compagno and Last, (1999) and Jones et al., (2017) reported that fish had five gill openings on the underside of the front half of the disc. In the same way as those researchers, we also observed five gill slits on each side of the ventral surface of the cownose rays (Figure 1D).

Heart: The heart (h) was located slightly behind the gills and just in front of the cavity. That localization differed from the guitarfish and stingrays, whose heart were located between the gill slits on both sides (Mylniczenko & Culpepper, 2017; Taskin et al., 2022). The heart of the specimen was conical in shape and consisted of four chambers called sinus venosus, atrium, ventricle, and conus arteriosus (Figure 1E).

Liver: In the specimens, the liver (lv) consisted of two lobes and the right lobe was slightly shorter than the left lobe. The liver had filled most of the pleuroperitoneal cavity and extended from the cranial wall of the pleuroperitoneal cavity to the pelvic inlet (Figure 1F). Like reported by Smith et al., (2004) the liver was the most prominent organ in the pleuroperitoneal cavity of elasmobranchs. In addition, the color of the liver was light brown in our study. Hamlett, (1999) also reported that lipids constitute 80% of liver tissue and that a healthy liver appears light brown in newly opened cadavers due to its high lipid content, suggesting the presence of high lipids in the liver of the ray in our study.

The gallbladder's placement was seen on the dorsal surface of the liver, close to the cranial edge, and between the right and left liver lobes. The gallbladder was described in the literature of Smith et al., (2004) that it was a greenish sac at the junction of the two lobes of the liver.

Oral Cavity: The oral cavity was observed between the mouth and the esophagus. To reach the oral cavity, the heart was reflected by opening the pericardial cavity. The oral cavity was distinguished from the esophagus by its pale mucosal color and the papillae on the mucosa (Figure 1C).

The teeth were large, flat plate-like, and laterally enlarged hexagons in the specimens. Teeth was seen as arranged in 10 rows on both jaws. In each row, there were three large teeth in the middle and three smaller teeth on each side (Figure 1C). Our findings were consistent with Jones et al., 2017 who they reported that cownose rays showed 5-13 tooth series in the upper jaw and 6-13 tooth series in the lower jaw.

Esophagus: The esophagus (e) was a short, wide musculomembranous tube that extended from the oral cavity to the stomach. The cranial part ran below the pericardial cavity, between the gill leaves, while the caudal part ran in the pleuroperitoneal cavity at the craniodorsal of the liver. The esophagus was found different from the oral cavity and stomach due to its dense musculature (Figure 2A, B).

Stomach: The stomach (st) had the appearance of a J-shaped bag that extended from the esophagus to the duodenum. Following the shape of the stomach, there was a descending cranial section on the left side of the median line and an ascending caudal section that passed to the right side of the median line and was connected to the duodenum (Figure 2C). Smith et al., (2004) described those descending and ascending sections as cardiac and pyloric sections, respectively.

Duodenum: As mentioned by Mylniczenko & Culpepper, (2017) and Taskin et al., (2022), we observed that the duodenum (du) was a short, curved passage between the stomach's pyloric sphincter and the valvular intestine (spiral valve) (Figure 2C).

Valvular Intestine (Spiral Valve): The valvular intestine (vi, spiral valve) was found as a caudal section of the intestines and situated to the right of the median line and extends from the duodenum to the colon. Numerous transversal folds were seen on the mucosa of the spiral valve (Figure 2C). It was suggested that those folds increase the inner surface area of the organ and allow the food to be digested to stay in the alimentary canal longer. Thus, it caused more effective digestion (Mylniczenko & Culpepper, 2017).

Colon: The colon (co) was the last part of the alimentary canal before it opens into the cloaca of the specimens. It was a short, straight, and wide tube between the spiral valve and the cloaca (Figure 2C, D). Cloaca was the part where the digestive and urogenital systems end in common. In addition, according to (Romer, 1970), rays were one had a true cloaca among fish, was present only in elasmobranchs (sharks and lobe-finned fish).

Rectal Gland: The rectal gland (rg) was a blunt-ended tube-shaped gland located on the median line, caudal to the spiral valve, opening into the colon (Figure 2C). It was reported that this gland was a special salt-secreting tissue (Ferreira et al., 2010) and involved in the osmoregulation of sodium and chloride ions (Hamlett, 1999).

Spleen and Pancreas: The spleen (sp) was observed as a brownish-red ellipsoidal organ located to the left of the median line, between the descending and ascending portions of the stomach (on the lesser curvature of the stomach). In addition, the pancreas was a light brown-pink-colored small organ consisting of two lobes located on the short edge of the duodenum (medial surface of the fold) (Figure 2A-C). According to Smith et al., (2004), the spleen and pancreas are adjacent to the pyloric stomach and in a healthy cownose ray, the spleen was bright red or maroon and the pancreas beige. Their findings were also consistent with our observation.

Kidneys: The kidneys (kd) were flattened dorsoventrally in shape and brownish red (Figure 2D). Although Mylniczenko & Culpepper, (2017) has reported that the anterior part of the kidneys is covered by the Leydig gland in males and by the uterus in females, in the present study, it was determined that the kidneys were located in front of the pelvic inlet, against the dorsal wall of the cavity and symmetrically on both sides of the dorsal aorta.

Ovaries: It was reported that in most rays, only the left ovary was developed and functional (Koob & Callard, 1991). Consistent with this, the ovaries of our specimens were located to the left of the median line, close to the cranial end of the pleuroperitoneal cavity, caudal to the stomach, and cranial to the uterus (Figure 2C). Koob and Callard, (1991) and Hamlett et al., (1999) also stated that follicles may also develop in the right ovary, but we did not observe the right ovary or any follicular development.

Oviduct: The oviduct (ovi) was found as a short canal between the ovary (ov) and the uterus (u), where the ovum was retained and transferred to the uterus (Figure 2C). Due to the development of the left ovary, we only observed the left oviduct. In like manner, as mentioned in the research of Smith and Merriner, (1986), that only the left reproductive tract was functional in cownose rays.

Uterus and Epigonal Organs: The uterus (u) was located caudal to the ovary. Similar to the oviduct, due to the development of the left ovary, only the left uterine horn was observed in the specimens. However, it was reported that the development of both uterine horns could exist in some species (Mylniczenko & Culpepper, 2017). In the present study, the uterus opened with an aperture, a vaginal opening, in common with the colon. (Figure 2C). Fisher, (2013) also reported that the colon and uterus ended at the cloaca jointly in cownose rays.

The epigonal organs (ep) were seen in pairs on either side of the median line, close adjacency to the ovary. The epigonal organs had positioned medial to the gonads and had similar color with them in fresh cadavers (Figure 2C). Similarly, Fisher, (2010) found that ovary embedded in epigonal organ and showed that these organs were in similar color and placement while Burgos- Vazquez et al., (2018) reported that the epigonal organ was located on the lateral side of each ovary.

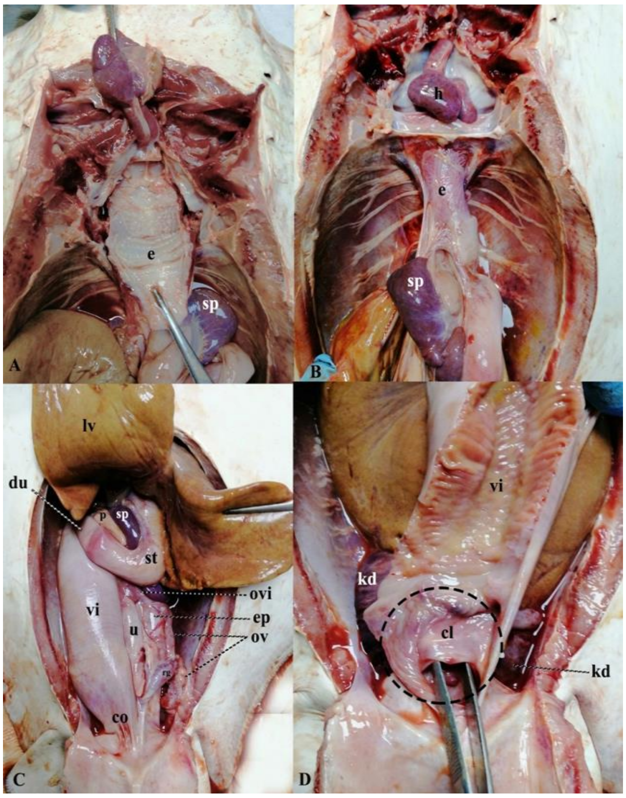

Figure 2. Internal organs of cownose ray fish, craniocaudal inspection from up to down. A: Esophagus (e), spleen (sp); B: Heart (h); C: Liver (lv), stomach (st), duodenum (du), pancreas (p), valvular intestine (vi), colon (co), rectal gland (rg), uterus (u), oviduct (ovi), ovaries (ov), epigonal organ (ep); D: Valvular intestine (vi), kidneys (kd), cloacal area (cl) penset insertion to the uterus.

CONCLUSION

As in other species, anatomical dissection and necropsy are supplementary parts of the elasmobranch examination. This manuscript was designed to provide researchers with an atlas and a dissection guide showing the anatomical positions of organs of cownose rays.

As a result, the basic morphologies of the cownose rays, which are in danger of extinction, were examined in the study. The organs' location, relations with the other organs, and characteristics were revealed. It was concluded that the study would contribute to the lack of knowledge about this species of fish and to understanding the impact of this species on other species and its role in the ecosystem. With the present study, the dissection of cownose rays will contribute to the approach to organs and the interpretation of images during imaging methods such as ultrasonography and radiography

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