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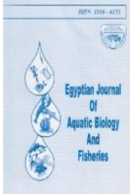

Comparative osteological studies on skull structure and shape of some coral reef fishes adapted to different feeding habits

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ABSTRACT

The present study aimed to compare the diversity of head skeletal structure and shape between some coral reef fishes with different feeding habits, by using the technology of radiography (x-ray). This study was conducted to clarify the osteological structure, size, and shape of skulls in three coral reef fishes (*Acanthurus sohal*, *Novaculichthys taeniourus* and *Fistularia commersonii*). These three coral reef fishes were collected from Hurghada, Red Sea, Egypt in 2021. Skulls were separated, prepped, and radiographed with X-rays. The pictures were reviewed by 15 radiology specialists and registrars who used DR varex workstations to assess whether the bones were visible or not. Results showed that the morphology and anatomy of skulls in coral reef fishes have undergone variation in size, shape, and degree of skeletal mobility according to specialization for various modes of feeding. The skull of herbivorous fish, *A. sohal* is a triangle in shape. The width of the skull is larger than its length. It has a small mouth with short, stout jaws provided with very tiny smooth lobed incisor teeth. The skull of carnivorous fish, *N. taeniourus* is composed of multiple movable blocks of bones. The mouth is slightly larger with robust jaw bones; the jaw has 2 pairs of stout, canine teeth positioned anteriorly on the upper and lower jaws and there's no canine in the hindmost region of the jaws. The head in planktivorous fish, *F. commersonii* consists of a long, tubular snout that constitutes almost one-third of the total body length. The snout ends in a small mouth; the lower jaw is slightly larger than the upper jaw with the absence of jaw teeth. The present study concluded that examination of the skull in some coral reef fishes with different feeding habits resulted in a significant diversification to evolutionary modifications of skull anatomy in fishes.

INTRODUCTION

Coral reefs are one of the world's most diversified ecosystems, with fish playing an important role **(Triki & Bshary, 2019** and **Maaty *et al*., 2021)**. The northern Red Sea is a unique water, has a variety of habitats, hosting some of the most productive and diverse coral reefs **(Fine *et al*., 2019; Ghallab *et al*., 2020 & 2022)**. The Red Sea inhabits more than 200 species of stony corals, about 2000 mollusk species and 1270 fish species **(Lieske & Myers, 2004)**. The Red Sea shore is a particularly important sector in Egyptian fisheries, both in terms of overall catch and the presence of a large number of commercially important species **(Mohammad, 1999).** It has long been recognized that understanding fish biology requires knowledge of their diet and eating behaviors **(Rao & Durga-Prasad, 2002** and **El-Deeb *et al*., 2016).**

Reef-dwelling fish have highly developed mouths, jaws, and teeth that are adapted to certain types of food sources found in coral reef settings. Not unexpectedly, ingesting and digesting plant matter necessitates specialized feeding structures and digestive systems that differ significantly from those of meat eaters **(Shalaby, 2017).**

Except for Mediterranean Sea, Acanthuridae is a family of bony fishes found across the tropical and subtropical world. *Acanthurus sohal* (Forsskali, 1775) is a marine species endemic to the Red Sea and the Persian Gulf, where it is considered common. It is known to be a highly territorial herbivorous fish that is aggressive, inflicting damage with its sharp scalpel-like spines at the base of caudal fin **(Froese & Pauly, 2018** and **Shalaby, 2020).**

Labridae is a successful family of roughly 575 marine perciform fishes that are most commonly found in reef settings **(Parenti & Randall, 2000).** *Novaculichthys taeniourus* feeds on benthic communities of molluscs, echinoderms, polychaetes and crustaceans. Often turns over rocks with its jaws to get to the invertebrate animals beneath **(Randall, 1983).**

The cornet fish (Family: Fistulariidae) is extremely long, cylindrical body with long tubular mouth and filamentous tail. They live in most habitats, especially coral reefs, singly or in small groups and swim close above the bottom. The cornet fish is a planktivorous, feed on zooplankton such as different stages of crustaceans and small fish **(Lieske & Myers, 2004** and **Shalaby, 2017).** The stomach contents of *F. commersonii* were studied in Mediterranean Sea and showed that the cornet fish feeds preferably on zooplankton in young stage and feeds on schooling species living in the water column in large one (**Bariche *et al*., 2009).**

Fishes have a skull that is morphologically complex and highly dynamic **(Ferry-Graham & Lauder, 2001).** Adult teleost fishes have cranium musculoskeletal system comprising of roughly 60 linked skeletal components moved by a nearly equal number of muscles **(Aerts, 1991).** Fish skeleton anatomy is thought to be exceedingly complicated, with wide variation in size, form, and grade of skeletal motion among species **(Lauder, 1985** and **Sanford & Wainwright, 2002).** Although the bulk of feeding-related anatomy is cranial, certain species use non-cranial structures for nutrition, such as the scales on the body's flanks **(Southall & Sims, 2003).**

Therefor the present study aimed to provide comparative description on the osteology of skull structure according to different feeding habits between some coral reef fishes (surgeon fish, *A. sohal*, wrasse fish, *N. taeniourus* and cornet fish, *F. commersonii*) inhabiting Egyptian Red Sea water at Hurghada, for attempting to correlate the relationship between structure and shape of skull and their different modes of feeding strategy.

MATERIALS AND METHODS

1. Studied fish species (Fig. 1)

A total of 11 specimens; 5 surgeon fish, *Acanthurus sohal* (Forsskali, 1775)**;** 4 wrasse fish, *Novaculichthys taeniourus* (Lacepede, 1801) and 2 cornet fish, *Fistularia commersonii* (Ruppell, 1838) were collected from Hurghada, Red Sea, Egypt during the period from January to December 2021. Long lines nets were the primary way of catching fish. Fishes were examined fresh or preserved in a 10% formalin solution for taxonomic identification according to **Randall (1983)** and **Lieske & Myers (2004).** The following measurements were recorded for each fish: body length (TL), head length (HL); head width (HW); eye diameter (ED); upper jaw length (UJ) and the lower jaw length (LJ). The heads of the collected specimens were separated from the rest of the body and then, the following studies were carried out.

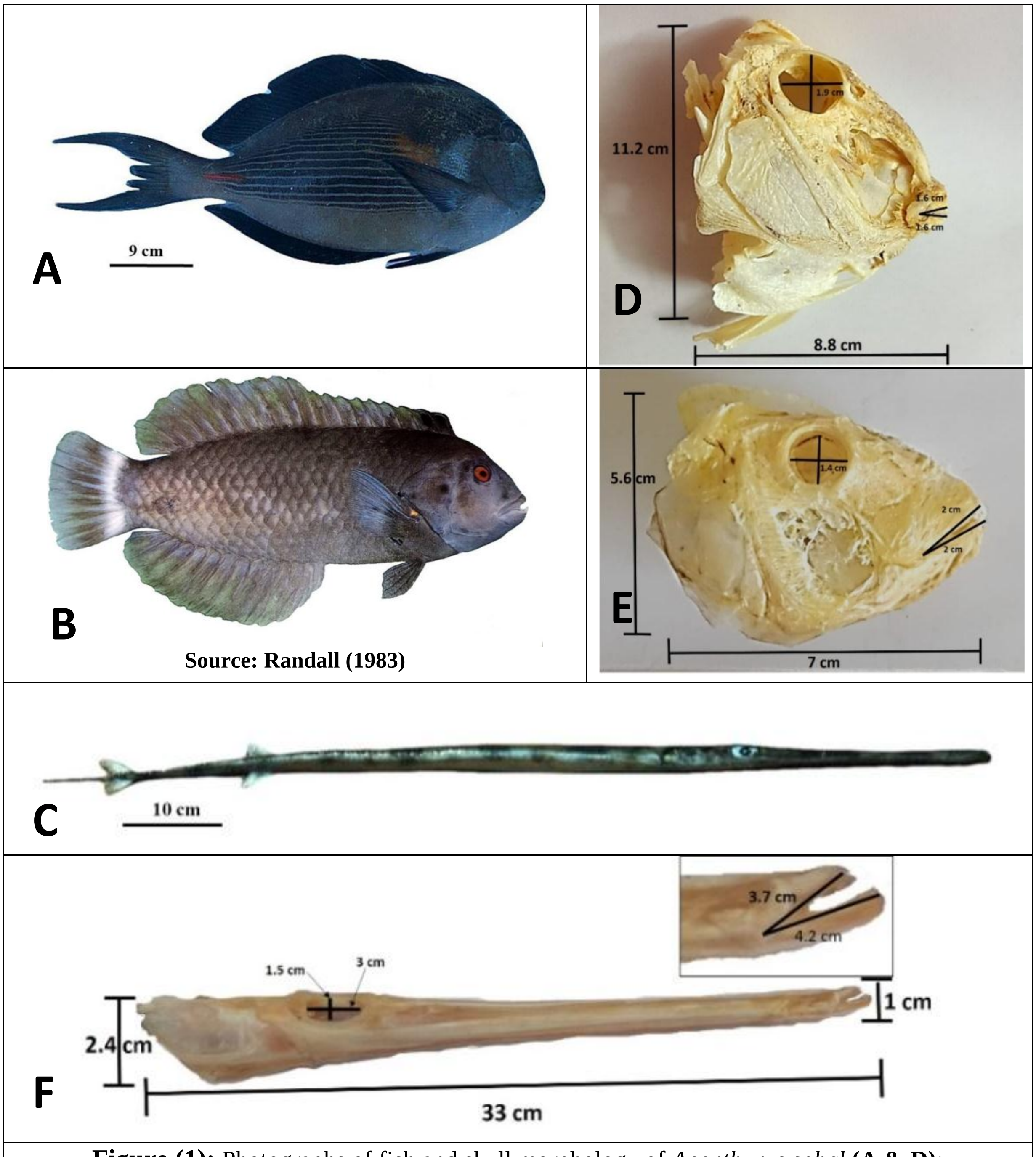

Figure (1): Photographs of fish and skull morphology of *Acanthurus sohal* **(A & D)**; *Novaculichthys taeniourus* **(B & E)** and *Fistularia commersonii* **(C & F)**.

2. Morphological and osteological studies:

For studying the morphology, each skull was soaked into the water for at least 1-2 hour to rehydrate the tissues. The skull transferred into 5 % potassium hydroxide (KOH) + 0.5 % hydrogen peroxide solution (H_2O_2) for 4-5 hours to remove the soft tissues from the skull. The solution was replaced every 1 hour. Then, specimen transferred into 10% solution of H_2O_2 in 0.5% KOH solution for 12 hours, and it should be exposed to light and air to dry.

The prepared skulls were examined to identify the morphological features then photographed using a digital camera (Kodak, 14 megapixels) and described according to **Taylor & Van dyke (1985)**. For more studies, the skulls have been provided for the purpose of exposing to radiography (x -ray)–the radiography device (DR varex).

RESULTS

1. Skull morphology:

The skull of *A. sohal* (as an herbivorous surgeon fish) is triangle in shape. The skull width (11.20±0.2 cm representing 23.33% of the total length) is larger than its length (8.8±0.17cm, representing 18.3% of the total length). It has small mouth with short, stout jaws (1.6±0.10cm, each representing 18.18% of the head length) provided with very tiny smooth lobed incisor teeth. The orbit is rounded, recorded 1.9±0.11cm in diameter and represented 21.5% of the head length (**Table, 1** and **Fig. 1B).**

The skull of *N. taeniourus* (as a carnivore wrasse fish) is composed of multiple movable blocks of bones (7±0.3 cm represents 23.30% of the total length). The mouth is a slightly large with robust jaw bones; the jaw (2±0.4 cm each represent 28.6% of the head length) has 2 pairs of stout, canine teeth positioned anteriorly on the upper and lower jaws and there's no canine at the hindmost region of jaws. The orbit is also rounded as in surgeon fish but 1.4±0.02 cm in diameter and represent 20% of the head length (**Table, 1** and **Fig. 1D).**

The head of *F. commersonii* (as a planktonic cornet fish) consists of a long tubular snout and constitutes almost one third of the total body length (31.4% of TL). The snout, which ending in small mouth, lower jaw (4.2±0.2 cm represent 12.7% of the head length) is slightly larger than the upper jaw (3.7±0.15 cm represent 11.20% of the head length). Jaws teeth of *F. commersonii* are absent. The orbit is oval its length (3±0.02 cm represent 9.10% of the head length) (**Table, 1** and **Fig. 1F).**

Table (1): Morphometric characters of three coral reef fishes, collected from Egyptian Red Sea water at Hurghada, during the period from January to December 2021.

Characters		Fishes		
		A. sohal	*N. taeniourus*	*F. commersonii*
Head length	Average ±SD	8.8±0.17	7±0.3	33±0.5
HL/TL	%	18.3%	23.30%	31.4%
HW	Average ±SD	11.2±0.2	5.6±0.09	2.4±0.04
HW / TL	%	23.33%	18.67%	2.28%
UJ	Average ±SD	1.6±0.1	2±0.04	3.7±0.15
UJ/ HL	%	18.18%	28.6%	11.2%
LJ	Average ±SD	1.6±0.1	2±0.04	4.2±0.2
LJ/ HL	%	18.18%	28.6%	12.7%
OD	Average ±SD	1.9±0.11	1.4±0.02	3±0.0215
OD / HL	%	21.5%	20%	9.1%

TL: Total length; **HL**: Head length; **HW**: Head width; **UJ**: Upper jaw**; LJ:** lower jaw; **OD**: Orbit diameter

2. Skull anatomy:

<u>2.1. Skull of *Acanthurus sohal*</u>

The upper jaw of ***A. sohal*** is made up of the toothed premaxilla (pmx), which is attached to the maxilla (mx) below and behind it. The lower jaw is made up of dentary (dt), articular (art), and angular (an) ossifications. Dentary is significantly bigger than articular and angular. Behind the lower jaw is the quadrate (qu), which has a significant dip posteriorly. An elongate bone behind the top end of the maxilla most likely represents the palatine.

The hyomandibula (hy) and preopercle (pop) are well maintained in the suspensorium. The hyomandibula is wide dorsally where it joins the skull and tapers ventrally where it meets the anterodorsal margin of the preopercle. The preopercle has a fairly curved shape. The distinction between the opercle (op) and the subopercle (sop) is hazy.

The top margin of the parasphenoid is clearly preserved in the lower part of the orbit, but its ventral projection is less well marked but appears to be of considerable depth. Below the parasphenoid is a slightly upwardly curved arch of bone that is most likely the remnants of the infraorbital series of bones, and more anteriorly is an unclear hint of a big lachrymal (la).

The lateral ethmoid (le) and the rather well preserved frontal (fr), which has some surface sculpturing and sensory canals, define the top area of the orbit. The sphenotic (sph) bone is visible under the posteroventral portion of the frontal, but the other otic bones are obscure. The top supraoccipital (soc) margin and the posterior curvature of the skull are clearly visible (**Fig. 2 A-C).**

2.2. Skull of *Novaculichthys taeniourus*

The skull's skeleton of ***N. taeniourus*** (carnivorous wrasse fish) showed that the premaxilla (pmx) attached to the skull and can be protruded; upper and behind this bone is the maxilla (mx), which is connected to it. The upper jaw connected with the lower jaw ventrally by the quadrate bone (qu). Behind the maxilla is an elongate bone that probably represents of the palatine (Pl). The maxilla frequently rotates to assist in pushing the premaxilla forward into a protruded posture. The lower jaw is a composite structure comprised of dentary, articular, and angular ossifications.

The hyomandibula (hy) and preopercle (pop) of the lower jaw are well preserved ventrally surrounding its quadrate joint. The hyomandibula (hy) and preopercle (pop) are both intact. The hyomandibula articulates with the skull dorsally and ventrally with the anterodorsal border of the preopercle. The preopercle (pop) bone is curved and located anterior to the opercle (op) bone, which has more ossifications than the opercle and the subopercle (sop) bone. The boundaries between the opercle (op) and the subopercle (sop) are hazy. The opercle (op) is a large bone that covers the gill area.

The anterior area of the orbit is well defined by the lachrymal (la) bone; the upper part of the orbit is well defined by the lateral ethmoid (le), and the frontal (fr) bone is generally well maintained. The posterior curve of the skull is clearly seen with the upper margin of the supraoccipital (soc) which has less ossification (**Figs. 2 D & E).**

2.2. Skull of *Fistularia commersonii*

The rostrum of ***F. commersonii*** (planktivorous cornet fish) is made of the premaxillary bone, with the suspensorium (pterygoid series, palatine, and quadrate) and neurocranium stretched to form a long tube with a small mandible and premaxilla at its apex. The maxilla follows the premaxilla. The lower jaw is composed of only dentary bone. At the end of dentary, small angular and articular bones are present. The toothless mouth is quite small (only 12 % of the head length). The formation of the long tube occurred due to the elongation of the nasal and frontal bones from the skull and the dentary and quadrate bones from lower jaw (**Figs. 2 F-H).**

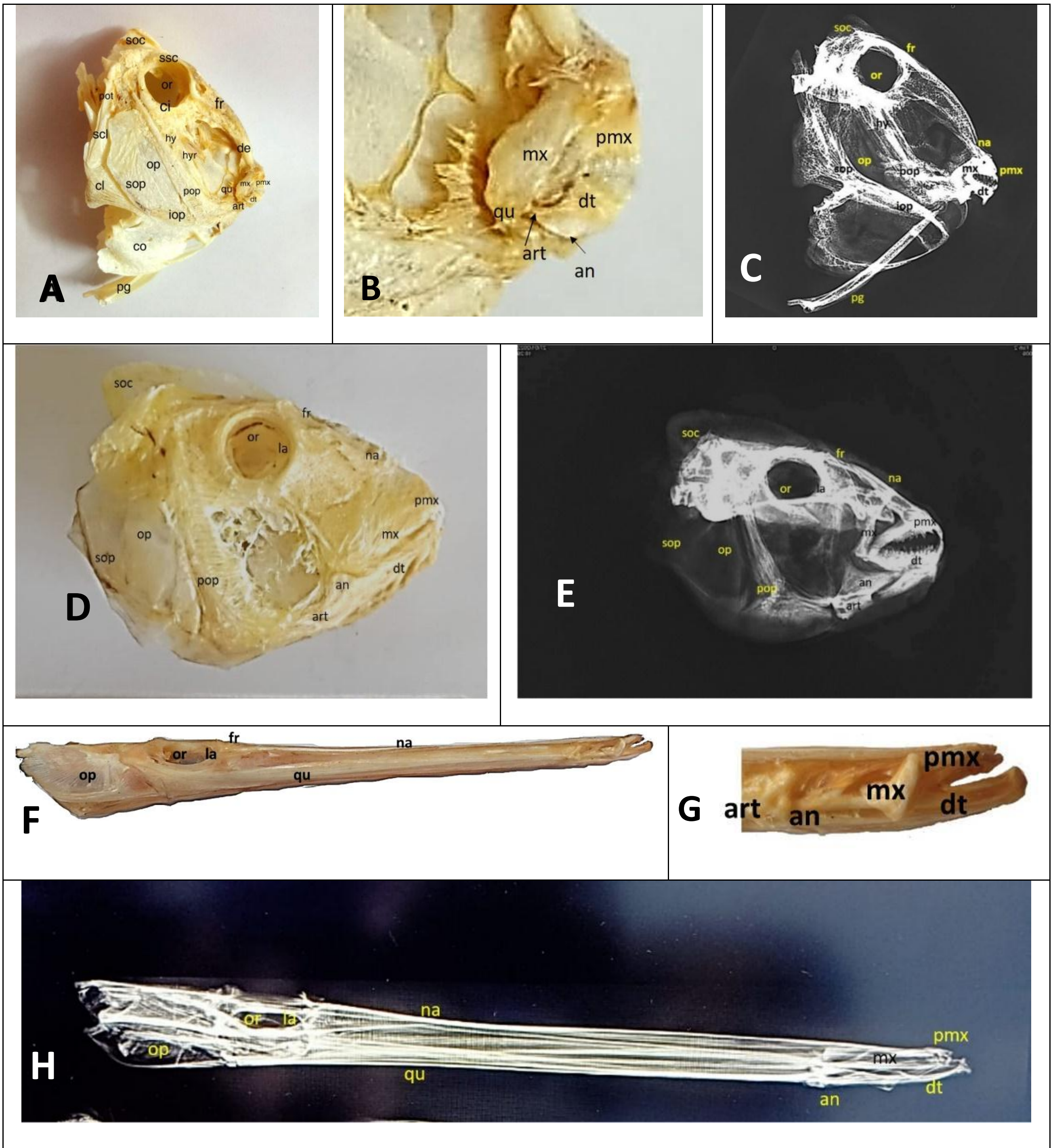

Fig. (2): (**A**) Skull of *A. sohal*, (**B**) Enlargement of jaws bones, (**C**) Radiography of the skull. (**D**) Skull of *N. taeniourus*, (**E**) Radiography of the skull. (**F**) Skull of *F. commersonii* (**G**) Enlargement of jaws bones (**H**) Radiography of the skull.

an: angular; **art**: articular; **dt**: dentary; **fr**: frontal; **hy**: hyomandibula; **iop**: interopercle; **la**: lacrimal; **le**: lateral ethmoid; **mx**: maxilla; **na**: nasal; **op**: opercle; **or**: orbit; **pg**: pectoral girdle; **pmx**: premaxilla; **pop**: preopercle; **qu**: quadrate; **soc**: supraoccipital; **sop**: subopercle.

DISCUSSION

The morphological examination of the skull in the studied three species (*A. sohal, N. taeniourus* and *F. commersonii*) exhibited the presence of great modifications related to their function during specific feeding by using their jaws as to adapt to catch their food.

Acanthurus sohal was herbivorous fish that mostly eat filamentous benthic algae and debris (**Dias *et al.*, 2001** and **Francini-Filho *et al.*, 2010)**. *Novaculichthys taeniourus* is predatory wrasse feed mainly on invertebrates found in reef environment, small fishes and crustaceans (**Sanderson, 1990** and **Westneat, 1990 & 1991).** The cornet fish *F. commersonii***,** feed preferably on zooplankton sucked from the water column and sometimes in different stages of crustaceans and small fishes (**Lieske & Myers, 2004** and **Shalaby, 2017).**

These various feeding techniques have an effect on the structure, shape, and size of the cranium of studied fish species. Because of the utilization of suction, the introduction of jaw protrusion, and other kinematic aspects of the skull, feeding in fishes is far more difficult than just opening the mouth and shutting it over a prey item. Although lifting the top jaw is a crucial aspect of mouth opening, the majority of the increased gape between the upper and lower jaws is produced by moving the lower jaw ventrally around its quadrate joint **(Schaefer & Rosen, 1961** and **Lauder, 1982).**

Short, strong jaws for a forceful bite are used by surgeon fish, *Acanthurus sohal*, to remove chunks of coral or algal mat that they pull off of reefs. The eating and feeding habits of surgeon fishes have been studied **.** Incorporating information on the eating mechanism and emphasizing the extreme shorten the biting jaws. Most Acanthuridae species are algivorous, with *Zebrasoma* spp. and *Acanthurus* spp. consuming different micro-algae and macro-algae **(Montgomery *et al.*, 1989; Purcell & Bellwood, 1993** and **Wainwright & Bellwood**, **2002)**.

Following the extreme small jaws and mouth-opening, the gill-bases of surgeon fishes are more anterior and flatter than those of predatory fish, increasing the volume of the oropharyngeal cavity and therefore allowing greater space for food-accumulation. The expansion of the basal section of the gill-arches extends the "oral basket," allowing for the temporary accommodation of a large number of food particles, which are later sorted by taste receptors and either rejected or eaten. These findings are consistent with earlier research by **Fishelson & Delarea (2013).**

A line of serrated teeth runs down the premaxillae and dentary of examined species in the current study. *Acanthurus* has flat, lateral teeth with noticeable dentition. This observation was similar to that obtained by **Wainwright & Bellwood (2002).** The eating activity of *A. sohal* is characterized by strong teeth. The role of the "retention plates," which are intimately linked to the teeth on both jaws, becomes vital during eating. It appears that in this case, when the teeth merely conduct early perforation of the algae, the plates shut, keeping the algae tight, while the fish shakes its head to remove the needed piece from the source. These findings are consistent with **Fishelson & Delarea (2013).**

The present study showed that, jaws of carnivorous fish, *N. taeniourus* are beset with one or two outer uniserial canine teeth. They are specialized for catching and holding of the prey and preventing its escape out of buccal cavity. Teeth are conical in shape, neither enlarged canines nor a crushing dentition because these fish don't eat the mollusk food, which need crushing the shells. These modifications are in conformity with those of carnivorous fish described by **Khalaf-Allah (2013)** and **Shalaby (2017).**

In the present study, the skull in carnivorous wrasse fish**,** *N. taeniourus***,** has multiple movable blocks of bones that function in prey capture and show flexibility and diversity in how they capture prey. The mouth is large known for its behavior of turning over rocks and preying upon the exposed crabs and other invertebrates. These findings were consistent to that obtained by **Randall *et al*. (1990).**

Wrasses fish exhibited great variability in feeding-related features, and the various components of the cranium exhibited a wide range of diversity. For example, the movement of the premaxilla, dentary, and moveable maxilla leads to jaw protrusion, which is used in suction by wrasse fishes to grab food hanging in mid-water, despite considerable changes in cranial kinematics across species. The capture of connected prey requires some changes in feeding kinematics and the utilization of biting. These findings were consistent with those indicated by **Ferry-Graham *et al*. (2002)** and **Wainwright *et al*. (2004).** Wrasses may use biting and grasping activities to acquire prey rather of relying entirely on inertial suction. Wrasse jaw has been interpreted on several occasions as being specialized for biting as mentioned by **Tedman (1980).**

In the present work, the mouth of cornet fish, *F. commersonii* is tubular, long (trumpet-like) and ending with small oblique mouth opening which very efficient for sucking up. Similar results were detected by **Shalaby (2017)**. **Bond (1996)** mentioned that, tubular mouth was found in most of the plankton-feeders, in which the method of feeding may be by suction, as in the case of trumpet fish and other Syngnathiform fish.

In the present study, jaws teeth in plankton feeder, *F. commersonii* are absent since there is no need for suction of plankton. Similar results were detected by **Bond (1996)**.

The skull morphology of plankton feeders, *F. commersonii*, varies the most, and its rostrum is an extended sensory organ that senses prey density via electroreception. Rostral protraction is another intriguing and immediately discernible exception from usual fish skull architecture. Elongation of the rostrum can be beneficial during nourishment in a variety of ways, including reaching or paralyzing prey and sensory adaptations for prey monitoring **(Allen & Riveros, 2013).**

Elongation can occur in the premaxilla, (e.g., Istiophoridae, Xiphiidae), the maxilla (e.g., Lepisosteiformes), the neurocranium/suspensorium (e.g., syngnathiformes), and/or the mandible (in many taxa), or as in this study dentary and quadrates of the lower jaw, frontals and nasals of the skull. The rostrum of billfishes is entirely made up of premaxillary bones **(Fierstine, 1990)**. Gars attack prey with enlarged rostra, as well as elongation of the dentary in the lower jaw and the premaxilla and frontal bones in the skull **(Lauder 1980** and **Porter & Motta, 2004).**

By comparing our findings on cornet fishes to those of **Weisel (1973)** on paddle fishes, we discovered that they are both plankton feeders with rostrums that sense food density via electroreception. However, while their toothless mouths are relatively huge, paddle fishes have tiny mouths in comparison to cornet fishes. In both of paddle and cornet fishes, the gill rakers are incredibly lengthy, filtering away plankton as they travel through the water.

In syngnathiform fishes reported by **Bergert & Wainwright (1997)**, the rostra are likewise extended, but with the suspensorium and neurocranium, it elongates to create a long tube with a little mandible/premaxilla at its apex. Syngnathiform's elongated tube-mouths are advantageous for swiftly rotating the head, pushing the jaws toward prey, and exerting high suction pressures. These findings are congruent with the current cornet fish investigation.

CONCLUSION

The present study concluded that examination of the variation in the structure, size and shape of the skull in studied coral reef fishes (herbivorous fish, *A. sohal*; carnivorous fish, *N. taeniourus* and planktivorous fish, *F. commersonii*) with their different feeding habits resulted in significant diversification to evolutionary modifications of skull anatomy in fishes.

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