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**Report of the** 

# REGIONAL WORKSHOP ON THE IDENTIFICATION OF DEEP-SEA CARTILAGINOUS FISHES OF THE SOUTHEASTERN ATLANTIC OCEAN

Cape Town, South Africa, 23–26 June 2015

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#### **Preparation of this document**

This report gives a full account of the regional workshop on the "Identification of Deep-sea Cartilaginous fishes of the Southeastern Atlantic Ocean" which was held at the Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa, from 23 to 26 June 2015.

The workshop was funded by the project "Support to the implementation of the International Guidelines on the Management of Deep-Sea Fisheries in the High Seas" (GCP/GLO/323/NOR).

It was aimed at improving the capabilities of scientists from countries facing the South and Central Eastern Atlantic Ocean, in the identification of a range of deep-sea cartilaginous fish species caught in the region by using an established species identification process and through the correct use of the identification tools developed by the FAO Deep-sea Fisheries and FishFinder Programmes.

The main objectives of the workshop were to improve the participants' knowledge on (a) the anatomical features and taxonomy of the orders of deep-sea sharks occurring in the Southeastern Atlantic Ocean, (b) the use of the taxonomic keys included in the reference text material (e.g. FAO Catalogues and Identification Guide) and (c) the processing and identification methodologies on a selection of deep-sea shark, batoid and chimaera specimens. Moreover, a biological data collection protocol was illustrated thus allowing for a better reporting of cartilaginous fish specimens.

The report provides the record of the presentations, lectures and the practical sessions held during the workshop.

**FAO**. 2016. Report of the Regional Workshop on the Identification of Deep-sea Cartilaginous Fishes of the Southeastern Atlantic Ocean. Cape Town, South Africa, 23–26 June 2015. FAO Fisheries and Aquaculture Report No.1141 Rome, Italy.

#### Abstract

The regional workshop on the "Identification of Deep-sea Cartilaginous fishes of the Southeastern Atlantic Ocean" was held at the Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa, from 23 to 26 June 2015. It was attended by 15 participants from a wide range of countries and fields of expertise, including taxonomy and bio-ecology of cartilaginous fishes. The general objective of the workshop was to improve the capabilities of scientists from countries facing the South and Central Eastern Atlantic Ocean in the identification of a range of deep-sea cartilaginous fish species caught in the region. The participants were introduced to the anatomical features and taxonomy of the orders of deep-sea cartilaginous fishes occurring in the Southeastern Atlantic Ocean, to the use of the taxonomic keys included in the reference text material (e.g. FAO Catalogues and Identification Guide) and to the methodologies of processing and identifying a selection of specimens. Moreover, a biological data collection protocol was illustrated thus allowing for better reporting of shark specimens.

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# Abbreviations and acronyms

ABNJ	areas beyond national jurisdiction
EAF	ecosystem approach to fisheries
EEZ	exclusive economic zone
FAO	Food and Agriculture Organization of the United Nations
IUCN	International Union for Conservation of Nature
RFMO/As	regional fisheries management organizations and arrangements

#### **Report of the training workshop**

#### **Opening of the workshop**

1. Within the framework of the FAO Deep-sea Fisheries Programme a regional workshop on the "Identification of Deep-sea Cartilaginous fishes of the Southeastern Atlantic Ocean" was held at the Department of Agriculture, Forestry and Fisheries, Cape Town, South Africa, from June 23 to 26, 2015.

2. The workshop was attended by 15 scientists representing a wide range of countries facing the South and Central Eastern Atlantic Ocean (Appendix 1).

3. The workshop was opened by Edoardo Mostarda, FAO consultant for the Deep-sea High-Seas Fisheries and FishFinder Programmes, who welcomed the participants and thanked the staff of the Department of Agriculture, Forestry and Fisheries for making the Department's facilities available and for collecting the specimens during the demersal fishing surveys.

4. Subsequently, Edoardo Mostarda asked each participant to introduce themselves and presented the agenda of the workshop (Appendix 2). It was explained that the general objective of the workshop was to train scientists from countries bordering the Southeastern Atlantic Ocean in the identification of deep-sea cartilaginous fishes and sample processing.

5. The participants were told that a number of specific objectives were to be reached by the end of the workshop, such as:

- Learning the anatomical features of the deep-sea species belonging to the main shark, skate and chimaera *taxa* occurring in the Southeastern Atlantic Ocean.
- Using the taxonomic keys included in the reference text material (e.g. FAO catalogue and identification guide).
- Processing and identifying a number of deep-sea cartilaginous fish species.
- Understanding how, and being able, to: (i) take basic measurements; (ii) take biological samples, i.e. for age, growth and molecular studies; (iii) take photographs; and (iv) preserve specimens in the field.

#### **Background information**

#### The work carried out by FAO to improve the identification of marine organisms

6. The first presentation was delivered by Edoardo Mostarda. He clarified the role of FAO in conservation and fisheries management issues, the importance of systematics in fisheries management, and the activities of the FAO FishFinder Programme.

7. It was explained that, in order to achieve food security for all, FAO deals with all the practices that provide a source of food, income and livelihood to people around the world. In particular, it was highlighted how important fish is as a source of food, providing 20 percent of animal protein to about 3 billion people with a yearly production of more than 150 million tonnes. However, the depletion of numerous stocks underlines the growing need for improved management including policy, planning, data collection, research, laws, enforcement and regional cooperation.

8. The work of the FAO Fisheries and Aquaculture Department centres on the "sustainable management and use of fisheries and aquaculture resources". The department strongly endorses the development and implementation of an ecosystem approach to fisheries (EAF) including mainstreaming biodiversity and ecosystem concerns in fisheries management. Greater awareness is placed on the effects of fishing also on non-target species and habitats, and the department is also committed to ensuring biodiversity conservation. Fishery-induced changes can influence the structure and functioning of marine ecosystems and generate a loss of productivity and stability of entire ecosystems.

9. It was stressed that proper identification of species is an important component of fishery resources management and biodiversity preservation. That is, it is difficult to manage what is unknown and to detect changes in the species composition of an area if no clear picture is available of what is present.

10. Two examples of the implications of erroneous management actions based on inaccurate species identification were presented. In the 1970s, fishery managers in the Unites States of America planned to use data on the Brazilian Spanish mackerel to manage the population occurring in their waters. However, Collette *et al.* (1978) showed that the Brazilian population represented a distinct species (*Scomberomorus brasiliensis*). The species found in United States waters is *S. maculatus*, a much smaller fish, reaching a maximum size of 77 cm fork length compared with 125 cm in *S. brasiliensis* and it matures at a smaller size (Collette and Nauen, 1983). Managing the United States species with the Brazilian data could have resulted in unnecessary economic impacts on the fishing industry and inadequate conservation measures for *S. maculatus*.

11. The yellowfin tuna was known by 27 names until Gibbs and Collette (1967) showed that all referred to a single worldwide species. Much time and money has been unnecessarily spent gathering meristic, morphometric, anatomical, distributional, and life-history data on tunas in each area where they were found. After Gibbs and Collette's findings, managers have been able to use data on food or larval development from other regions to aid them in their work.

12. The main issues that make identification of fish in fisheries often difficult were addressed. First, species descriptions are published in a large variety of specialized journals usually unavailable to the fishery workers, and many of them are based on a few museum specimens rather than on representative samples of fresh material. Moreover, fishery data collectors have to identify many species at the same time and to do this they need multi-specific identification guides, which are often lacking. Finally, nomenclature is often not available for most regions or countries, and the fishery workers are confronted with a wide, confusing and often contradictory spectrum of names, most of them of dubious value and furthermore information on distribution, habitat, biology, and fisheries of individual species are all too often lacking entirely.

In order to improve the quality of fishery statistics, FAO developed (in 1972) the Species Identification 13. and Data Programme (now called FishFinder Programme). It was stressed that, after 40 years, there is still a need to improve marine resources identification in many regions where the percentage of catches reported to the species level is still low. The objectives of the programme are to improve the identification of marine organisms of actual and potential interest to fisheries by developing and disseminating tools to facilitate species identification in fisheries and by providing a global and coherent system of scientific and common nomenclature. Priority is assigned to resources of major commercial importance or threatened species and to developing countries/regions facing difficulties in species identification. The main activities of the programme are: securing the best and most up-to-date information (calling upon knowledgeable specialists in taxonomy); compiling information on species distribution in order to produce distribution maps: drawing reliable and accurate illustrations of marine organisms and their anatomical details; and to producing and distributing, through different media, species identification information for fishery purposes. The principal outputs of the programme are publications such as species catalogues, regional catalogues, field guides, pocket guides, CD-ROMS, synopses, fact sheets available on the Web, species distribution maps and scientific illustrations.

#### Current knowledge on cartilaginous fishes biodiversity

14. Subsequently, Dr. Dave Ebert presented an overview of the diversity of cartilaginous fish species both at a global and regional level, e.g. Southeastern Atlantic Ocean.

15. The public's perception of sharks often conjures up images of a large fearsome toothy predator, with its large dorsal fin cutting through the water surface. However, the reality is that sharks come in a variety of sizes and shapes, from the whale shark (*Rhincodon typus*), the world's largest fish, to the dwarf pygmy sharks (*Squaliolus* spp.), and these enigmatic fishes occupy most marine, and some freshwater, habitats. In addition, the batoids and chimaeras, along with the sharks, form a distinctive group of fishes collectively referred to as

the chondrichthyans. There are more than 500 species of sharks, along with almost 650 batoid and 50 chimaera species, bringing the overall total to about 1 200 species of sharks and shark-like fishes.

16. In terms of identification, the diversity of sharks and their relatives has increased markedly in the past decade with more than 200 new species described since 2000. Since 2007, 147 new species have been described, an average of 24.5 new species per year. This represents almost 20 percent of all shark species that have been described, and compares with about 200 species that were described in the previous 30 years (1970–1999). Most of the new species discovered in the past decade have come from the Indo-Australian region, followed by the southern African and western North Pacific regions. Many of these newly discovered species are deep-sea inhabitants, mostly at depths in excess of 200 m. The discovery of new species combined with the taxonomic resolution of species complexes has led to a scientific renaissance in chondrichthyan taxonomy, and it highlights the importance of taxonomy for their proper identification and management.

17. The Southeastern Atlantic Ocean, e.g. FAO Fishing Area 47, has a relatively high diversity of cartilaginous fish species. In fact, about 205 species are known to occur in this region. Of these, 61 species are endemic to Southern Africa.

18. Data on the conservation status of Southern African chondrichthyans, according to the International Union for Conservation of Nature (IUCN) were presented. Of the 205 species occurring in the region, 8 species have not been assessed, 62 species are Data Deficient, while 57 species are either Vulnerable, Endangered or Critically Endangered.

19. Fifty sharks, 20 batoids and 8 chimaeras occur in the deep-sea, representing about 7 percent of all known chondrichthyan species. However, these numbers are likely to be an underestimation since there are several undescribed species and a few species complexes under investigation that should eventually resolve the systematics of those species.

20. The Southeastern Atlantic deep-sea shark fauna is represented by 6 orders, 17 families, 30 genera, and at least 50 species. The most species-rich group of deep-sea sharks are the Squaliformes with at least 32 species, representing about 64% of the deep-sea shark species in this region. Most are in the families Etmopteridae (9 species) and the Centrophoridae and Somniosidae with 6 species in each family. The Carcharhiniformes are represented by 10 species, 8 of which belong to the family Scyliorhinidae. All of the other shark orders only have four or fewer species representatives.

21. The deep–sea batoid fauna is represented with 3 orders, 4 families, 10 genera, and at least 20 species. The most specious group of deep–sea batoids are the skates (Rajiformes) that have two families (Arhynchobatidae and Rajidae), 8 genera and 18 species represented.

22. The deep-sea chimaera fauna has representatives of two families (Chimaeridae and Rhinochimaeridae), and includes five genera, and at least 8 species.

#### The work carried out by FAO on deep-sea fisheries

23. Dr. Mostarda presented the work of FAO on deep-sea fisheries, the approach used to develop the identification tools, and an overview of the species catalogue and identification guide.

24. Most fisheries take place in each state's exclusive economic zone (EEZ). This zone stretches from the coastline out to 200 nautical miles and over this area each state has special rights, including the right to harvest fish resources. The state is also responsible for monitoring its fisheries and ensuring the sustainability of fishing operations. Beyond the 200 nautical miles boundary, the ocean regions do not fall under the jurisdiction of any one state and are known as the Areas Beyond National Jurisdiction (ABNJs). The vessels fishing in these areas must comply with the measures adopted by the states under which they are flying. In addition, the ABNJs of a number of regions are managed by inter-governmental organisations known as Regional Fisheries

Management Organizations (RFMOs) committed to managing the fish resources occurring in their area of competence in a sustainable manner.

25. Fisheries in the ABNJs target two main ecological groups, the pelagic and highly migratory species (e.g. tunas, etc.) living in the upper water column layer and the demersal species living in close contact with the seafloor and often at high depths, that is the deep-sea. Most of the latter species form large spawning and feeding schools on or near topographic features such as seamounts, ridges, banks and canyons.

26. In the Southeastern Atlantic Ocean ABNJ, the Southeast Atlantic Fisheries Organisation (SEAFO) is the RFMO committed to ensuring the long-term conservation and sustainable use of all living marine resources, and to safeguarding the environment and marine ecosystems in which the resources occur. In SEAFO's area of competence bottom fisheries, e.g. bottom trawl, longlines and pots, target four bony fish species (*Hoplostethus atlanticus, Beryx* spp., *Dissostichus eleginoides*, and *Pseudopentaceros richardsoni*) and one crab species (*Chaceon erytheiae*).

27. In recent years, the deep-sea bottom fisheries have been a source of growing concern because unmanaged and uncontrolled deep-sea bottom fisheries are unsustainable for deep-sea fish species since they target a number of long-lived, slow-growing, or late-maturing species that can only sustain low fishing rates. Furthermore, the sustainable harvest of these fish species is difficult to achieve considering the limited data and information available on these species to support management decisions. Moreover, some of the fishing gears used to catch these species such as bottom trawls and longlines, contact or are likely to contact the seafloor and can produce significant adverse impacts on the ecosystems. These deep-sea ecosystems are populated by organisms such as cold water corals, sponges and other invertebrates which form complex biogenic structures which other species use as habitat, food or shelter from predation. These organisms are also typically slow growing and long lived and are very vulnerable to disturbance. The benthic ecosystems that include organisms with these characteristics are referred to as Vulnerable Marine Ecosystems (VMEs) and the organisms as VMEs indicators. Other vulnerable deep-sea organisms include cartilaginous fishes.

28. The result of these concerns has been expressed by a number of calls to take action. In particular, the United Nations General Assembly (UNGA) adopted Resolution 61/105 which called on high seas fishing nations and RFMOs to take urgent action to protect VMEs from destructive fishing practices including bottom trawl fishing, in areas beyond national jurisdiction. Moreover, in 2007 the Committee on Fisheries, a global forum convening every two years at FAO in Rome and during which governments discuss major international fisheries and aquaculture problems and issues and recommendations are addressed to governments, regional fishery bodies, NGOs, FAO and international community, requested that FAO assist states and RFMOs in sustainably managing deep-sea fisheries.

29. FAO responded to this call by drafting the FAO International Guidelines for the Management of Deepsea Fisheries in the High Seas (2009) developed with the involvement of countries, the fishing industry, NGOs and scientists and researchers.

30. The Guidelines' objective is to facilitate and encourage the efforts of States and regional fisheries management organizations and arrangements (RFMO/As) towards a sustainable use of marine living resources exploited by deep-sea fisheries and the protection of the marine biodiversity that these ecosystems contain.

31. The guidelines, among other recommendations, attach particular importance to the collection and reporting of reliable and accurate catch data, which, in an EAF context, should not be limited to the target resources but also take into account the associated species affected by the fishing activity.

32. With the aim of providing support on specific issues for the implementation of the Guidelines, FAO developed the programme on Deep-sea fisheries in the high seas. One of the programme's components is aimed at developing improved tools and identification guides for deep-sea vulnerable species to be used onboard deep-sea fishing vessels.

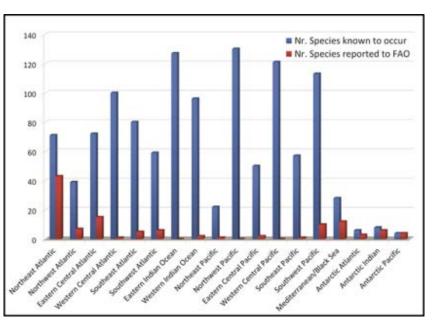
33. A workshop on "Deep-sea Species Identification" held in Rome in 2009 organized in response to the need for a strategy for the development of appropriate deep-sea species identification tools for fishery purposes, recommended that a series of identification guides be developed for certain vulnerable groups of

species affected by bottom gear, with an initial focus on three of the most impacted groups: cartilaginous fishes, corals and sponges.

34. The need to improve the identification of deep-sea sharks, batoids and chimaeras is clear. A comparison of the number of caught species reported to FAO in the last decade to the number of known species per region reveals the fact that only a small portion of these is reported to the species level (Figure 1).

#### Figure 1

# Number of deep-sea cartilaginous fish species reported to FAO and known to occur in FAO fishing areas



35. The Indian Ocean was chosen as the first region for developing the identification tools and two species catalogues and an identification guide were produced (Ebert, 2013, 2014; Ebert and Mostarda, 2013). Subsequently, a workshop aimed at improving the capabilities of scientists from the region in the identification of deep-sea sharks was organized in 2014 (FAO, 2015).

36. Finally, the structure of the identification guide and species catalogue was illustrated to the participants. The former publication includes accounts for 37 shark, 9 batoid and 4 chimaera species, selected as being the more difficult to identify and/or commonly caught. It also includes short accounts for 24 species that are easily misidentified with the more common species occurring in the region. It was stressed that the guide is not a simple collection of factsheets, but it includes a guide to the orders to all cartilaginous fishes, a guide to the families for the Squaliformes and a guide to the genera for the Rajiformes.

37. The most characteristic species are included with short accounts displaying their main characters as captions and summarized information on their distribution, size, depth range and other important details. The less characteristic species, that can be easily misidentified with a number of similar species, have a more comprehensive species account, where high importance is given to the characters that separate them from the other ones.

38. The Species catalogue includes 10 orders, 23 families, 45 genera, and 78 species of cartilaginous fishes occurring in the Southeastern Atlantic. It provides accounts for all orders, families, and genera and all keys to *taxa* are fully illustrated. A species representative account of each genus is also provided and includes: valid modern names and original citation of the species; synonyms; the English, French, and Spanish FAO names for the species: a lateral view and often other useful illustrations; field marks; diagnostic features; distribution, including a GIS map; habitat; biology; size; interest to fisheries and human impact; local names when available; a remarks section; and literature. The volume is fully indexed and also includes sections on

terminology and measurements including an extensive glossary, a list of species by FAO Statistical Areas, a glossary, and a dedicated bibliography.

#### Use of identification keys included in the guide and technical terms

39. Dave Ebert illustrated the basic terminology used to describe a shark, skate and chimaera specimen and the taxonomic keys included in the identification guide.

40. Sharks (Selachii) are an easily identifiable group of more than 500 species worldwide characterized by having 5–7 gill openings and the pectoral fins not attached to the head. Most shark species have 2 dorsal fins, with the exception of the members of the order Hexanchiformes, which have a single dorsal fin and also 6 or 7 gill openings. A number of species, which are mostly deep-sea, have spines in front of either one or both dorsal fins. The spines can be very prominent, and thus visible, or difficult to detect. Sharks also have paired pelvic fins and an anal fin, which is lacking in the order Squaliformes, one of the most important groups of deep-sea sharks. As in all cartilaginous fishes, male sharks have copulatory organs present on the pelvic fins for internal fertilization of eggs.

41. Batoids (Batoidea) are flat-bodied cartilaginous fish including more than 650 species worldwide. Most batoids have 5 ventral gill openings, but the Hexatrygonidae have six. Most batoids have a disc-like body, with the exception of the guitarfishes and sawfishes. Many batoid species have developed their pectoral fins into broad flat wing-like appendages, while the anal fin is absent. The eyes and spiracles are located on top of the head. Batoids have a ventrally located mouth and can considerably protrude their upper jaw (palatoquadrate cartilage) away from the cranium to capture prey. Bottom-dwelling batoids breathe by taking water in through the spiracles, rather than through the mouth as most fishes do, and passing it outward through the gills.

42. Chimaeras (Holocephali) are separated from the sharks and batoids by many important anatomic characters. Externally they have no spiracle, only one external gill opening on either side, a symmetrical tail and their gill filaments are free at the tips like those of bony fishes.

43. The taxonomic keys included in both the identification guide and species catalogues are known as dichotomous keys. These can be structured in different ways but are usually characterized by a series of questions arranged in "couplets". Each time a question is answered, the user is directed to the new question-couplet. This continues until the name of the species (or other taxon) is given. The structure of the key is such that each question is actually like a tree branch that has smaller branches proceeding from it.

44. The key to the Orders of deep-sea cartilaginous fishes of the Southeastern Atlantic Ocean was illustrated and the main characters included in this key were explained.

#### Session 1 – Practicum: testing each participants' knowledge of the technical terms

45. After the morning lectures, the practical work began in the laboratory of the Department of Agriculture, Forestry and Fisheries set up with a large table over which a number of specimens were placed (Plate 1).

46. The participants were asked to observe a specimen of shark (*Centroscymnus coelolepis*), skate (*Dipturus doutrei*) and chimaera (*Chimaera notafricana*) and to fill out the first encounter forms provided for each taxonomic group (Appendixes 3, 4 and 5).

47. As for the shark specimen, most participants were able to record its characters correctly. However, some of them encountered difficulties in determining whether it had an anal fin, dorsal-fin spines, precaudal

Ebert, D.A. 2013. Deep-sea Cartilaginous Fishes of the Indian Ocean. Volume 1. Sharks. FAO Species Catalogue for Fishery Purposes. No. 8, Vol. 1. Rome, FAO. 256 pp.

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FAO. 2015. Report of the Regional Workshop on the Identification of Deep-sea Cartilaginous Fishes of the Indian Ocean. Albion, Mauritius, 10–13 June 2014. FAO Fisheries and Aquaculture Report No. 1091. Rome. 41 pp.

48. With regard to the skate specimen, major difficulties were experienced by most participants when they had to determine the presence or absence of the orbital, scapular, nuchal, malar, alar, tail and interdorsal thorns. The instructors clarified the latter and other characters, such as the hardness or softness of the snout and the tail shape.

49. Finally, as most participants were not familiar with the chimaera specimen, the instructors described its main characteristics.

#### Plate 1

#### Practical session.



#### Session 2 – Lecture: review of the terminology and major groups of deep-sea Chondrichthyans

50. In consideration of the difficulties experienced by the participants during the practical session, it was decided to review the basic external terminology in use for sharks, batoids and chimaeras. xx. Subsequently, Dave Ebert provided an overview of the cartilaginous fish *taxa* included in the identification guide. Particular attention was given to the taxonomic keys to the orders, families and genera.

51. The Hexanchiformes were the first order that was presented. The members of this order have 6 or 7 gill slits and 1 dorsal fin. Two families occur in the Southeastern Atlantic, the Chlamydoselachidae and Hexanchidae, with 2 species each.

52. The Squatiniformes and Pristiophoriformes are two very characteristic orders. Members of the former have a ray-like flattened body, a terminal mouth and pectoral fins not attached to the head, while the Pristiophoriformes are characterized by a saw-like elongated snout. Each order is represented by one species in the Southeastern Atlantic.

53. The Lamniformes are a diverse group of sharks with 5 gill slits and two dorsal fins. Specifically, they are characterized by having no nictitating eyelid and a ring intestinal valve as opposed to the Carcharhiniformes that have both the nictitading eyelid and a spiral intestinal valve. The Lamniformes are represented in the Southeastern Atlantic by 4 families and 4 species.

54. The main characteristics of the Squaliformes were highlighted. Members of this order have 5 to 7 gill slits, no anal fin, a non-ray like body shape, and a short and not saw-like shape of the snout. Seven families of Squaliformes occur in the Southeastern Atlantic.

55. The bramble sharks (Echinorhinidae), are represented in the Southeastern Atlantic by one species, *Echinorhinus brucus*. This is a large and sluggish shark with a stout cylindrical body, large and thornlike denticles, a broad flat head and small dorsal fins with no spines and it can be separated from all other Squaliformes families by the position of the first dorsal fin which originates posteriorly to pelvic-fin origins.

56. The rough sharks (Oxynotidae) are represented in the Southeastern Atlantic Ocean deep-sea by one species (*Oxynotus centrina*). These sharks have a compressed body, triangular in cross-section, very high dorsal fins with spines, and rough skin.

57. The dogfish sharks (Squalidae) include two genera, *Cirrhigaleus* and *Squalus*. The genus *Cirrhigaleus* is only known from very few records of *Cirrhigaleus asper* from the Eastern Cape Province, South Africa. The genus is most common in the Southwestern Indian Ocean. The two genera differ in the length of the secondary lobe of the anterior nasal flap, which is larger in *Cirrhigaleus* than in *Squalus*. The importance of taking photographs of the underside of the head was stressed, in order to be able to compare the distance from snout tip to nostril with that from nostril to front of upper labial furrow. This characteristic is important for separating species within the genus *Squalus*.

58. The gulper sharks (Centrophoridae) are comprised of two genera, *Deania* and *Centrophorus*, with 3 species, each reported from the Southeastern Atlantic Ocean. These species tend to have large green eyes, upper teeth relatively broad and bladelike, lower teeth that are low and wide, and dorsal fins with spines. The genus *Centrophorus* is one of the most taxonomically complex and confusing elasmobranch groups. For example, three species, *C. granulosus*, *C. acus* and *C. niaukang*, described from different regions were found only recently to be different life stages of a single species, i.e. *C. granulosus* (White *et al.*, 2013<sup>1</sup>). The two genera can be separated by looking at the snout length.

59. The lanternsharks (Etmopteridae), characterized by the presence of black markings and light organs on the flanks, caudal fin and underside of body, are represented in the Southeastern Atlantic Ocean deep-sea by 2 genera, *Centroscyllium* and *Etmopterus*, with 1 and 8 species, respectively. The best characteristics used to identify these species are the shape, length and position of the markings (when visible), and the shape and arrangement of the dermal denticles along the dorsal surface of head, trunk and caudal peduncle.

60. The sleeper sharks (Somniosidae) are a relatively small family represented in the Southeastern Atlantic Ocean deep-sea by 5 genera and 6 species. Most of the species are of moderately large size and their uniform dark brown to black coloration makes them look alike. The characteristics that are used to separate the different species are the shape of the teeth, the presence or absence of small dorsal-fin spines, the length of the snout and the shape of the lower teeth.

61. The kitefin sharks (Dalatiidae) are a group of deep-sea sharks with more oceanic habits. Four genera and 4 species occur in the deep waters of the Southeastern Atlantic Ocean. Most of the species are caught occasionally.

62. The main features of the order Carcharhiniformes were illustrated. Particular attention was given to the species of the genus *Apristurus*, belonging to the catsharks (Scyliorhinidae). These species are of very difficult identification and are characterized by a flattened and spatulate head, very long labial furrows, rearsited dorsal fins and a uniform coloration. They can be divided into two species groupings (*Apristurus brunneus*-group and *A. spongiceps*-group) based on a number of differences. The characteristics of a species representative of each grouping were presented. The characteristics of the other most common and important catshark species were illustrated.

63. The most important deep-sea batoid fish *taxa* were presented. The Southeastern Atlantic fauna includes two orders, the electric rays (Torpediniformes) and skates (Rajiformes). The former order is represented by a newly described species, *Tetronarce cowleyi*. This species can be easily identified by the fact that it has an oval disc, a massive and stout tail and pelvic fins with a single lobe.

64. The skates include two families, the Arhynchobatidae and Rajidae. These can be easily distinguished by looking at their snout which is soft and flabby in the members of the former family and rigid and stiff in the species belonging to the latter one. The Arhincobathidae are represented by one species, *Bathyraja smithii*,

<sup>&</sup>lt;sup>1</sup> White, W.T., Ebert, D.A., Naylor, G.J.P., Ho, H.-C., Clerkin, P., Veríssimo, A. & Cotton, C.F. 2013. Revision of the genus *Centrophorus* (Squaliformes: Centrophoridae): Part 1—Redescription of *Centrophorus granulosus* (Bloch & Schneider), a senior synonym of *C. acus* Garman and *C. niaukang* Teng. *Zootaxa*, 3752: 35–72.

while the Rajidae include seven genera and seventeen species. The guide to the Southeastern Atlantic genera and main species were illustrated.

65. Finally, the main characteristics of the species belonging to the order Chimaeriformes were presented. Two families (Chimaeridae and Rinochimaeridae) and five genera occur in the Southeastern Atlantic. These can be easily separated by looking at their snout which is short and blunt in the members of the former family and elongated and tapering in the latter one. The Chimaeridae accounts for 4 species, including the recently described *Chimaera notafricana*, and three *Hydrolagus* species currently under revision. The Rhinochimaeridae are represented by 4 species belonging to three genera.

#### Session 2 – Practicum: identification of deep-sea cartilaginous fish species

66. The participants moved to the laboratory and were divided into 4 groups of 4-5 people. Each group gathered around one of the tables equipped with a measuring board and laboratory tools such as scissors, forceps, scalpels and callipers (Plate 2).

#### Plate 2



#### Practical session.

67. The participants were tasked with the identification of a number of species. The species collected were: *Pliotrema warreni, Squalus acutipinnis, Squalus* cf. *mitsukurii, Centrophorus squamosus, Deania calcea, Centroscyllium fabricii, Etmopterus sculptus, Centroscymnus coelolepis, Zameus squamulosus, Apristurus saldanha, Apristurus microps, Holohalaelurus regani, Scyliorhinus capensis, Bathyraja smithii, Cruriraja hulleyi, Dipturus pullopunctatus, Dipturus doutrei, Dipturus springeri, Rajella leoparda, Rajella caudaspinosa, Hydrolagus* sp., Chimaera notafricana, Rhinochimaera africana.

68. Each group selected a shark, skate and chimaera specimen and wrote down, on the first encounter forms, each species' characteristics. Then, based on the latter, they were asked to use the identification guide and to follow the different steps necessary to reach a correct identification at the species level.

69. The instructors made sure that the participants used the keys to the orders and families included in the identification guide.

70. Each species was also measured (total length), and sexed based on external examination. The instructors clarified every doubt expressed by the participants regarding particular external anatomical features, e.g. denticles (shape and arrangement), thorns (placement on dorsal surface of skates), teeth (shape and counting of the rows), and dorsal-fin spines (origin and length).

71. By using the identification guide, and with the support of the instructors who clarified some characters whose interpretation was subjective, each group was able to reach a correct identification of a high number of specimens. Among the shark species, the identification of the Leafscale gulper shark *Centrophorus squamosus* and Gulper shark *C. granulosus* was relatively easy, with all participants being able to reach the family level

by looking at the different shapes of the upper and lower teeth. The former species was identified by observing the rounded pectoral-fin rear tip together with the extremely high and leaf-shaped denticles, as opposed to the low denticles and pointed pectoral-fin rear tips of *C. granulosus*.

72. Other easily identifiable species were the Sixgill sawshark *Pliotrema warreni* with its characteristic saw-shaped snout and six gill slits; the Izak catshark *Holohalaelurus regain* with its typical coloration of intricate reticulations and u-shaped markings; and the Yellowspotted catshark *Scyliorhinus capensis* with its pattern of numerous bright yellow spots and brown bands on the back.

73. However, the participants needed the help of the instructors when trying to identify a number of species whose diagnostic characters were more difficult to detect.

74. Problematic shark species were the ones belonging to the family Etmopteridae. The main issue was the recognition of the black markings and light organs on the flanks and tail that are characteristic of this family. Moreover, most participants struggled to detect and interpret other characteristics such as the arrangement of the denticles and the length of the flank and caudal markings. The dogfish *Squalus acutipinnis* and *S. cf. mitsukurii* were also difficult to identify at the species level due to their slight differences in the snout length, shape of medial nasal barbels and coloration.

75. Other challenging species were the ones belonging to the family Rajidae, despite the inclusion in the identification guide of a key to the families and genera. The latter key was successfully used by the participants to reach the genus level but some characters had to be better explained by the instructors. For example, the legskates are characterized by anterior pelvic-fin lobes elongated, limb-like, and separated externally from the posterior fin-like lobes by a deep notch, whereas all other skate species have always a membrane connecting the anterior and posterior pelvic-fin lobes. The only legskate species available for identification, *Cruriraja hulleyi* was examined but most participants needed to compare its pelvic fins with the ones of other skate species to discern their different shape. With regard to the *Dipturus* species, *D. doutrei* and *D. springeri* were mostly identified at the genus level while *D. pullopunctatus* was identified at the species level due to its characteristic dorsal coloration. Finally, the participants needed the help of the instructors in the identification of the *Rajella* species.

76. After the practical session, the groups were asked to meet and review the work done during the day and to present the characteristics of one of the identified species to the other participants. One representative of each group was nominated to present these results (Plate 3).



Plate 3 **Presentation of the day's work by the participants.** 

Session 3 - Lecture: methodologies of biological data collection in the field

77. Dave Ebert made a presentation on the methodologies of data collection from sharks, skates and chimaeras in the field. Each participant was provided with a set of guidelines edited by Dave Ebert and Paul Clerkin summarizing these methodologies (see Appendix 6).

The following topics were covered:

#### Length data

78. Length is probably the most important parameter to measure because it correlates with maturity, age and it can also help in the identification of the species under investigation. It is also easy for people to take in the field or estimate.

79. For sharks, the most important measurements are the total length (from the snout tip to the tip of the tail), the pre-caudal length (from the snout tip to the upper caudal-fin origin, taken when the caudal fin is damaged) and the fork length (from the snout tip to the intersection of the lower and upper caudal-fin lobes). The fork length should not be taken from species that have a poorly defined fork. For skates, the disc width is used as a standard measurement, while for chimaeras the chimaera length (from the snout tip to the posterior end of the supracaudal fin, excluding the caudal filament) and pre-caudal length (from the snout tip to the anterior end of the supracaudal fin) are taken.

#### Sex determination, maturity ranking and reproduction parameters

80. Male sharks, batoids and chimaeras all have tubular processes called claspers arising from the trailing margins of the pelvic fins. Claspers are the male's reproductive organs, used to internally fertilize the females. Female cartilaginous fishes do not have claspers, just smooth edged pelvic fins.

81. Male shark specimens can be ranked as **new-borns** showing an umbilical scar; **juveniles**, with short and uncalcified flexible claspers; **adolescents**, with claspers extended but still not calcified; and **adults** with elongated and calcified claspers.

82. Male batoids can be ranked as **juveniles** with very small claspers, shorter than pelvic fins, **adolescents** with extended but not calcified claspers and **adults** with elongated and calcified claspers.

83. Male chimaeras can be ranked as **neonates** with a very soft body and undeveloped sexual organs, **juveniles** with small and flexible claspers, **adolescents** with claspers beginning to elongate but still flexible and **adults** with elongated and stiff claspers.

84. Female shark specimens can be ranked as **new-borns**, showing an umbilical scar; **juveniles**, with ovaries that lack differentiation and with an oviductal gland not differentiated from the uterus; **adolescents**, with smaller ovaries, some differentiation of oocytes but lacking mature oocytes, oviductal gland undeveloped and uterus narrow; **mature**, with large yolky oocytes and shell/oviductal gland distinctly differentiated from the uterus; **pregnant**, with pups in the uterus and **spent**, with a developed uterus and the eggs still regenerating.

85. Female batoids can be ranked as **juveniles** with undeveloped ovaries, shell gland and uterus, **adolescents** with small ovaries without vascularization but differentiated and **adults** with vascularized ovarian eggs, differentiated shell gland and pendulous uterus

86. Female chimaeras are different from other cartilaginous fishes in having two oviducts, each of which exits the body independently of the vent. They can be ranked as **neonates** with a very soft body and undeveloped sexual organs, **juveniles** with non-dilated oviduct openings appearing as deep dimples posterior to vent, **adolescents** with fleshy post-anal pad starting to swell and oviduct opening that is small and not swollen, **mature** with a well developed fleshy post-anal pad and oviduct openings that are large, dilated and swollen, **pregnant** with egg cases, and **spent** a stage that is difficult to evaluate due to the fact that mature female chimaeras have eggs at all stages of development and appear to lay multiple sets of eggs throughout the year.

87. A number of parameters can be taken on both female and male elasmobranch specimens. A maturity parameter for females is the width of the oviductal gland measured with callipers. The diameter of the largest oocyte and the number of eggs are also counted for both the right and left side. With males, the length of the inner and outer clasper edges are taken.

88. When dealing with a female with pups, record the following information: the position of the pups in the uterus (the ones closest to exiting are named L1 and then L2, L3, etc.), their length, sex, weight of pups and yolks.

#### Taking genetic samples

89. Genetics is a useful tool in defining a species as distinct on a molecular level. There are three different ways of taking a tissue sample for molecular studies, depending on whether:

- the specimen has to be discarded;
- the specimen has to be preserved and become a voucher specimen;
- the specimen is alive.

90. If the specimen has to be discarded, the best place to take a sample from is the liver, the largest organ in the body. Another option is the muscle tissue close to the vertebral spine.

91. If the specimen has to remain undamaged, the tissue sample should be taken from the area just behind the pelvic fins and anus.

92. From live specimens, it is best to take a clip of tissue from the pectoral fin or from the muscle tissue just below the dorsal fin.

93. When taking a tissue sample, make sure to sterilize the equipment (scissors, forceps, scalpels, etc.). Do not handle tissues with bare hands (this in order not to contaminate the sample). Take a sample the size of a pea and cut it into three slivers. Put each sample in a vial with pure 100 percent ethanol. Shake the vial well to allow the ethanol to penetrate into the tissue, and make sure you record a number on the vial that matches the number on your data sheet and thus the photographs, morphometric data, etc. It is very important to take photographs of the specimen when taking genetic samples.

#### Collection of vertebrae and spines for age studies

94. Vertebrae and spines can be removed and then analysed to study the age of cartilaginous fishes. Their section shows a number of alternating dark and light bands similar to the rings found in the horizontal cross-section of trees. Often, the rings represent the changing of the seasons (mainly winter and summer), but other times the rings are annual. The collection of samples every month allows for the determination of the interval between the bands.

95. About ten vertebrae must be removed from the vertebral spine region of shark specimens, at the first dorsal- and pectoral-fin level. Spines should be taken from directly in front of each dorsal fin and cut out with a "V" cut. Both vertebrae and spines should be tagged (identifying number corresponding to all the recorded data), bagged and frozen.

96. Batoids vertebrae should be removed from the torso area, roughly between the wings, under the nuchal region. Spines and thorns do not need to be taken from skates and rays.

97. The laboratory procedure for sectioning and reading the vertebrae and spines was described.

#### Photographic documentation

98. Taking photographs of cartilaginous fish specimens is important as it can allow other experts to properly identify a species. Photographs are easy to share and usually show the individual in the best possible conditions.

99. A series of photographs must be taken of each shark specimen. In particular, the following photographs should be taken:

- A full lateral view of the specimen facing to the left. It is important to have the entire shark in the picture, avoiding cutting parts of the caudal fin and snout. Make sure that this photograph shows a straight-on view and not a ventral or dorsal view. An effort should be made to have the surface of the dorsal fins parallel to the ground.
- A lateral, dorsal and ventral view of the head from the snout tip to the gills.
- A close up of the pelvic-fin region, dorsal fins (both in the same photograph in order to compare the relative size and shape of the fins), caudal fin and pectoral fin. A photograph of the ventral side including both the pectoral and pelvic fins can be useful.
- A close-up of the mouth region showing the labial furrows.

100. Additional photographs should be taken to show any unusual or descriptive feature, e.g. teeth, denticles.

- 101. With regard to the batoids, the following photos must be taken:
  - Total upper and lower views
  - Close-ups of details, such as upper and lower side of head, mouth-nasal region, dorsal and caudal fins (if present), serrated tail spine(s) in stingrays, details of obvious thorn patterns on upper side of disc and tail. Colour pattern details like eye-spots, should also be taken.
- 102. As for chimaeras, the following photos must be taken:
  - A full lateral view of the specimen facing to the left.
  - Close-up of the head, the spine and first dorsal fin, ventral view of the chimaera, caudal fin, tenaculum, pre-pelvic tenaculum and pelvic claspers.

103. Every photograph should include a ruler or an object of known size in order to have a metric scale. It should also include an identification tag with information such as the species name (when identified), the date, sex and ID number.

#### Preservation, tagging and bagging of a specimen

104. The specimens that are to be kept must not be dissected. They must be injected with 100 percent formalin in a number of different spots along the side of the body and in the abdominal cavity. After injecting the specimen, it should be soaked in a 10 percent formalin/water solution for up to two weeks, but also a couple of days should be sufficient. Protective gear (long gloves, eye protection glasses, gas mask, protective clothes) must be used as formalin is highly toxic. The soaked specimens must be rinsed in water, wrapped in cloth, bagged, tagged and shipped to the research group. For long-term preservation, the specimen treated with formalin should be kept in a 70 percent ethanol/water solution.

#### Session 3 – Practicum: identification and biological data collection on deep-sea shark species

105. Dave Ebert showed all participants how to take biological samples from a shark, skate and chimaera specimen (Plate 4). The three specimens were dissected and the different organs were shown and described. Then the maturity stage was determined, oviductal gland measured, vertebrae and spines removed, spiral valve turns counted, tissue sample taken, and stomach contents analysed.

#### Plate 4

#### Demonstration of biological data collection methodologies.



106. After the demonstration, the participants were tasked with the identification of other species. Moreover, each group performed a standard biological data collection procedure on the specimens under the supervision of the instructors including taking photographs based on the indications provided during the morning lecture.

#### Session 4 – Practicum: individual review of the identification and data collection methodologies

107. On the last day, the participants continued practising the identification and biological sample collection procedures on a number of specimens. All of them demonstrated the ability to use the identification guide and the keys, and to collect biological data from the specimens.

#### Session 4 – Lecture: iSharkFin software and identification guide and final exercises

108. In the afternoon, after the practical sessions, Ms. Barone presented the iSharkFin (http://www.fao.org/fishery/ipoa-sharks/iSharkFin/en) software and the SharkFin Guide (in press) for the identification of shark fins.

109. The main contents of the SharkFin Guide were presented, including its objectives, the criteria for the inclusion of the species and the relevant information provided by the fact sheets. Then the participants were involved in a hands-on exercise that is, the identification of a dried fin through the use of the identification keys of the fin types included in the guide.

110. The practical methodology on how to collect photo samples and genetic samples from shark fins was also described, illustrated and discussed.

111. The iSharkFin software was distributed and installed on the laptops of several participants. The main steps required for its correct use were described, with a particular emphasis on the definition of the main four

landmarks/points that the user has to place on the fin layout. The software was immediately tested by the participants who used some photo samples and followed the semi-automatic procedure.

112. Finally, the participants were tasked with solving three exercises (see Appendix 7) aimed at understanding their ability to use the identification guide and the keys therein included. All participants were able to solve the exercises.

113. The participants showed great interest in the topics of the workshop, and their active participation enabled its success.

114. The training workshop closed with a group photograph (Plate 5).

#### Plate 5

### Group photograph.



#### Appendix 1 List of participants

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#### Appendix 2 Agenda

#### Day 1: Tuesday 23 June 2015

09:00

- Opening address by FAO
- Self-introduction of participants
- Overview of the workshop objectives, activities and expected outputs
- Importance of information gathering and role in fisheries management

11:00

- Current knowledge on deep-sea Cartilaginous fishes biodiversity in the southeastern Atlantic
- Presentation of the deep-sea cartilaginous fishes identification products (activities, approach used and future projects)
- Use of identification keys included in the guide and technical terms

#### 12:30 Lunch

13:30

Practical session:

• Identification of genera and species

16:15

• Wrap up discussion (Questions, clarifications, review of day's work)

#### 17:00 Day Closure

#### Day 2: Wednesday 24 June 2015

09:00

Lectures:

• Identification of major groups of deep-sea Chondrichthyans

10:45

Practical session:

• Identification of genera and species

#### 12:30 Lunch

13:30

Practical session:

• Identification of genera and species (continued)

15:30

• Wrap up discussion (Questions, clarifications, review of day's work)

#### 17:00 Day Closure

#### Day 3: Thursday 25 June 2015

09:00

Lectures:

- Identification of major groups of deep-sea Chondrichthyans
- Introduction to taking photographing and saving specimens in the field
- Taking and storing biological samples/what happens then?

#### 10:45

#### Practical session:

• Identification of genera and species

#### 12:30 Lunch

13:30

#### Practical session:

• Identification of genera and species (continued)

15:30

• Wrap up discussion (Questions, clarifications, review of day's work)

#### 17:00 Day Closure

#### Day 4: Friday 26 June 2015

#### 09:00

#### Practical session:

- Practice taking basic measurements and biological samples
- Practice taking photographs and saving specimens

#### 11:00

#### Practical session:

• Review day's practicum with each person going over a specimen from start to finish, e.g. identify their species, take basic measurements, photograph, decide whether it should be kept, preserving it, and taking general biological samples.

#### 12:30 Lunch

13:30

• iSharkFin: an innovative software for the identification of shark species from the fins

#### 15:30

• Wrap up discussion (Questions, clarifications, review of the workshop's work)

#### 16:30 Closing of the workshop

#### Appendix 3 Shark ID sheet – first encounter form

When a species is encountered for the first time, fill out the appropriate sections of this form and include a drawing to the best of your ability.

- Anal fin? Y/N
- Snout shape: (blunt/sharp, long/short), describe:
- Number of gills:
- Number of dorsal fins:
- First dorsal fin shape (long/short, tall/low, flag-like/sail-like, tapering?), describe:
- Second dorsal fin shape (long/short, tall/low, flag-like/sail-like, tapering?), describe:
- First dorsal fin placement relative to other fins:
- Second dorsal fin placement relative to other fins:
- First dorsal spines (size and shape):
- Second dorsal spines (size and shape):
- Upper teeth shape (blades, daggers, cuspids, hooks, comb, other), draw/explain:
- Lower teeth shape, draw/explain:
- Caudal fin notched?
- Precaudal pits?
- Subcaudal keel?
- Coloration or markings:
- Length (TL), sex, maturity:
- Short description of other features

#### **Drawing:**

### Appendix 4 Flat Chondrichthyan ID sheet– first encounter form

When a species is encountered for the first time, fill out the appropriate sections of this form and include a drawing to the best of your ability.

- Ray, skate, guitarfish, angelfish, sawshark, other:
- Snout (hard or soft nose):
- Pectoral (wing) attachment/shape:
- Mouth (terminal, subterminal)
- Prebranchial gills (present/absent):
- Tail size/shape/how it ends:
- Fins: placement, size, presence/absence
- Pelvic
- First dorsal
- Second dorsal
- Thorns count (skates, see figure):
- Rostral
- Malar patch (males): present/absent?
- Alar patch (males): present/absent?
- Orbital
- Scapular
- Nuchal
- Tail
- Interdorsal
- Spine (rays)?
- Size, sex, maturity:

#### **Drawing:**

#### Appendix 5 Chimaera ID sheet– first encounter form

- Anal fin? Y/N
- Nose size/shape (short, long, plow):
- Body (robust/slender):
- Spine longer than first dorsal fin? Y/N
- Pectoral fins overlap pelvic fins? Y/N
- Second dorsal straight or undulation? Y/N
- Shape of each fin (draw):
- Eye size:
- Cheek lines branching:
- Coloration or markings:
- Length (BDL), sex, maturity:

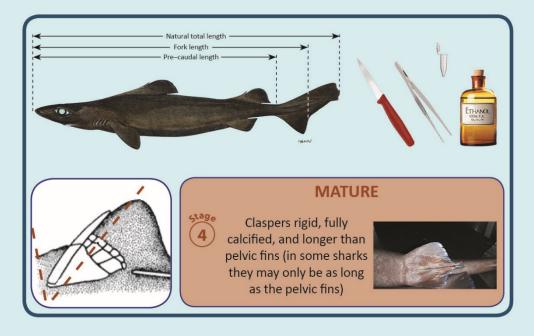
#### **Drawing:**

Appendix 6 Biological data collection manual



Food and Agriculture Organization of the United Nations

# **Biological Data Collection Manual**



An illustrated manual for collecting biological data from deep-sea cartilaginous fishes

> by P.J. Clerkin and D.A. Ebert

# **HOW TO USE THIS MANUAL**

This manual includes a set of explanatory and fully illustrated sheets describing the correct methodologies required to:

- · Take length and weight measurements;
- Determine the sex and maturity stage of the specimens;
- Collect age structure samples, such as otoliths, scales, spines and vertebrae, for age and growth studies;
- Collect genetic samples for further studies aimed at defining a species as distinct on a molecular level;
- Preserve specimens for further studies aimed at defining a species on a morphological level;
- Take photos that can help experts in the identification of the specimens.

Each section includes:

- · Graphics showing the equipment necessary to collect the required information;
- A step-by-step guide to the procedure that the user should follow;
- A guide on how to record the collected the information on the data sheets.

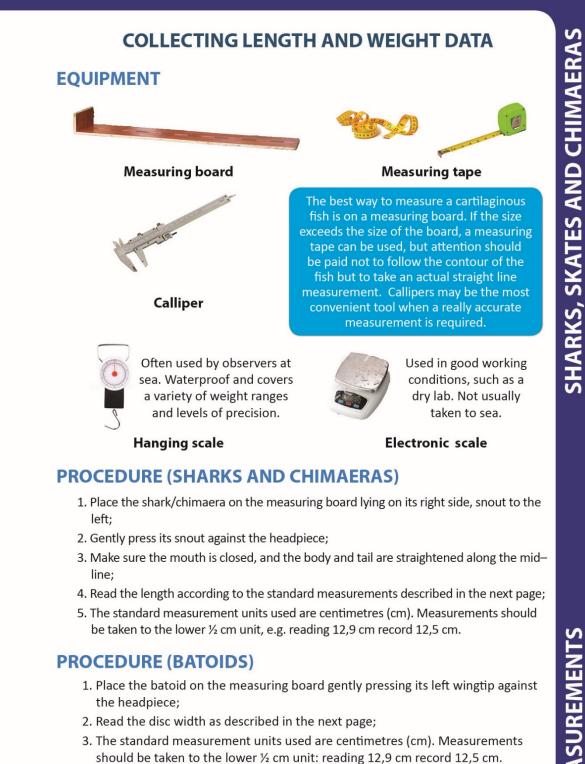
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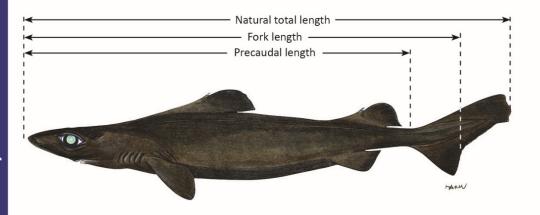
The fish should be measured while it is fresh and wet. If the fish is in rigor mortis (stiffness after death) should be flexed gently before it is measured.

# STANDARD MEASUREMENTS FOR SHARKS

There are three standard measurements taken for sharks. These are:

NTL = Natural total Length (tip of snout to extreme end of tail in a straight line). FL = Fork Length (tip of snout to fork in tail).

PCL = Precaudal Length (tip of snout to precaudal notch).



# **STANDARD MEASUREMENTS FOR BATOID FISHES**

Disc width

There is a standard measurement taken for batoid fishes:

DW = Disc Width (wingspan in skates and rays).



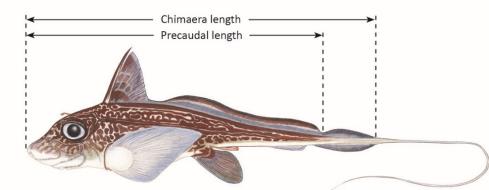
MEASUREMENTS

## STANDARD MEASUREMENTS FOR CHIMAERAS

The most common measurements used for chimaeras are the following:

**CL = Chimaera length** (length from tip of the snout to the posterior edge of the supracaudal fin, excluding the caudal filament).

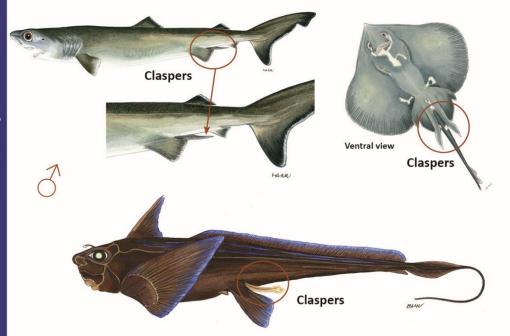
**PCL = Precaudal length** (length from tip of the snout to the anterior edge of the supracaudal fin, excluding the caudal filament).



## SEX DETERMINATION IN SHARKS, BATOIDS AND **CHIMAERAS**

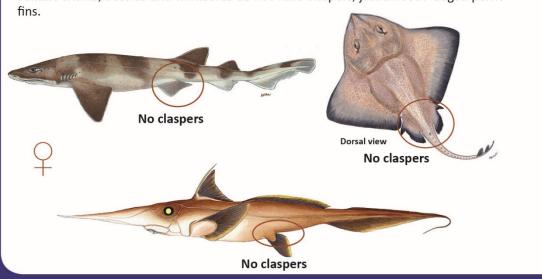
The sex of sharks and batoids can be determined by looking at the paired pelvic fins underneath the middle of the shark, as shown in the following drawings.

Male sharks, batoids and chimaeras have tubular processes called claspers arising from the trailing margins of the pelvic fins. The claspers are the male's reproductive organs, used to internally fertilize the females. In juvenile males, the claspers will be present but may be small. If you are unsure, do not guess.



Female sharks, batoids and chimaeras do not have claspers, just smooth-edged pelvic





## **MATURITY STAGE DETERMINATION**

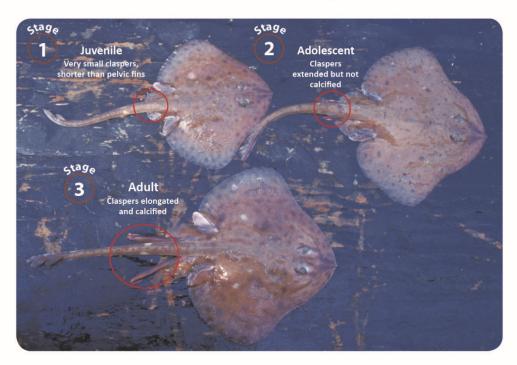
## **MATURITY RANKING OF MALE SHARKS**

Clasper length is an important parameter in accessing a species' length at maturity. Male specimens, can be ranked as:



## **MATURITY RANKING OF MALE BATOIDS**

Males are considered mature when the claspers are elongate and calcified.



## MATURITY RANKING OF MALE CHIMAERAS

Like all cartilaginous fishes, male chimaeras possess a pair of appendages called claspers attached to their pelvic fins. These claspers are used during copulation to transfer sperm into females' oviducts. In addition to claspers, male chimaeras have two secondary sexual characteristics used to ensure copulation. Males have a frontal tenaculum, a small projection medially located on the anterior portion of their heads, and prepelvic tenacula located within slit–like pockets just anterior to the pelvic girdle. By evaluating these external features, we can efficiently rate a male chimaera's maturity without damaging scientifically valuable specimens.

Stage

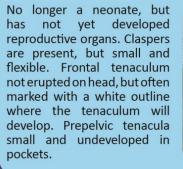
Newly hatched and distinguishable by its very soft body. Sexual organs undeveloped and flesh translucent. Small and undeveloped. Claspers are present, but tiny.

## NEONATE



## JUVENILE

Claspers small and flexible



stage

stage

3

4

Reproductive organs are developing, but the individual is not yet capable of mating. Claspers are beginning to elongate, but are flexible and not yet calcified. Frontal tenaculum in the process of erupting from the head and the prepelvic tenacula are developing in their pockets.



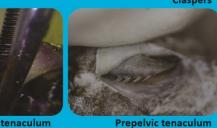
## ADULT

**ADOLESCENT** 

A fully mature individual capable of reproducing. Claspers are elongated (though not necessarily surpassing the posterior edge of the pelvic fin) and are stiff and calcified. Distal ends have a fleshy tissue covered in denticles and are sometimes bulbous when the animal is ready for copulation. Frontal

tenaculum is fully erupted and can articulate away from the body. The prepelvic tenacula are developed, calcified and hard, bear denticles along their distallateral edge, and articulate out of their pockets.





Frontal tenaculum

SEX DETERMINATION

### **MATURITY RANKING OF FEMALE SHARKS**

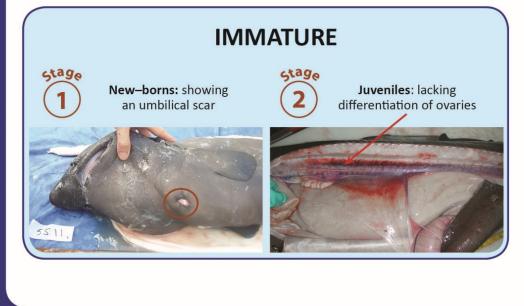
The maturity stage of female sharks has to determined after dissection and assessment of the development of the reproductive apparatus.

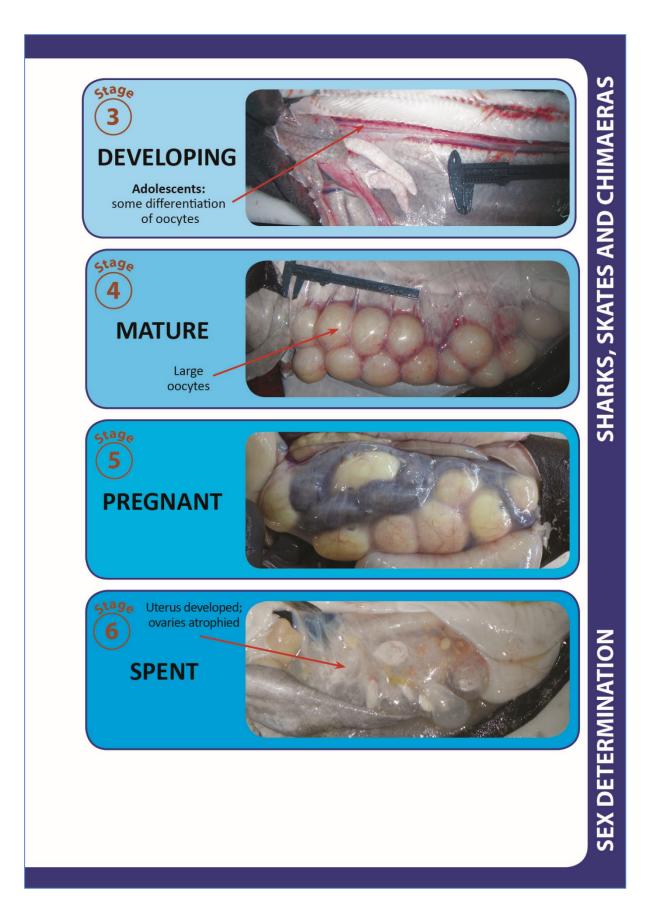
## **EQUIPMENT**



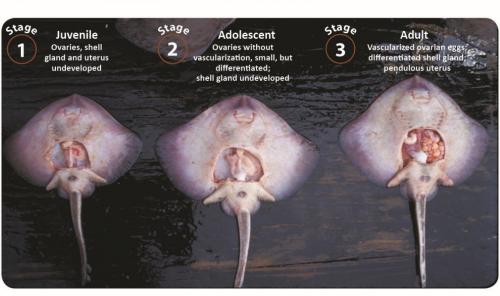
## PROCEDURE

- 1. If the specimen is small, using scissors (blunt tip inside the shark) open the fish by making a cut parallel to the spine forward from the anus;
- 2. For large specimens, make an incision with a knife along the length of the body from pectoral fin to pelvic fin, then cut dorsally from each end of that incision. Fold the resulting flap of flesh up to open the body cavity. A shallow incision along the inside of the dorsal surface will prevent the flap of muscle from returning to its original position;
- 3. Move stomach, liver and intestines to the side;
- 4. The gonads can be located close to the spine below the intestines;
- 5. Determine the fish's maturity stage based on the following scale:





## **MATURITY RANKING OF FEMALE BATOIDS**



## **RECORDING INFORMATION ON SEX**

#### On the data sheet write down:

- **M** (for fish determined to be male)
- F (for fish determined to be female)

The maturity stage should be annotated by writing the number corresponding to the estimated stage close to the above mentioned codes.

For example, a pregnant female should be annotated as **F5**, a developing male as **M4**.

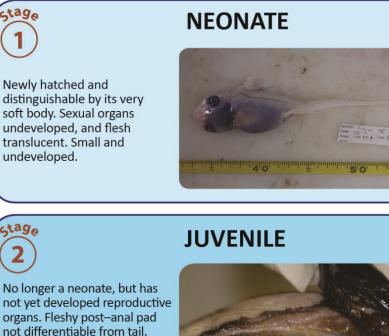
If the gonad is checked but is too small or immature to confidently determine the sex, the observer should record: I Indeterminate.

Finally, use  ${\bf U}$  Unknown when, due to lack of time or damage to the fish, the sex can't be determined.

Biological Data Collection Form			Observer ID John May					50 Time of net deployment and retrieval:	Date <b>26/3/15</b>	
Gear type <b>Bottom trawl</b>		Fishing depth/Bottom depth 650/650			Time of net deployment and retrieva					
Code	Species name	Species code	Total Length (cm)	Other measurement <sup>2</sup> (cm)		Sex	100000000000000000000000000000000000000	and the second	Otholits	
132-2	Etmopterus spinax		42.4	38.2 SL	0.8	F	F4		-	
		_								

## **MATURITY RANKING OF FEMALE CHIMAERAS**

Female chimaeras are different from other cartilaginous fishes (sharks and rays) in having two oviducts, each of which exit the body independently of the vent. These external orifices develop and dilate as the animal matures, providing researchers with a qualitative metric to evaluate female maturity without having to damage scientifically valuable and often rare specimens. If the species is common and/or will not be collected, then internal evaluation may be performed following the protocols used for sharks.



# stage

No longer a neonate, but has not yet developed reproductive organs. Fleshy post-anal pad not differentiable from tail. Oviduct openings not dilated, without papule, and appear as deep dimples posterior to vent.



## tage 3

## **ADOLESCENT**

Reproductive organs are developing, but the individual is not yet capable of mating. Fleshy post-anal pad starting to swell, differentiable from the rest of the tail, but not yet completely developed. Oviduct opening small or starting to dilate, but not yet textured or swollen.



SEX DETERMINATION

## MATURE

A fully mature individual capable of reproducing. Fleshy post–anal pad well developed, swollen, well defined from tail, and often darker in color with a lightly colored fold. Oviduct openings large and dilated, often swollen and textured with papule.



## Stag

stage

4

## PREGNANT

All chimaeras are oviparous and lay eggs. This maturity ranking is based on the presence of egg cases in the mother. This can be evaluated via x-ray, spontaneous abortion upon capture, or dissection. Feeling an egg case through the body cavity is not a reliable method of evaluation since many food items are egg case-shaped (e.g. squid); therefore, visual confirmation is necessary.

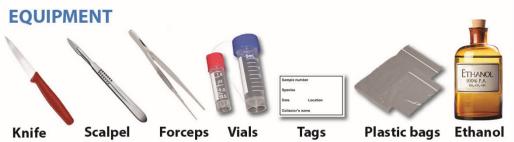
# Stage

## **SPENT**

Mature female chimaeras have eggs at all stages of development and appear to lay multiple sets of eggs throughout the year. This makes the spent status difficult to evaluate.

## **COLLECTION OF TISSUES FOR GENETIC ANALYSIS**

Genetics is a useful tool in defining a species as distinct on a molecular level.

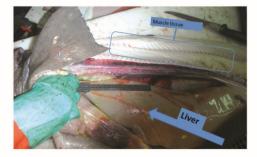


## **PROCEDURE FOR SHARKS**

#### Collection from a shark to be discarded

- 1. Make an incision along the length of the body from pectoral fin to pelvic fin, then cut dorsally from each end of that incision. Fold the resulting flap of flesh up to open the body cavity. A shallow incision along the inside of the dorsal surface will prevent the flap of muscle from returning to its original position;
- 2. Cut a small piece of tissue (the size of a pea) from the liver or trunk using clean scissors or a new scalpel blade;
- 3. Hands of the collector should be cleaned of mucus and scales between handling fish, and scissors/knife should be rinsed between samples.

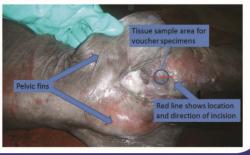
Make sure that the equipment (scissors, forceps, scalpels, etc.) be sterilized; do not handle tissues with bare hands in order not to contaminate it. Samples cannot be obtained from specimens that have been exposed to or fixed in formalin



#### Collection from a shark to be kept

If a specimen is designated for collection, taking muscle tissue will minimize damage to the specimen while supplying valuable genetic information;

- 1. The most discrete location from which to collect a sample is just posterior of the pelvic fins, on the ventral surface of the body;
- Be certain this area is free of any contaminating materials, then make a small incision in line with the body about 1–2 cm in length (or about the width of a finger nail);
- 3. Use a sterilized knife or scalpel and a pair of forceps to remove a peasized sample of muscle tissue.



#### **Collection from a live shark**

A dermal punch is the preferred method for collecting a genetic sample from a live animal, because it yields a better tissue sample and is less damaging to the animal. When using a dermal punch on a live sharks the best location from which to obtain a muscle tissue sample is the trunk area just below the dorsal fin. This is the easiest area to sample and is the least damaging to the shark.



A fin clip produces genetic material that is very hard and, therefore, difficult for geneticists to process. It should only be used when taking a genetic sample from a live animal and a dermal punch is not available.

To take a fin clip from a shark, you will need a strong blade and/or clippers. A scalpel will not work. Make two cuts into the pectoral fin in a V–shape to remove a pea–sized wedge.



Since the animal is alive, remember to stay clear of its mouth.

## STORAGE

Place the tissue sample into a small glass or plastic vial containing high strength (95%) ethanol. The ethanol will preserve the tissue and the DNA at room temperature, so does not need to be refrigerated. It is crucial that wet tissue samples be completely immersed and not exposed to air (vial should be filled to the top).

A minimum 10:1 ratio of preservative to tissue is desired.

Label each vial with a permanent (Sharpie) marker. Ensure each sample can be identified later by including the following information on each label: locality, sample number, collection date, and species.

The date of collection, detailed locality information (accurate description of locality is critical – include GPS info if possible), collector(s) name, species, type of collection (e.g. fin clip), length, and sex, should be written on data sheets.

Samples must be kept out of extreme sun/heat (e.g. dashboards, hot warehouses), especially those in ethanol, as this may damage the DNA.

## **PROCEDURE FOR BATOIDS**

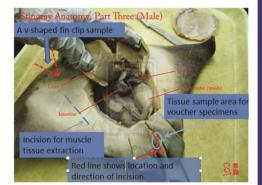
#### Collection from a batoid to be discarded

- Place the ray or skate on its back and make a series of incisions to open the thoracic cavity (see figure);
- 2. Cut a small piece of tissue (the size of a pea) from the liver using a clean or new scalpel blade. Make sure to select a clean, healthy looking section of the liver;
- 3. The liver is the largest organ in a ray or skate's body. It is brown, green, or tan, and appears as two large lobes in the body cavity. The liver will probably be the first and largest internal organ you see when dissecting a ray or skate from its ventral surface;
- 4. If the specimen is intended for discard and a muscle sample is to be collected, muscle from the wing can be accessed by making an incision into the wing and removing some of the inner tissue (do not take dermal tissue).

#### Collection from a batoid to be to be kept as a specimen

If a specimen is designated for collection, taking muscle tissue will minimize damage to the specimen while supplying valuable genetic information.

- The most discrete location from which to collect a sample is just posterior of the pelvic fins, on the ventral surface of the body (see figure).
- Be certain this area is free of any contaminating materials, then make a small incision in line with the body about 1–2 cm in length (or about the width of a finger nail).



3. Use a sterilized knife or scalpel and a pair of forceps to remove a pea-sized sample of muscle tissue.

#### **Collection from a live batoid**

A dermal punch is the preferred method for collecting a genetic sample from a live animal. because it yields a better tissue sample and is less damaging to the animal. When using a dermal punch on a live sharks the best location from which to obtain a muscle tissue sample is the trunk area just below the dorsal fin. This is the easiest area to sample and is the least damaging to the shark.

A fin clip produces genetic material that is very hard and, therefore, difficult for geneticists to process. It should only be used when taking a genetic sample from a live animal and a dermal punch is not available.

To take a fin clip from a shark, you will need a strong blade and/or clippers. A scalpel will not work. Make two cuts into the pectoral fin in a V–shape to remove a pea–sized wedge.

Since the animal is alive, remember to stay clear of its mouth.

SHARKS, SKATES AND CHIMAERAS

## **PROCEDURE FOR CHIMAERAS**

### Collection from a chimaera to be discarded

The primary location from which to collect a genetic sample is the liver. The liver is a soft tissue and is easy for geneticist to process. The liver is the largest organ in a chimaera's body. It is brown, green, or tan, and appears as two large lobes running the length of the body cavity. Select a clean, healthy looking section of the liver and excise a pea-sized sample.

If the specimen is intended for discard and a muscle sample is to be collected, muscle from the trunk can be accessed by making an incision down the length of the body and then making incisions dorsally at the pectoral and pelvic fins. An incision along the muscle penetrates the peritoneum and insures clean tissue for collection.

#### Collection from a chimaera to be to be kept as a specimen

If the specimen is designated for collection, taking muscle tissue will minimize damage to the specimen while supplying valuable genetic information. The most discrete location from which to collect a sample is just posterior of the pelvic fins, on the ventral surface of the body. Since this area is hidden by the pelvic fins, it is the less noticeable. First, be certain this area is free of any contaminating materials, then make a small incision in line with the body about 1–2 cm in length (or about the width of a finger nail). Use a sterilized knife or scalpel and a pair of forceps to remove a pea–sized sample of muscle tissue. If you have a dermal punch, insert it into the incision and scoop out a genetic sample by angling the dermal punch and gouging out a pea–sized sample of muscle tissue.

#### **Collection from a live chimaera**

A dermal punch is preferable because it supplies better tissue and is less damaging to the animal. When using a dermal punch on a live chimaera, the best location from which to obtain a muscle tissue sample is the muscle of the tail, just posterior of the trunk. This is the easiest area to sample and least damaging to the chimaera.

A fin clip produces genetic material that is very hard and, therefore, difficult for geneticists to process. It should only be used when taking a genetic sample from a live animal and a dermal punch is not available.

To take a fin clip from a chimaera, make two cuts into the pectoral fin in a V-shape to remove a pea-sized wedge.

## **COLLECTION OF VERTEBRAE AND SPINES**

Sharks lack bones, but do have hard structures namely vertebrae and fin spines. These vertebrae and fin spines can be cross sectioned and their rings can be counted to get an estimate of age (just like counting the rings in a tree).

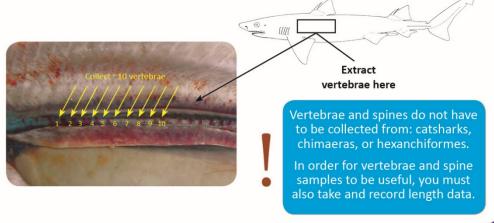
Vertebrae and spines are not required from every specimen. The most important thing is to collect vertebrae and spines from a variety of sizes and, therefore, different ages. Since males and females often grow at different rates, it is important to collect vertebrae and spines from a mix of males and females. For these reasons, we break the sizes into 10 centimetre bins, 0–10 cm, >10–20 cm, >20–30 cm, and so on. For each species encountered, collect vertebrae and spines from up to five males and five females from each size bin. Once you collect a vertebrae and spine sample, record it (by tally) on the data collection form for the appropriate species, sex, and size bin.

EQUIPMENT Knife Scalpel Forceps Tags Plastic bags

### **PROCEDURE FOR EXTRACTING SHARK VERTEBRAE**

Collect vertebrae from the shark's torso area, roughly between the pectoral fins (see figure).

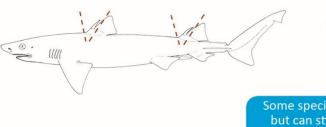
- Cut into the body cavity and excise about 10 vertebrae through the roof of the body cavity (cutting through the vertebrae can be difficult and it is helpful to cut through the small space in between the individual disks);
- 2. Trim excessive flesh away from vertebrae, but leave some muscle as a tissue sample for heavy metal analysis;
- 3. Tag and bag vertebrae with the same unique serial number assigned to the specimen and freeze.



## **PROCEDURE FOR EXTRACTING SHARK SPINES**

Collect spines from the same specimens from which you have collected vertebrae.

- Take spines from directly in front of each dorsal fin and cut it out with a "V" cut (see figure);
- 2. Since the spines often extend deep into the muscle, it is best to cut all the way down to the vertebrae;
- 3. If both spines are damaged, discard them both and use a different candidate as a specimen for spines;
- 4. ALL fin material must be removed from fin spines. Freeze spines in the same bag as the associated vertebrae. Spines and their associated vertebrae can share the same unique serial number.



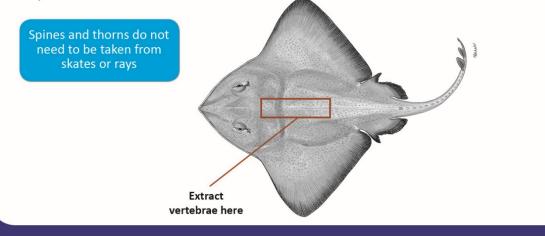


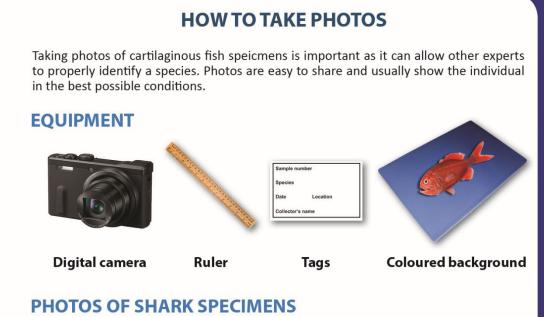
Some species do not have fin spines, but can still be aged through their vertebrae. For these species, only collect vertebrae.

## **PROCEDURE FOR EXTRACTING BATOID VERTEBRAE**

Collect vertebrae from the skate/ray's torso area, roughly between the wings, under the nuchal region (see figure).

- 1. Cut a rectangle out of the back around the nuchal thorns (if present) and excise roughly 10 vertebrae;
- 2. Trim excessive flesh away from vertebrae, but leave some muscle as a tissue sample for heavy metal analysis;
- 3. Tag and bag vertebrae with the same unique serial number assigned to the specimen and freeze.





- 1. Place a ruler or other measuring scale alongside the specimen; if no ruler is available, then some other object to show a size relationship.
- 2. A handwritten label that includes the sample number, the date, location, and other relevant capture information, and may include the person's name is desirable.
- 3. Plain coloured or an artificial background contrasting the specimen's colour is fine.
- 4. The following photos should be taken:

A **full lateral view** of the specimen facing to the left. It is important to have the entire shark in the picture making sure that the entire caudal fin and snout be included (make sure that this photo shows a straight on view and not a ventral or dorsal view and an effort should be made to have the dorsal fins surface parallel to the ground).



PHOTOGRAPHIC DOCUMENTATION

A lateral, dorsal and ventral view of the head from the snout tip to the gills.



A close up of the **pelvic-fin region**, **dorsal fins** (both in the same photo in order to compare the relative size and shape of the fins), **caudal fin** and **pectoral fin**. A photo of the **ventral side** including both the pectoral and pelvic fins can be useful.



Take photos of any detail that looks unusual or descriptive. Close–ups of the teeth are also helpful, especially for the sharks of the genus *Carcharhinus*.



**Dermal denticles** 



Teeth



Parasites

## **PHOTOS OF BATOID SPECIMENS**

The following photos should be taken:

#### Total upper and lower views.



Close–ups of details, such as upper and lower side of head, mouth–nasal region, dorsal and caudal fins (if present), serrated tail spine(s) in stingrays, details of obvious thorn patterns on upper side of disc and tail, colour pattern details like eye–spots, should also be taken.

## PHOTOS OF CHIMAERA SPECIMENS

The following photos should be taken:

- 1. A full lateral view of the specimen facing to the left. The photo should:
- a. Include tag, and size reference;
- b. Include entire animal from snout to filamentous tail ending;
- c. Have spine and first dorsal fin erect with spine visible so you can compare its height to that of the first dorsal fin;
- d. Have second dorsal unfolded and even. Shape of this fin can be important to species identification;
- e. Pectoral fins should lie naturally and not block view of first dorsal fin, second dorsal fin origin, for aspects of the head;
- f. Caudal fin should be laid out straight;
- g. Anal fin (if present) should be made visible.

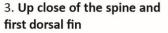


#### 2. Up close of the head a. Extend from snout tip to

dorsal spine. b. Show snout shape, canals

of face, eye shape and position, and mouth.





a. Show relative heights. b. Size and shape of spine and fin.

c. From spine (erect) origin to origin of second dorsal fin.



#### 4. Ventral view of chimaera

a. Include snout, pectoral fins, pelvic fins, some aspects of the tail.

b. Show the relative size and shape of the pectoral fins and the pelvic fins.



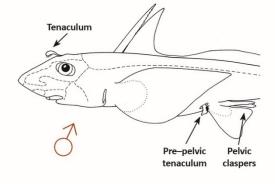
5. Caudal fin

a. Show anal fin if present. b. Show end of second dorsal. c. Show dorsal and ventral heights and lengths of caudal fin.

d. Include filament.



Claspers are important descriptive features for many species. The percent of specimens with developed claspers are relatively low so when possible it is important to completely photograph as thoroughly as possible. Chimaeras have 3 kinds of appendages for copulation, each can be species specific.



#### 7. Tenaculum

a. Located on the anterior part of the head.

b. Include shape and thornlike denticles. c. Use a pair of forceps or a

pen to pull the tenaculum away from the head.



a. Located in from the pelvic fins in small pockets b. Push out by hand or use forceps when fresh.

c. Include size, shape, and aspects of the teeth-like denticles.



a. Located behind pelvic fins.

b. Get multiple angles and include branching.



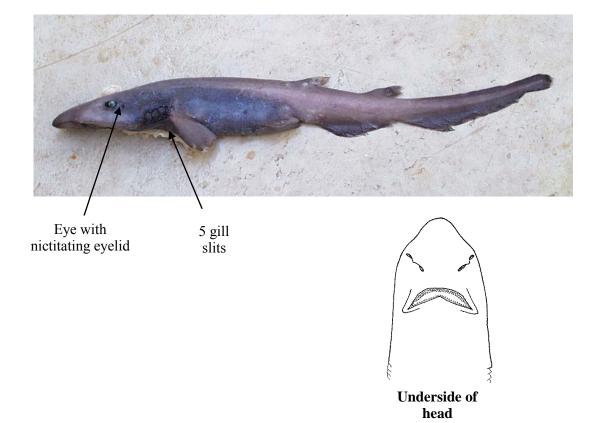


### Appendix 7 Exercises

#### Use of the dichotomous keys included in the catalogue and guide

- 1) Try to identify the specimen based on the following characteristics:
  - Shark-like appearance
  - No anal fin
  - Snout short
  - First dorsal fin anterior to pelvic fins
  - Body low
  - Caudal fin with no subterminal notch
  - Anterior nasal flaps with secondary lobe small and narrow to absent
  - First dorsal fin originates in front of pectoral-fin free rear tips
  - Nostril closer to snout tip than to upper labial furrow
- 2) Try to identify the specimen in the photo also based on the following characteristics:
  - Anterior pelvic-fin lobes not limb-like
  - Dorsal surface medium grey to brownish often with blackish spots
  - Tail length about equal to or usually longer than precaudal length.
  - Median row of tail thorns may distinctly continuous to origin of first dorsal fin.
  - One to several rows of thorns extending from shoulder region to origin of first dorsal fin, with thorns of mid-row equal in size or larger than those of lateral rows
  - Anterior disc margin slightly convex
  - Tail length from cloaca to tip about equal, or slightly shorter than disc length from snout tip to cloaca
  - Snout rigid, not flexible





3) Try to identify the specimen in the photo:

I5514E/1/03.16