UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea Magistrale in
BIOLOGIA MARINA

VALUTAZIONE DELLA STRUTTURA DI POPOLAZIONE E DELLE RELAZIONI TROFICHE DEI BATOIDEI NEL NORD ADRIATICO

ASSESSMENT OF THE POPULATION STRUCTURE AND TROPHIC RELATIONSHIPS OF BATOIDS IN THE NORTH ADRIATIC SEA

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Anno Accademico 2019/2020

A chi non teme i muri perché sa di poterli abbattere.

SUMMARY

RIASSUNTO

Gli ecosistemi sono fondati sulle interazioni tra organismi e potenzialmente ogni specie può influenzarne altre. Ci sono varie teorie che spiegano i sistemi biologici: una di queste sostiene che i predatori apicali sono in grado di influenzare la biodiversità di un ecosistema tramite un controllo top-down. La scomparsa di grandi predatori innesca generalmente l'aumento di meso-predatori e determina disequilibri nelle interazioni e/o cascate trofiche.

L'ambiente marino registra da oltre un secolo una graduale ma costante riduzione di squali, prevalentemente selaci. Si ritiene che questo fenomeno abbia determinato l'incremento di batoidei.

Un esempio di sistema sovrasfruttato è il Nord Adriatico: da secoli e in particolare dall'inizio del '900, i selaci sono stati oggetto di pesca intensiva. Già dopo il primo Dopoguerra si è assistito ad un aumento nello sbarco di razze, ed essendo organismi che si trovano in alto nella rete trofica, è importante andare a valutarne la popolazione e le relazioni trofiche per cercare di migliorare la gestione della pesca e evitare impatti sul resto dell'ecosistema.

Ci siamo chiesti come si fosse stabilizzata la popolazione dei batoidei in un sistema sovra-sfruttato come il Nord Adriatico occidentale e abbiamo valutato l'ipotesi diffusa dei pescatori riguardo un aumento di razze relativo agli ultimi 10 anni e infine abbiamo valutato le relazioni trofiche con gli altri organismi.

Per risolvere queste ipotesi abbiamo utilizzato tre approcci distinti: l'analisi dei dati dei mercati ittici di Ancona, Chioggia e San Benedetto del Tronto correlati alla "Local Ecological Knowledge" (LEK), l'analisi dei contenuti stomacali di campioni raccolti nell'area di Ancona e infine era in programma anche l'utilizzo di sistemi non invasivi come

il Baited Remote Underwater Video (BRUV) per la valutazione e misurazione della popolazione dei batoidei, ma non è stato possibile portare a termine questa parte date le restrizioni da Coronavirus.

Le serie di dati dei mercati ittici hanno aiutato a capire i cambiamenti nelle popolazioni e l'impatto di alcune forzanti come la pesca sulle risorse marine. Visto che abbiamo ottenuto solo dieci anni di dati, per sopperire alla mancanza del dato storico, abbiamo utilizzato la LEK tramite interviste strutturate ai pescatori di alcune città del Nord Adriatico (Ancona, Senigallia, Fano).

Confrontando i dati di mercato delle 3 città è stato possibile notare che la biomassa di razze vendute è effettivamente aumentata negli ultimi 5 anni. I dati di Ancona e San Benedetto ci hanno permesso anche una divisione specie-specifica delle tre razze più comuni: *Raja asterias* (razza stellata), *Raja clavata* (razza chiodata) e *Raja miraletus* (Razza occhialina). I dati mostrano come la razza chiodata abbia subito un grosso crollo dopo il 2014 e allo stesso tempo come la razza stellata stia aumentando a livello di biomassa. C'è stato quindi uno *shift* nella popolazione di razze (da *R. clavata* a *R. asterias*), dato confermato anche dalle interviste.

I pescatori ci hanno riferito che la presenza delle razze fino a qualche anno fa era limitata a 10-15 miglia dalla costa e che era generalmente *R. clavata* ad essere pescata. in corrispondenza delle piattaforme estrattive. Negli ultimi anni invece sono pescate con frequenza anche a 3-4 miglia dalla costa, con la differenza che si tratta di *R. asterias*.

Una osservazione interessante del presente studio è la presenza comune di *Aetomylaeus bovinus*, precedentemente considerato raro nella zona per un probabile errore di identificazione con *Myliobatis aquila.* Questo dato è scaturito sia dalle interviste sia dai campionamenti effettuati, in quanto tutti i 4 individui di Myliobatidae, appartenevano alla specie *A. bovinus*.

Studiare la dieta degli organismi è essenziale per definire il loro ruolo trofico all'interno di un ecosistema. Sono stati campionati al mercato ittico di Ancona 75 individui appartenenti a 4 famiglie (Rajidae, Myliobatidae, Dasyatidae, Torpenidae) e quella con la percentuale di campionamento più alta (Rajidae: 86.6%) ci ha permesso di quantificare la dieta, valutare l'effetto del sesso e della lunghezza e calcolare il livello trofico. Per quanto riguarda Myliobatidae e Dasyatidae, la bassa frequenza nei campioni ci ha permesso di stimare esclusivamente il livello trofico.

I risultati dell'analisi della dieta hanno confermato che le razze sono predatori voraci e generalisti: le loro preferenze si basano per lo più su crostacei (granchi %IRI=48.03) e pesci (%IRI=48.51). L'effetto della dimensione e del sesso sulla dieta hanno identificato che la lunghezza non influisce significativamente ma spesso influenza la composizione di prede e, in particolare, sulla diversità di prede in quanto l'indice di Shannon-Wiener diminuisce all'aumentare della grandezza, indicando una probabile specializzazione degli individui di taglia maggiore.

Per quando riguarda il sesso, nelle femmine l'importanza dei granchi nella composizione della dieta corrisponde ad un %IRI del 54.2% mentre nei maschi si mantiene sul 30%.

La grossa importanza dei granchi nella dieta dei batoidei in generale è stata confermata anche da altri studi condotti in Mediterraneo, ma non è ancora chiaro se si tratti di predazione diretta o è data dall' abbondanza di crostacei nell'area di campionamento. Ma, poiché le differenze nell'abbondanza di granchi tra maschi e femmine non sono ancora state evidenziate, abbiamo supposto ci possa essere una specializzazione delle femmine nell'alimentazione sui granchi probabilmente legata al profilo lipidico di questi ultimi, che potrebbe apportare alla dieta componenti utili durante il periodo riproduttivo.

L'utilizzo del BRUV era programmato con un totale di almeno 9 deployments tra Ancona, Fano e Chioggia. Il sistema di stereo-visione avrebbe permesso di conoscere la taglia media degli individui e la loro distribuzione. Purtroppo, le restrizioni COVID hanno ritardato le attività, ma nel frattempo abbiamo testato e perfezionato sia la calibrazione delle stereo-videocamere sia il processo di deployment e retrieval del sistema.

In futuro sono programmati altri deployment con il BRUV per introdurre l'utilizzo di questo sistema innovativo per la valutazione di popolazioni di meso-predatori in Nord Adriatico e continueremo gli aspetti riguardanti analisi di contenuti stomacali e interviste ai pescatori.

1. INTRODUCTION

1.1. GENERAL OVERVIEW

Ecosystems are built around interactions webs in which every species could potentially influence others, and for this reason evaluating trophic relations it's really important [1]. Such interactions, which include both biological processes (e.g., predation, competition, and mutualism) and physicochemical processes (e.g., the nourishing or limiting influences of water, temperature, and nutrients), link species together in a highly complex network [2]. The description of the interaction among species, usually occurs when the system is perturbated, but even in this case the response could require years to become evident. In addition, the effect of the reduction of apex predators has the problem that it could be observed when they are not in the system anymore, that is when the capacity to restore a top-down control has been lost [3].

It is possible to consider an ecosystem as a "system" shaped by the apex consumer. Predation is really important to explain meso-predator population dynamics and community change in ecosystems: for example, large sharks are predator of smaller sharks and rays. Top-down control is an important determinant of ecosystem structure and function.

Studies regarding the oceanic top-down control showed that it has important socio-economic, conservation, and management implications as meso-predators and invertebrates assume dominance, and the recovery of overexploited predators is impaired [3]. Changes in predators' abundance can have far-reaching consequences for ecosystem structure, functioning, and resilience [4], [5], with also shift in species and trophic cascades.

Predators, as we said, are important for various biotic interactions, and with the predator-prey relationship we can keep track of preys' abundances or biomasses.

Anthropogenic changes have greatly altered the abundance of large predators at high trophic levels [6]. In fact studies in the Gulf of Mexico, Pacific and Indian Oceans have demonstrated that this depletion resulted in the release of meso-predators [7], [8] and trophic cascades happened for the same reason in the coastal northwestern Atlantic - like in the Chesapeake Bay.

There, in the N-W Atlantic, the study made by Myers *et al.* (2007) [9] showed that the intensified shark exploitation happened in last decades resulted in a overgrowth of specific species of batoids (e.g. cownose ray, *Rhinoptera bonasus*) which resulted in a trophic cascade as the organism was foraging on bivalves, one of the greatest source for fisheries in the Chesapeake Bay. The overgrowth of such species, usually characterized by late sexual maturity, was anomalous, but explainable only by the implying the higher predation by great sharks that prevailed in the past.

Possibly the same could happen or is already happened in the Adriatic Sea with similar ecological consequences.

In fact, Ferretti *et al.* (2007) observed that large predators in Mediterranean declined dramatically in abundance over the last 2 centuries and 5 species may be considered functionally extincted.

Many historical records show the Mediterranean Sea as having an abundance of large sharks [10]. Sharks were (and still are) considered a pest by fishers [11] or an impediment because feeding on bony fishes [12]. In the early 20th century many coastal fisheries regularly targeted or landed sharks [13]–[17].

This concept is strictly linked to the notion of “trophic cascade”, that is the propagation of impacts by consumers on their prey downward through food webs [18].

The loss of apex consumers also reduces food chain length [2].

In general, competition and predation releases are more evident when not confused by high levels of fishing mortality [19]. Stronger predator or competitor releases in the Mediterranean Sea might have occurred historically in the 19th and early 20th centuries, when large predatory sharks (e.g. *Sphyrna lewini* (hammerhead), *Isurus oxyrinchus* (mako), *Lamna nasus* (porbeagle) and *Carcharodon carcharias* (white sharks)) were in decline [10], but fishing had not yet expanded to industrial levels. At that time, angel sharks (*Squatina squatina*), spurdogs (*Squalus blainville*), smooth-hounds (*Mustelus mustelus*), and skates (*Raja* spp.)were so abundant to sustain targeted fisheries [20], and the main Adriatic fish markets recorded increases in elasmobranch landings [21][22].

Concerning trophic cascades and release of meso-predator, it is possible to talk about “Mechanisms of fear”: Sherman *et al*. (2020) [23] studying rays in reef ecosystems, revealed that their behavior was affected by predator abundance. Sharks have been often suggested to play key role in control of reef ecosystem but also influencing genetics, movement and conditions of rays [24]. For this reason, in many areas where sharks declined, a meso-predator release has been reported, as rays and skates appeared to be the preferred food for some sharks.

Without predators, prays forage more boldly, and they dedicate more time and resources to reproduction. Although causative link is not fully demonstrated, several studies have shown the increase in meso-predator abundance following the decrease of top predator [8], [25]. The analysis of long-term data series of elasmobranch in Mediterranean Sea, showed a drastic reduction in abundance. This is mainly related to trawl fisheries, which not only destroy seafloors but also have strong effects on demersal communities because uses unselective nets.

A key step in ecosystem approaches to fisheries management is trying to quantitatively describe the diet and foraging habitat and predator–prey interactions of top predators or meso-predator in a community. This was also one of the aims of this work concerning the Adriatic Sea.

The Adriatic Sea, especially the Northern part, is a heavily exploited basin and its health and marine communities are influenced by the anthropogenic pressure (e.g., fishery and nutrient input) [20].

In particular, in the area it has been observed a decline in landings of rays and skates since the beginning of ‘90, but never with a clear trend [26].

On the other hand, during last decade fishermen perceived an increase in rays population, both in bycatch and target fishing, with many juveniles near the shores and nursery areas in correspondence of sandy substrates or oil platforms (working as Fish Aggregating Devices (FAD) [27], [28]).

Elasmobranchs in general are characterized by late sexual maturity, low fecundity and low growth rate. For these reasons they are very sensitive to even low levels of exploitation [29]. In the Adriatic Sea we can assume that there were some ecological factors that triggered the release of small elasmobranchs in past.

The area has a long history of human-induced changes, and it has been exploited for thousand years for fisheries. Its continental shelf and accessible fishing grounds allowed the development of large fisheries for shellfish and groundfish, but fisheries developed unevenly between the western and eastern sides of the basin. Italian waters are exposed to extremely high exploitation pressure from high-capacity fishing fleets while the eastern part is characterized from a lighter fishing exploitation [30].

In 2013, Ferretti *et al.* [30], demonstrated that in the Adriatic Sea happened a shift in communities, after a prolonged human impact. They combined catch data from five trawl surveys from 1948 to 2005, noticing that 11 species of sharks ceased to be detected after 2000, while in general there were few dominant species and many sporadic species. Comparing Hvar and MEDTIS surveys, they concluded that elasmobranchs declined by 94.5% over less than 60 years, with higher decrease in sharks than rays. Another interesting data was that populations of rays not only decreased in number but changed in species composition: the thornback ray (*Raja clavata*), which was the most abundant in 1940s, recorded a huge decline and the brown skate (*R. miraletus*) increased. Significant increases were registered also for eagle rays (*Myliobatis aquila*) and marbled torpedoes (*Torpedo marmorata*), which both are declining again in past few years.

In the North West Adriatic, there is a higher fishing intensity along all the shores than in the East part, especially between Ancona and Chioggia (Fig. 1.1). Italy records nearly twice the amount of otter trawlers (1361) than Croatia (800) [31].

As this area has been overexploited in last century, it is possible to imagine we already had a meso-predator release that occurred probably during first after-War World I: we have references from D'Ancona in 1926 that catches increased for small elasmobranchs [22] so the lack of top predators probably occurred at the beginning of 1900.

At this time, the area of the Northern Adriatic Sea is settled without the shaping function of apex predators, but still fishermen are perceiving an increase in rays.

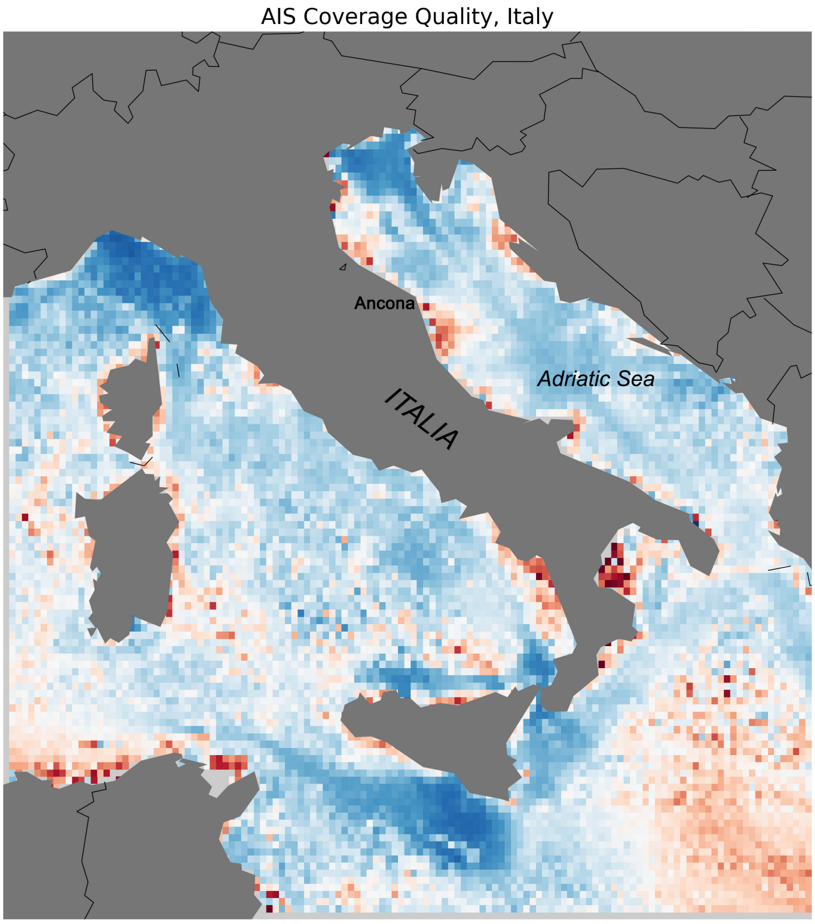


Figure 1.1- Automatic Identification System (AIS): map of the footprint of fisheries showing the higher fishing effort on the Italian side of the Adriatic Sea. Uses fishing hour and kW-hours [32].

The Adriatic fleet is one of the most important of the whole Mediterranean Sea, hence it could be important to keep track of their work and assess rays and skates population in order to better understand how it's changing and better manage this resource to prevent trophic cascades (like what happened in N-W Atlantic) or variations in predator-prey interaction in a delicate area such as North Adriatic Sea. In fact, with the decreasing of large predators in the area, fishermen remained the only predator for rays and skates.

1.2. OVERVIEW ON THE NORTH ADRIATIC SEA

1.2.1. *DESCRIPTION OF THE AREA*

The Mediterranean Sea constitutes 0.81% (2.514 million km^2) of the total water surface of the earth and is surrounded by twenty-two different countries. It extends from the Straits of Gibraltar to the Bosphorus and reaches its maximum depth (5 121 m) in the Ionian Sea. [33]
The Mediterranean Sea can be divided into two main basins: western and eastern separated by the Sicilian Channel. Within these basins, it is possible to define regional seas.
The eastern basin is characterized by a great oceanographic variability on the surface with temperatures of 16°C in winter and up to 29°C in summer and salinities of 39‰.
The distribution of marine organisms in the environment is usually related to bottom characteristics, nutrients abundance and oceanographic conditions. [33]
In fact, the biodiversity of the basin decreases from west to east, probably due to physical conditions such as the presence of threshold-strait or canal effects (Gibraltar, Sicily-Tunisia, Bosphorus, and Suez). The diversity in number of species shows the same negative eastward gradient that has been found for nutrients [33]. A minimum of biodiversity is present in the Adriatic Sea and Black seas [34].
The Adriatic Sea in particular is a semi-enclosed basin within the larger semi-enclosed sea. It is characterized by the largest shelf area of the Mediterranean, which extends over the Northern and Central parts where the bottom depth is between about 75 and 100 m, except for the Pomo/Jabuka Pit (200-260 m) in the Central Adriatic. The Southern Adriatic has a relatively narrow continental shelf and a marked slope, reaching the maximum depth of 1223 m.

We can divide the Adriatic Sea in 3 main basins: Northern, Central and Southern Adriatic.

For the purpose of fishery management, the GFCM (General Fishery Commission for the Mediterranean Sea) divided the whole Mediterranean in 30 GSA (Geographical Sub-Areas), and the Adriatic basin is divided in two GSA: the GSA 17 (North and Central Adriatic, the area concerning this work (Fig. 1.2)) and the GSA 18 (Southern Adriatic) [35].

The Italian pelagic fleet is distributed in ports along the Adriatic coastline from Trieste to Vieste and operates in GSA 17 and 18. The fleet is composed primarily of 'lampara' vessels (purse seiners operating at night with the use of light attraction) and midwater pelagic pair trawlers ('volante'), which were introduced in 1959 and presently is the dominating fleet [35].

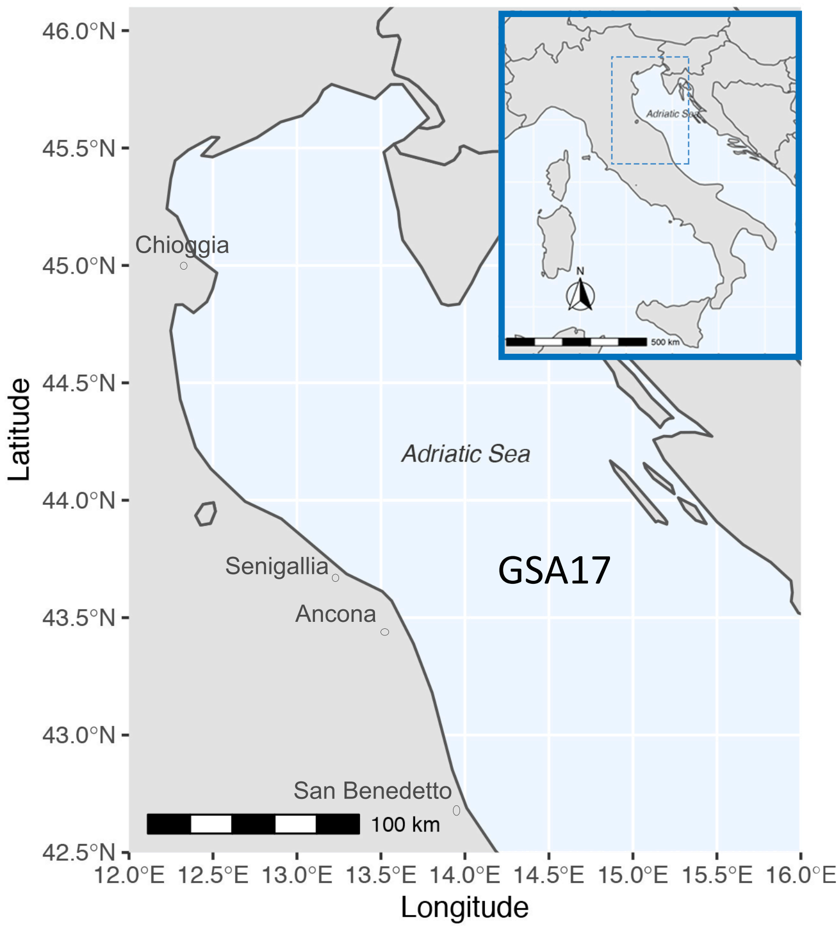


Figure 1.2 - Map of the Northern Adriatic Sea and principal cities with fishery traditions

The Northern Adriatic Sea (Fig. 1.2) is constituted by continental shelf (500 km long, and 200 km wide) and it is characterized by a very strong outflows of freshwater concentrated along the Italian corner. It is the shallowest region of the Mediterranean Sea.

Among the local rivers, the Po is the most important and one of the major driving forces of the currents as well as of the circulation in the whole northern shallow basin [36].

The Po and other river discharges originate a southward coastal current along the western coast (Western Adriatic Current, WAC), which fuels and sustains a cyclonic circulation, while on the eastern side of the basin, the weak, warm and salty Eastern Adriatic Current (EAC) flows along the eastern coast. At depth, a colder and denser water mass moves southward. A bathymetric controlled transverse current along the 50 m isobath, re-circulate the EAC into the WAC [37].

Concerning biological and oceanographic characteristics: the shallow depth, the moderate bathymetric gradient and the freshwater influx surely influence the circulation and presence of endemic species. All types of bottom sediments are found, muddy bottoms are mostly below a depth of 100 m moving southern, while in the Central and Northern Adriatic the shallower seabed is characterized by relict sand.

Due to its characteristics, especially the freshwater influx, the Northern Adriatic Sea is a eutrophic basin, contrasting the oligotrophy of the whole Mediterranean.

The high amount of nutrients results with fishery resources, and for this reason it is the most exploited Italian Basin [38], [39] and till the '80 it was also the most productive.

In the region surrounding the Po river, water column stratification, caused by freshwater buoyancy and heating of the sea surface, occurs from spring to mid-autumn. The winter cooling, sustained by cold north-easterly wind, causes intense mixing and the formation of dense waters [40], [41]. During the last 7-8 years this coastal region has been characterized by important eutrophication phenomena, due to urbanization and agriculture on the Po delta and with obvious consequences on local tourism [35].

Fishery represents one of the main source of ecosystems alteration [42] and is considered one of the major threats for biodiversity loss and collapse of populations [42]. Direct and indirect effects can interfere with abundance, habitats, trophic interactions and ecosystem functioning.

An example is the Chesapeake Bay – Virginia, US (which is a system similar to the Northern Adriatic Sea [43]) where the increasing agriculture on land surrounding the shores led to nutrient load in sea, determining eutrophication processes. Despite the human-induced changes, the oyster population (which was an important filter feeder) guaranteed the filtration of the exceeding phytoplankton, maintaining the turbidity low. After half-century of over-exploitation of the oyster fishery, the Chesapeake Bay became excessively eutrophic with many anoxic crises.

In the Adriatic Sea, the presence of cartilaginous fish species is scarce especially in the northern part. A total of 52 species of cartilaginous fish (Table 1.1) have been recorded in the Adriatic Sea, among them only 10 species are uniformly spread along the area [35].

ORDER	FAMILY	SPECIES
HEXANCHIFORMES	HEXANCHIDAE	*Heptranchias perlo* *Hexanchus griseus*
SQUALIFORMES	ECHINORHINIDAE	*Echinorhinus brucus*
	SQUALIDAE	*Squalus acanthias* *Squalus blainvillei*
	CENTROPHORIDAE	*Centrophorus granulosus*
	ETMOPTERIDAE	*Etmopterus spinax*
	OXYNOTIDAE	*Oxynotus centrina*
	DALATIIDAE	*Dalatias licha*
SQUATINIFORMES	SQUATINIDAE	*Squatina oculata* *Squatina squatina*
LAMNIFORMES	ODONTASPIDIDAE	*Carcharias taurus* *Odontaspis ferox*
	ALOPIIDAE	*Alopias vulpinus*
	CETORHINIDAE	*Cetorhinus maximus*
	LAMNIDAE	*Carcharodon carcharias* *Isurus oxyrinchus* *Lamna nasus*
CARCHARHINIFORMES	SCYLIORHINIDAE	*Galeus melastomus* *Scyliorhinus canicula* *Scyliorhinus stellaris*
	TRIAKIDAE	*Galeorhinus galeus* *Mustelus asterias* *Mustelus mustelus* *Mustelus punctulatus*
	CARCHARHINIDAE	*Carcharhinus plumbeus* *Prionace glauca*
	SPHYRNIDAE	*Sphyrna zygaena*
CHIMAERIFORMES	CHIMAERIDAE	*Chimaera monstrosa*
RAJIFORMES	PRISTIDAE	*Pristis pectinata*
	RHINOBATIDAE	*Rhinobatos rhinobatos*
	TORPEDINIDAE	*Torpedo marmorata* *Torpedo nobiliana* *Torpedo torpedo*
	RAJIDAE	*Dipturus batis* *Dipturus oxyrinchus* *Leucoraja circularis* *Leucoraja fullonica* *Raja asterias* *Raja clavata* *Raja montagui* *Raja miraletus* *Raja polystigma* *Raja radula* *Raja undulata* *Rostroraja alba*
	DASYATIDAE	*Dasyatis centroura* *Dasyatis pastinaca* *Pteroplatytrygon violacea*
	GYMNURIDAE	*Gymnura altavela*
	MYLIOBATIDAE	*Myliobatis aquila* *Pteromylaeus bovinus*
	MOBULIDAE	*Mobula mobular*

Table 1.1- Elasmobranchs present in the Adriatic Sea

1.2.2. *FISHING HISTORY*

The Adriatic fishing history goes back to at least Roman and Medieval times [20], even though until the end of the 18^{th} century, fishing was largely restricted to coastal zones. Subsequently, fisheries began to expand Chioggia (Northwestern Adriatic) was the biggest fishing fleet in the Adriatic which spread its activities southwards on both sides of the basin along coastal routes. The Northern Adriatic has always been overexploited due to its high resources. Even nowadays the fishing effort is still too high for the environment, resulting with eutrophication and mucilage phenomena [20].

From the late 19^{th} century until the end of the Second World War, fishing effort increased unevenly between the eastern and western sides. Italy operated most fisheries, which predominated in number and size of boats, number of fishers and gear efficiencies, while the other countries fished locally for subsistence. Trawling acquired popularity, initially with light gears towed by sail boats, and eventually after the 1920's increasingly with the use of combustion engines, mostly on the Italian side. After the Second World War, offshore fishing began, and the gap between western and eastern exploitation became even larger.

The Adriatic coastal ecosystem has been modified since Roman times [44], but it is over the last 50-60 years that coastal areas changed the most, due to urbanization and tourism.

Inland developments and increasing use of fertilizers and pesticides for agriculture increased the run-off of nutrients and chemical pollution, and so indirectly the eutrophication of the basin [45]. As a consequence, many species of plant and animals were eradicated from the area. Seagrass beds, for example, were common at least throughout the upper Adriatic before the 1800's, and two centuries of increased sedimentation have marginalized them just around the Istrian peninsula.

In addition to all these impacts, off-shore there are about 1000 dwells dislocated across the extended Adriatic shelf for extractive activity of natural gas [30].

Last decades have been really stressful for marine resources and several stock depletions have been experienced. An example is *Chamalea gallina*, which even with management plans continue to decrease [46] and the annual landing is presently 1/6 compared to the one recorded 25 years ago.

Finally trawling, dredging for shellfish and any other form of destructive fishing eliminated or reduced in size rocky biogenic formations that were once common in the Adriatic [47], [48].

1.2.3. *FISHING GEAR AND MAIN OCCURRENCES*

There are many kinds of fishing gears are used in the Adriatic Sea: the most used by the Italian fleet is the "volante". It is a mid-water pelagic trawl net towed by two vessels, mostly used in the northern and central areas. These vessels fish only by daytime and land their product every evening: the fishing trips last about 11-14 hours. Catches up to 15 tons per couple of boats per day have been recorded in the late seventies and early eighties, while presently the maximum catches are reduced to about 4 tons per day.

Until the mid-sixties the main gear was light attraction purse seine (lampara) and it is still in use in the Gulf of Trieste and south of Ancona. They fish by night in good weather conditions attracting fish with lights. Their activity could be suspended during the colder months [35]. In Croatia there is a fishing ban from 15th December till 15th January. Instead in the Italian side fishermen are limited to 3 days per week.

Classical bottom trawls (Fig. 1.3) are used to fish demersal species like red mullets, rays, sepias or squillas, while another bottom gear, the «rapido» is used for the demersal fishery. This gear is a dredge composed by an anterior rigid metallic framework, a wooden table acting as depressor and maintaining the mouth in close contact with the sea bottom, where a series of iron teeth penetrate into the sediment [49]. Along the Croatian coast bottom trawl fisheries is regulated by spatial and temporal fisheries regulation measures and about 1/3 of territorial sea is closed for bottom trawl fisheries over the whole year.

Rapido is used to catch flatfishes and batoids and Norway lobsters, or it is used offshore to fish mainly clams and other mollusks [49].

Figure 1.3- Typical Bottom Trawlers in Ancona's Fishery

Bottom trawls and Rapido trawls induce severe damages on non-target species.

The Adriatic Sea is one of the largest areas of occurrence of demersal and small pelagic shared stocks in the Mediterranean. The main small pelagic species are sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*), horse mackerel (*Trachurus* spp*.)* and mackerel (*Scomber* spp.).

On the continental shelf from 10-50 m depth, the dominant fish species in terms of biomass are red mullet (*Mullus barbatus*), sole (*Solea solea*), various species of flatfishes, gobies, batoids (mostly *R. asterias*), sharks (dogfishes and catsharks like *Squalus acanthias, Scyliorhinus canicular, Mustelus mustelus)* [35].

The continental shelf of the Adriatic Sea is also rich in invertebrate fauna, where some of the most abundant species are cuttlefish (*Sepia officinalis* and *S. elegans*), octopuses (*Eledone moschata, E. cirrhosa* and *Octopus vulgaris*), squids (*Loligo vulgaris* and *Alloteuthis media*), mantis shrimps (*Squilla mantis*), shrimps (*Solenocera membranacea* and *Parapenaeus longirostris*), Norway lobster (*Nephrops norvegicus*) and scallops [35].

In the Mediterranean Sea, as we said, almost no elasmobranchs are subject to directed fisheries, but nevertheless they constitute part of the bycatch in most local artisanal fisheries or bottom trawl. Catches of elasmobranchs primarily derive from two different fisheries: the pelagic artisanal fishery with longlines and gillnets, where smooth-hounds are the most common group, and the demersal trawl fishery, where rays and catsharks constitute the main groups [35].

In 2010 the fleet was composed of 131 mid-water pair trawlers (operating in pairs) and about 49 purse seiners. The actual number of vessels authorized to potentially be operative to use these gears in the fleet register through the fishing license is much higher. Total catches (not discriminated by species) of purse seiners in 2010 were 5,747 tons (65% in GSA 17) and of mid-water pair trawlers 44,393 tons (80% in GSA 17) [35].

1.2.4. *Morphology and Ecology of most common batoids in the Adriatic Sea*

Elasmobranchs are cartilaginous fishes, a subclass of Chondrichthyes. They include sharks (Selacii) and rays (Batoidea).

Batoids are the most varied group with more than 600 species. They have flat body and enlarged pectoral fins that are fused to the head, and gill slits that are placed on their ventral surfaces. They present 5 ventral gill slits, they lie under the pectoral fins on the underside, whereas the gill slits in sharks are located on the head sides. The anal fin is absent. The eyes and spiracles are located on top of the head. Electric rays, skates, and stingrays are greatly flattened and laterally expanded, with the pectoral fins united anteriorly with the sides of the snout. They possess a slender tail that is clearly demarcated from the disc, so the head and trunk form a circular, ovate, or rhombic disc. The majority of the batoids have 2 dorsal fins, but some electric rays, skates, and stingrays have either a single dorsal fin or no dorsal fins [50].

Batoids have a ventrally located mouth and can considerably protrude their upper jaw (palatoquadrate cartilage) away from the cranium to capture prey. The jaws have euhyostylic type suspension, which relies completely on the hyomandibular cartilages for support.

Jaw teeth are arranged in transverse rows, and like sharks are constantly replaced from inside the mouth; teeth are laterally fused to form large tooth plates in some of the more pelagic rays.
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 [50].

They can be more or less evenly covered with tooth-like placoid scales or dermal denticles (occasionally enlarged into thorns, bucklers, or spines) to completely lacking scales and scale-like structures.

Batoids, like sharks, have cylindrical copulatory organs or claspers that are used for internal fertilization of eggs in females. All batoids, except skates, are viviparous without placentas. Skates are oviparous, and deposit the fertilized eggs in rectangular, horn-like egg capsules (mermaid's purse) that are deposited on the bottom. Batoids vary greatly in size, but mostly are small to moderate in size, below 1 m and 60 cm wide.

They are generalized benthic predators that lack specialized food capturing and processing structures. These generalists consume a wide variety of infaunal and epifaunal benthic organisms ranging from polychaetes and other soft-bodied invertebrates to relatively small ray-finned fishes. Some electric rays (Torpedinidae) use their electric organs to stun large fishes that are swallowed whole; sawfishes use their rostral saws to disable or kill schooling fishes; eagle rays and cownose rays use their plate-like teeth to crush hard-shelled organisms such as oysters and clams. Manta rays have specialized filter plates associated with their gill arches and feed on zooplankton and nekton [50].

The order Rajiformes was mainly studied in this work: it comprehends skates with enlarged pectoral fins, head and trunk fused completely to sides and depressed body.

The order Myliobatiformes is also of interest: they are the common stingrays characterized from expanded pectoral fins, always fused with head and trunk, a whip-like tail, which can have one or several spines and no caudal fin.

Stingray species are progressively becoming threatened or vulnerable to extinction, particularly as the consequence of unregulated fishing. As of 2013, 45 species have been listed as vulnerable or endangered by the International Union for Conservation of Nature (IUCN). The status of some other species is poorly known, leading to their being listed as data deficient.

In the Adriatic Sea, according to the Food and Agriculture Organization (FAO) "Field Identification Guide To The Sharks And Rays Of The Mediterranean And Black Sea" [50], the main species captured are:

- ***Aetomylaeus** (=Pteromylaeus) **bovinus** (Geoffroy Saint-Hilaire, 1817)*

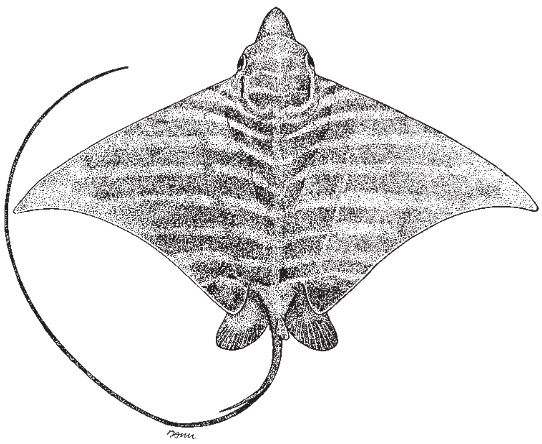


Figure1.4- A. bovinus *from the Field identification guide (FAO)*

These organisms (Fig.1.4) can reach 250cm of disk width, but usually between 60 and 100cm. It is a semi-pelagic species in coastal waters but in oceanic waters can reach up to 100m. It is ovoviviparous, it can have from 4 to 6 babies that at birth have 45 cm disk width. The gestation period is about 6 months. Front lobe of pectoral fin under snout, long and pointed in front. They mainly feed on gasteropods, crustaceans and occasionally teleosts. The species is distributed in the whole Mediterranean Sea, where is considered a threatened species in particular in the Northern Adriatic. Gillnets have been used for the catch of this species. Locally disappeared because of overfishing in the past.

- ***Myliobatis aquila*** *(Linnaeus, 1758)*

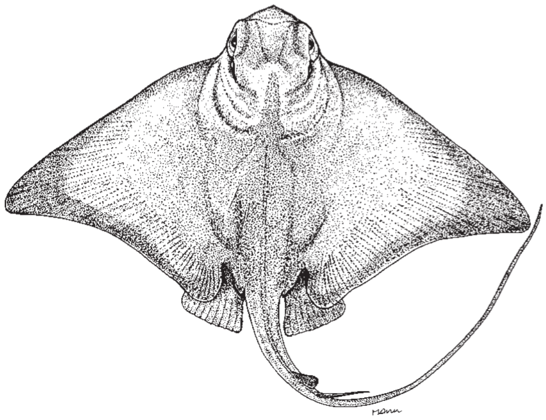


Figure 1.5- M. aquila *from the Field identification guide (FAO)*

The eagle ray (Fig. 1.5) can reach a disk width of 83 cm with a maximum total length of 260 cm.

Like *A. bovinus* is a semipelagic species in temperate coastal waters but occasionally can be oceanic from shallower waters up to 200m. The gestation period is 6 to 8 months and is ovoviviparous with 3 to 7 young. Maturity can be predicted also from the disk width: females are mature at 60cm and males at 40cm.

They mainly feed on gasteropods, crustaceans and occasionally teleosts. They are distributed in the whole Mediterranean sea and are occasionally caught by pelagic and bottom trawl.

- ***Pteroplatytrygon violacea*** *(Bonaparte, 1832)*

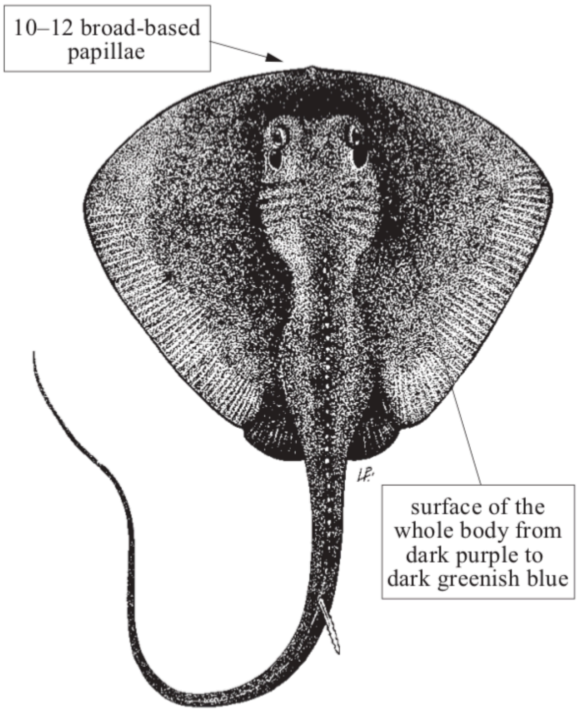


Figure 1.6- P. violacea *from the Field identification guide (FAO)*

The pelagic stingray (Fig. 1.6) can reach 80 cm disc width and at least 190 cm total length. It is a pelagic species in warm waters and it's quite common in the Adriatic Sea. It's an ovoviviparous species, with 5 to 6 young produced in summer.

This stingray feeds on decapods, squids, and pelagic fishes. It has a spine on the tail that is veleniferous.

Usually caught by drift nets and by drifting lines of hooks targeting tuna and swordfish. Discarded at sea because of their low or no commercial value.

- ***Dasyatis pastinaca*** *(Linnaeus, 1758)*

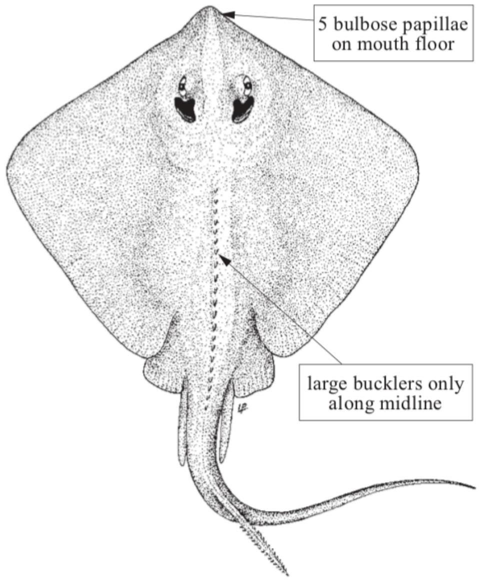


Figure 1.7- D. pastinaca *from the Field identification guide (FAO)*

Also called common stingray (Fig. 1.7), it reaches usually 40/45 cm disk width and 200cm of total length.

It is a benthic species over sandy and muddy bottoms from shallow waters to 200 m.

Ovoviviparous with 4 to 9 young born twice per year and after a gestation period of about 4 months. Females mature at 38 cm, males at 32 cm disc width.

Occasional bycatch in bottom trawl and gillnet fisheries.

- ***Raja asterias*** *(Delaroche, 1809)*

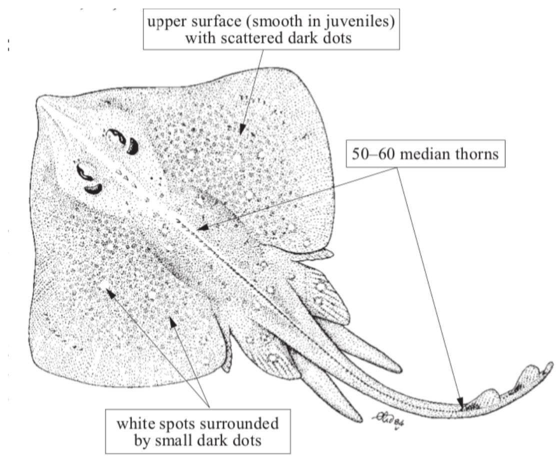


Figure 1.8- R. asterias *from the Field identification guide (FAO)*

The starry ray (Fig. 1.8) reaches 80cm of total length. It is a benthic species distributed in inshore waters on sandy bottom from 2 to about 200 m, more common between 20 and 50 m. Oviparous with about 30 to 112 egg-cases per year (depending on size of females) from 30 to 45 mm in length. Egg-cases laid mainly in summer and autumn at depth of 30 to 40 m; embryos development in 5 to 6 months. Young specimens of about 80 to 90 mm found in very shallow waters mainly in wintertime. Females mature at about 56 cm, males at 52 cm total length.

Distributed in the Mediterranean, it was less common in the eastern part but now it is the main species caught with bottom trawl.

Juvenile specimens are frequently caught by trammel net in very shallow waters (2–15 m) and discarded still alive. It has quite important commercial value in the northern Adriatic.

- ***Raja clavata*** *(Linnaeus, 1758)*

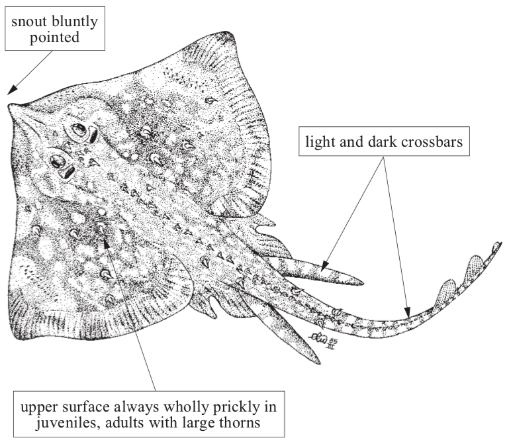


*Figure 1.9- * R. clavata *from the Field identification guide (FAO)*

Thornback ray (Fig. 1.9) reaches about 110 cm total length.

Benthic from shallow water to the bathyal zone (20–700 m). Oviparous, 140 to 170 egg-cases per year (60–90 mm length) laid mainly in winter and spring, development of embryo in about 5 months, with young hatching at 100 to 110 mm TL. Females mature at 85 cm, males at 75 cm TL.

It is distributed in whole Mediterranean including the Black Sea especially in the western part. Locally commercially important in the Mediterranean; bycatch of the demersal fishery. In past few years population shifted and R. asterias became the most popular ray in the Adriatic Sea.

- ***Raja montagui*** *(Fowler, 1910)*

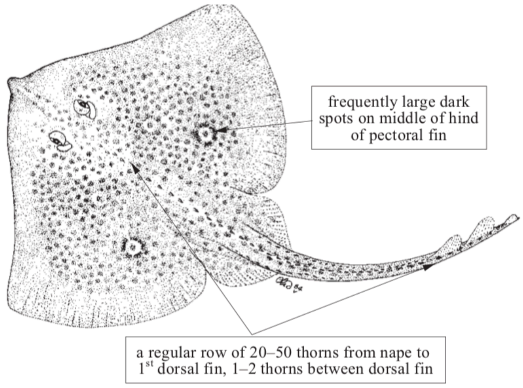


Figure 1.10- R. montagui *from the Field identification guide (FAO)*

The spotted ray (Fig. 1.10) reaches about 80 cm Total length. It's a benthic species found from shallower waters up to 600 m, but common at 100 m. It's distributed in the Western Mediterranean and has commercial value in the Northern Adriatic. Usually captured by bottom trawl fishery. Oviparous, 24 to 60 egg-cases per year laid in summer, embryos developing in 5 to 6 months with young hatching at size of 11 to 12 cm TL. Sexual maturity reached at about 60 cm TL.

- ***Raja miraletus*** *(Linnaeus, 1758)*

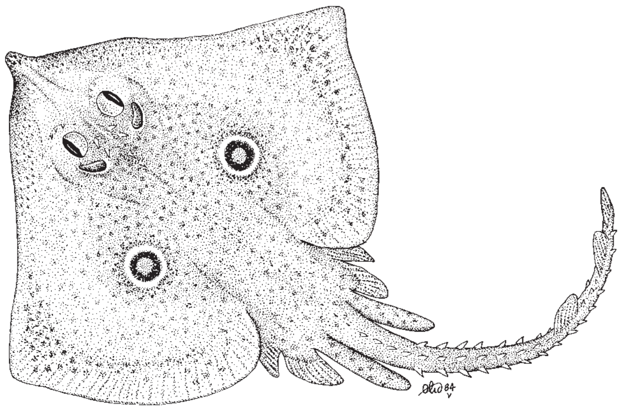


Figure 1.11- R. miraletus *from the Field identification guide (FAO)*

R. miraletus ("razza occhialina") (Fig. 1.11) it's slightly smaller than the others: it can reach 50/60cm of total length but usually is 25/30cm. This skate is benthic mainly on soft bottoms from 50 to 150 m. Males and females mature at 24 cm disk width. It is usually bycatched in bottom trawl fisheries.

- ***Raja radula*** *(Delaroche, 1809)*

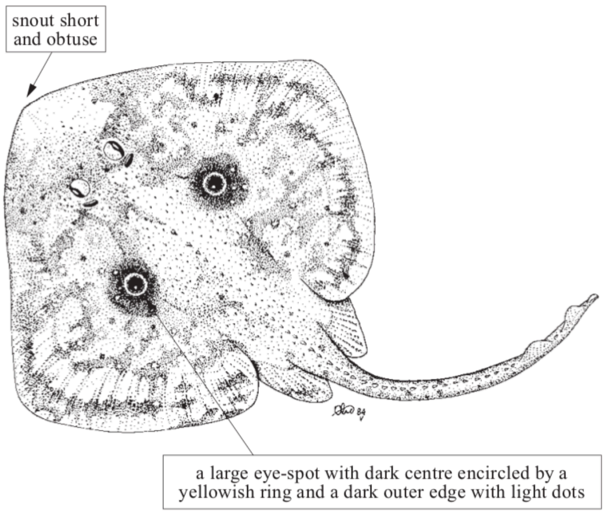


Figure 1.12- R. radula *from the Field identification guide (FAO)*

R. radula (Fig.1.12) reaches about 70 cm total length. It's a benthic species and can reach 350m. It is oviparous and the egg-case are laid throughout the year, mainly in spring and summer. Females mature at 34 cm, males at 30 cm of disc width. They are present in the whole Mediterranean but mainly in the western part and absent in the Black Sea. Bycatch in the bottom fisheries in coastal grounds. It is an occasional/rare species and needs to be investigated.

2. AIM OF THE STUDY

Studies concerning meso-predator population assessment, feeding habits and trophic relationships with other resources are essential seen their key role in ecosystems. In the northern Adriatic Sea, which is already depauperated due to the high fishing intensity all along the shores, the commonly perceived increase in a resource like the one of meso-predators could cause difficulties in resources placed at basal levels in the trophic network. There are already some studies concerning the diet composition and community of various batoid's species in the Adriatic Sea but none of them is made in recent years or in the upper zone.

In this work, the objective was to present a recent assessment on the feeding ecology and population of batoids in the northern Adriatic, using three different approaches, including one that appears to be innovative in the area.

We tried to answer to three main research questions:

1) How the population of meso-predators has settled in the Northern Adriatic Sea without the presence of top-predators?

2) Are rays and skates increasing or shifting in composition in the area?

3) Which are the trophic relations of meso-predators with other marine resources in this overexploited system?

We used: i) Local ecological knowledge (LEK) and fishery data, ii) analysis of stomach contents and iii) BRUV deployments.

More in detail, we tried to evaluate the diet composition and the trophic level of rays and skates trough the analysis of stomach contents of individuals collected in the North Adriatic, more precisely Ancona's Fishery. Using fishery data it was possible to better understand and evaluate short-term trends of individuals, shifts in species composition and the relation with other resources. As the trends covered only ten years of markets' data, we implemented the LEK in order to make a valuable use of the historical memory and present perceivings of fishermen, through the use of a structured interview and a photo identification guide.

Then with BRUV deployments, as a non-invasive technique, we wanted to assess the population and measure individuals. Unfortunately, due to COVID restriction, it was not possible to complete this aspect: we succeeded in the hard-working calibration of cameras on the BRUV and did pilot deployments in order to test the measurements, but further work is scheduled as soon as it possible.

We summarize the results of these approaches aiming to the possibility to evaluate diet composition, trophic level and shifts in population and we suggest further work to be made to understand if the increase of these species can affect in any way fishery resources and which could be future developments for the common fishery.

3. Methods and Material

3.1. Local Ecological Knowledge and Fishery Data

Ecosystems especially in marine environment are often subject to the impact of multiple anthropogenic stressors (e.g., pollution, eutrophication, ocean acidification, and fishing). Each of those can cause biodiversity loss, habitat degradation, and stock declines [51], [52][53]. Fishing activities, in particular those employing non-selective gear such as bottom trawling (as seen in this study) are considered one of the most important anthropogenic sources of marine ecosystem decline, causing both direct and indirect impacts on marine populations and habitats [54], [55]. These impacts are evident in the Mediterranean Sea, which combines a long history of exploitation with a high level of social, economic, and political complexity that present major challenges for effective marine management and conservation [52], [53].

Over the last decades, “Local Ecological Knowledge” (LEK) has emerged as an alternative approach to collecting information on species presence or abundances historical data are lacking [56]. However, up to now, the use of LEK in the Mediterranean Sea has been limited to collecting information and describing trends in fish diversity and abundances [57], and discarding of commercially important fish species in the bottom trawl fishery [58]. Here, we tried to apply LEK to examine the temporal change of batoids in the Northern Adriatic Sea, because if we want to deal with a fishery problem it is important to support data with the knowledge of people working in the sea every day.

For this reason, an interview was carried out with a photo identification guide, and results collected to better understand if the hypothesis sustained in this work could be supported from fishermen direct observation.

Interviews were carried out in Ancona, Fano and Senigallia, but more are scheduled in the next future in Ravenna and Rimini.

Another important part was the analysis of data from different fisheries in the Northern Adriatic Sea: Chioggia, Ancona, San Benedetto kindly gave us 10 years of data collected from 2010 to 2020 (Fig. 3.1). This helped to assess the biomass of the most commercialized rays and skates in past years. These species appear to be of quite important economic value, seen that in the Northern Adriatic there are many traditional food recipes.

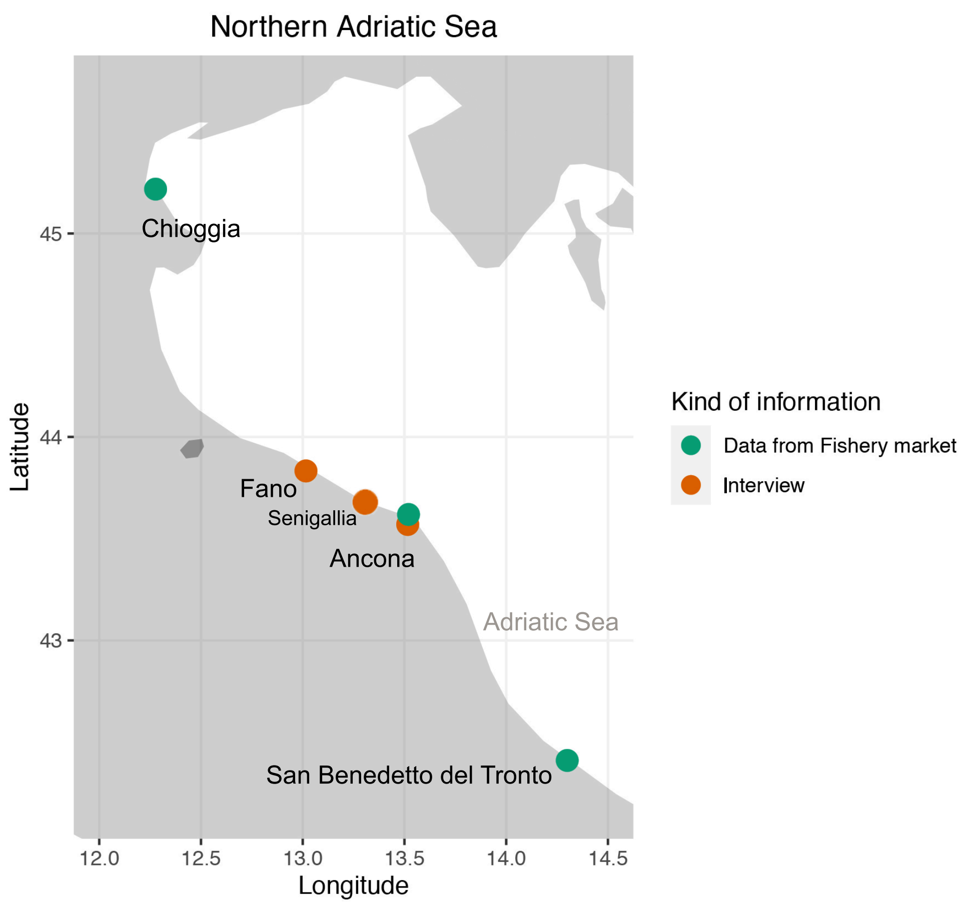


Figure 3.1- Map of the Northern Adriatic Sea with the cities from which we obtained interviews (orange) and data from fishery market (green).

3.1.1. *Study area*

The study was conducted from July to December 2020, in the most famous fishing ports of the Marche region (Italy, central Adriatic Sea): Ancona, Fano, and Senigallia. The area is characterized by sandy-muddy bottoms [59] with depths that do not exceed 100 m.

Fishing is intense in the Adriatic region, especially in the Italian side. The Northern part is a heavily exploited basin and its health and marine communities are influenced by the anthropogenic pressure.

Concerning fishery data to assess short-term trends, we obtained 10 years of market's data from Ancona, San Benedetto del Tronto and Chioggia, in order to complete studies with long-term trends such the ones from Ferretti *et al.* [91] and Barausse *et al.* [92].

3.1.2. *Interviews*

"Local Ecological Knowledge" (LEK) has emerged as an alternative approach to collecting information on species presence or abundances when historical data are lacking [56]. However, the use of LEK in the Mediterranean Sea has been limited to collecting information and describing trends in fish diversity and abundances [57] or discarding of commercially important fish species [58]. We tried to apply LEK to examine the temporal change of batoids in the Northern Adriatic Sea.

To overcome limitations in data availability it could be useful to take account of fishers' perceptions to document changes in marine ecosystems. Historical memories of experienced fishermen and skippers are still vivid and can provide valuable information on decades spent at sea fishing [58]. This approach could substantially contribute to improve the historical picture and understanding of the fisheries and associated fish communities, usually obtained from other sources. Useful information on description of fishing practices, reconstruction of the trends of exploited stocks and changes in the species population structure can be obtained.

However, there are some discrepancies due to human error in remembering past memories, but they can be easily overcome using LEK as non-only source of information. Fishermen's traditional ecological knowledge could have an important role in the management process because it constitutes a complementary source to integrate new knowledge in fisheries biology and marine ecology [58].

Information was gathered using a structured interview. In each port, we interviewed only otter trawl fishers, identified through their main associations or cooperatives. Fishers were selected on their availability to participate to our survey. An "Oral Consent Procedure" was followed: all potential interviewers were provided with the purpose of the study and with the usage of collected data before obtaining their consent. All involved fishers willingly agreed to participate in the survey. Interviews were kept anonymous and responses were coded with a numeric identifier making it impossible to disclose any personal sensitive data and track the individual fishers.

The interview was completed with a photographic guide with the main species that usually occurs in the area: *R. asterias, R. clavata, R. montagui, R. miraletus, R. radula, D. pastinaca, D. centroura, P. violacea, M. aquila* and *A. bovinus.* These species were selected according to literature knowledge about the most common species and most seen in the market.

First of all, we asked questions about each fisher: age, year he started fishing, which fishing gear was used, how many days per week he goes on a fishing trip and if/when he noticed an increase or decrease in rays catches. Then we used a photographic guide to identify and match the species and ask the abundance of each.

Hence, we used a qualitative class to observe abundances: “rare” if the species is observed less than 1-2 individual per month, “occasional” if 3-10 ind/ month, “common” if 10-50 ind/month, “very abundant” if more than 50. We also asked if the species was seen every fishing trip, every month or every season (and specify season).

3.1.3. *Fishery data and landings in Northern Adriatic*

Time series of species abundances are crucial to understand changes of marine communities driven by long-term anthropogenic pressures and, therefore, to develop strategies to effectively manage marine resources. Historical quantitative data can depict the decline and recovery of exploited stocks, fluctuations in population abundance in relation to environmental factors including climate changes, changes in species distribution, variation in community composition, predator-prey cycles, etc. [62].

Fishery-dependent data are known to present some limitations: they are usually not standardized on fishing effort, they can be affected by changes in fishing practices or exploited areas leading to misinterpretation of changes in resource abundance, they are often not species-specific and usually include only commercial species, they do not take into account discarded or illegal catches, they can be prone to misreporting, etc. Despite these limitations, historical fishery data on catches can provide useful and otherwise lacking information, thus filling the gap between discontinuous scientific surveys, including species not included in such surveys and broadening the time range of scientific data [63],[64], [65].

Life history characteristics, such as large size, slow growth rate, late maturity, and low fecundity result in low reproductive rates and make elasmobranchs more sensitive to exploitation than bony fish [66].

The goal of this study was to investigate the actual status of batoids in the northern Adriatic Sea in relation to fishing pressure and environmental factors, using (apart from interviews) also fishery data from the fish market of Chioggia, Ancona and San Benedetto del Tronto. The Italian side of the northern Adriatic is home to the major fishing fleet of the basin. Official markets data from Chioggia's fishery fleet were retrieved from the database, instead from Ancona and San Benedetto we directly asked to the director of each fish market who willingly agree to share them. Data (weight in kilograms) were available yearly from 2010–2020 for Ancona and San Benedetto, instead for Chioggia we had from 2010 to 2019. Ancona and San Benedetto gave us monthly data and rays are divided in "Razza chiodata" "Razza stellata" and "Razza occhialina", with all the total sold weight in kg, the price and minimum and maximum eur/kg.
On the contrary, Chioggia does not have specie-specific data but has all rays classified in the same group, hence we only had the total weight sold yearly.

We could use the data from 2010-2019 of each fishery to report trends in total sold weight of rays for three main fisheries. Furthermore, with data from Ancona and San Benedetto we could divide the species (with common names) into groups and see the trends in the sold weight from 2010-2020. Species for "Razza chiodata" "Razza stellata" and "Razza occhialina" are *R. clavata*, *R. asterias*, *R. miraletus* respectively. We also extracted the biomass sold of crabs, as they appear to be the preferred food for rays and skates.

3.2. ANALYSIS OF STOMACH CONTENTS

The analysis of stomach contents can be useful to assess the feeding habits and dietary composition in various organisms. Concerning rays and skates, they appear to be meso-predators so they may play influential roles in the food webs of demersal marine communities [67]. It has been hypothesized that skates may negatively impact commercially valuable ground fishes via ecological interactions such as competition and predation [68]–[71]. However, quantifiable studies on the diet composition and trophic relationships of skates are few [72][73]. Additionally, although some skate species are considered top predators [74]–[77] surprisingly few quantitative estimates of trophic level values exist to substantiate this perception [78]. Therefore, to better understand the ecological role of rays and skates, in this study we also present the trophic level values compared to the ones found on FishBase (https://www.fishbase.de), besides the diet composition.

3.2.1. *METHODS*

In order to analyze stomach contents, it was necessary a close cooperation with local fishermen (especially in Ancona, due to COVID restrictions). In this study, it was crucial to gain some trust from local fishermen and get into their fishing habits.

Once they understood the aim of the study, they appeared willing to help and started to notify us with landings of rays or bycatch of species that were not commercially useful (e.g., stingrays like bull ray, eagle ray, etc.). All the samples were caught with bottom trawling vessels.

Skates in Ancona are not targeted in fishing, but they have enough commercial value and so they are sold in the market.

Another important aspect was to record fishing data: kind of fishing gear, depth, distance from coast and date of capture (Fig. 3.2). This helped us with evaluating if there were correlations among size, depth, and distance from the coast.

Sample	Code	Species	date	Method	Depth(m)	Where	Sex	Weight (gr)	L_T (cm)	D_L (cm)
1	RA 1	*Raja asterias*	16/07/20	trawling	30	3 miles from Ancona	F	1300	47.5	37
2	RA 2	*Raja asterias*	16/07/20	trawling	30	3 miles from Ancona	F	1100	40.1	31
3	RA 3	*Raja asterias*	23/07/20	trawling	12	15 miles from Ancona	M	130	23.5	17
4	RA 4	*Raja asterias*	23/07/20	trawling	12	15 miles from Ancona	F	170	23	17
5	PV 1	*Pteroplatytrygon violacea*	23/07/20	trawling	12	15 miles from Ancona	F	7000	113	55
6	AB 1	*Aetomylaeus bovinus*	22/10/20	trawling	25	5 miles from Ancona	M	18000	182	103
7	AB 2	*Aetomylaeus bovinus*	21/10/20	trawling	18	4 miles from Portonovo	M	1500	72	39
8	RA 5	*Raja asterias*	21/10/20	trawling	15	4 miles from Ancona	M	130	28	19
9	RA 6	*Raja asterias*	21/10/20	trawling	15	4 miles from Ancona	M	116.5	27.5	18.5
10	RA 7	*Raja asterias*	21/10/20	trawling	15	4 miles from Ancona	F	142.7	28.5	21
11	RA 8	*Raja asterias*	21/10/20	trawling	15	4 miles from Ancona	M	111.3	26	18
12	RA 9	*Raja asterias*	21/10/20	trawling	15	4 miles from Ancona	F	113.4	26	17.1
13	RA 10	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	F	196.6	30	22
14	RA 11	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	F	195.1	30	20.7
15	RA 12	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	F	305.9	33	24.2
16	RA 13	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	F	237.4	31	23
17	RA 14	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	M	174.8	no tail	21
18	RA 15	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	F	157.7	31	21
19	RA 16	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	F	253.8	33	24.3
20	RA 17	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	F	1000	45.5	33
21	RA 18	*Raja asterias*	20/10/20	trawling	15	6 miles from Ancona	F	1600	47.5	36
22	AB 3	*Aetomylaeus bovinus*	29/10/20	trawling	25	6 miles from Ancona	F	1600	72	45
23	PV 2	*Pteroplatytrygon violacea*	04/11/20	trawling	30	11 miles from Ancona	F	8500	no tail	63
24	RA 19	*Raja asterias*	04/11/20	trawling	30	11 miles from Ancona	M	172.2	31.6	20.5
...	...	...	...	...	...	...	...	...	...	...

Figure 3.2- Example of metadata sheet for collection of samplings

All samples were caught mainly in the surroundings of Ancona (Fig. 3.2), in a range of distance from 3 to 18 nautical miles at varying depths (12-60 meters) on sandy and muddy bottoms.

Organisms were collected at the port right at the end of fishing trip or the next early morning.

In laboratory (Fig. 3.3) all samples were analyzed with same protocol:

- Photographed
- Sexed (based on presence of claspers)
- Weighted

- Measured (total length, disk width)

a) b)

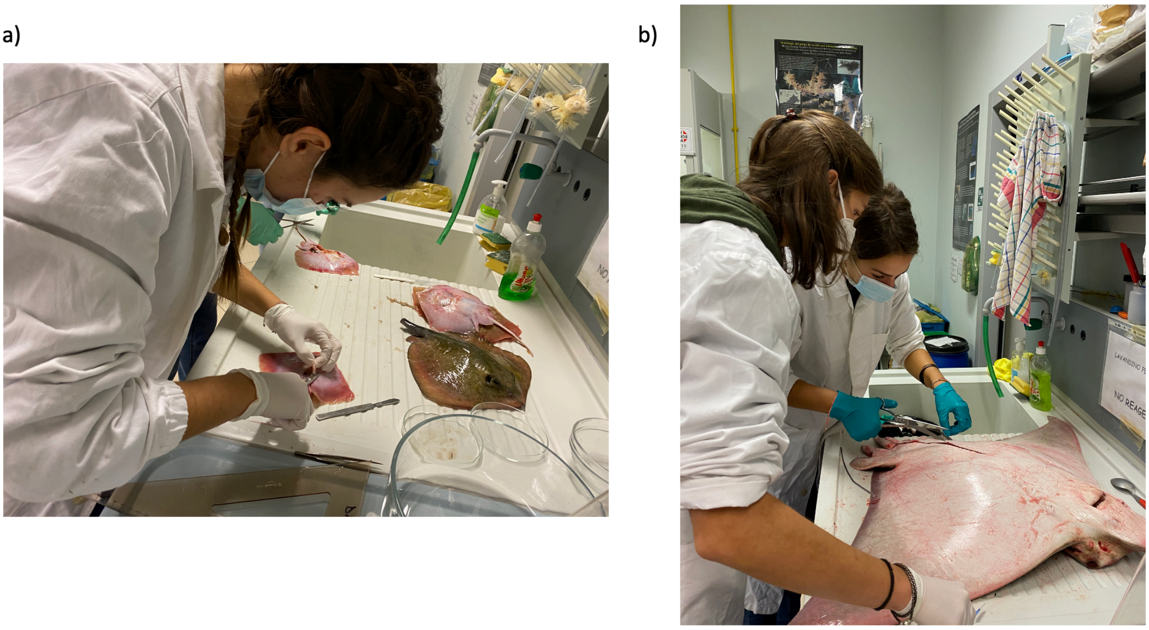


Figure 3.3-Lab activity of dissection of batoids, a) Raja asterias *and b)* Aetomylaeus bovinus.

After measurement (Fig.3.4 and 3.5), the specimens were dissected to obtain the stomachs, which were cut, weighted and emptied. We assigned the index Empty of Full (considering FULL a stomach with at least one piece of food). At the end the tissue was separated from the content and both stored in alcohol 70° for further analysis.

Stomachs content were subsequently dryed from alcohol residuals and analyzed. The prey items were sorted and divided in different categories. All categories were weighted and counted. In most cases, the prey count was based on the number of different typical parts: beaks or bones for cephalopods; carapaces, legs or chelipeds for crustaceans; otoliths and whole vertebral columns for fishes [79].

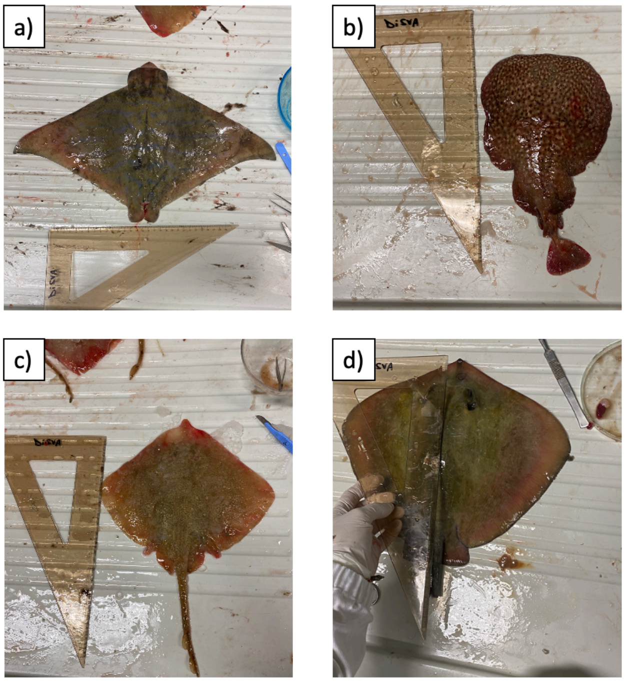


*Figure 3.4-Example of species before dissection: a)*A. bovinus*, b)*T. marmorata*, c)*R. asterias*, d)*D. pastinaca

Figure 3.5-Biggest individual of A. bovinus *sampled with close-up on mouth*

The determination of lowest taxonomic level was not possible in most cases because the level of digestion was far advanced.

3.2.2. *DATA ANALYSIS*

Due to the unbalance between samples in different families, the dietary composition was only assessed for Rajidae, while for the other families we only calculated the Trophic Level (T_L) [80], the Vacuity index (V_i=number of empty stomachs/total number of stomachs × 100) and the sex ratio in catches.

Rajidae were grouped into <u>five size groups according to the Total Length</u> (L_T).

In order to describe the diet, the following indexes were calculated: the vacuity index (V_i= number of empty stomachs/total number of stomachs × 100), relative frequency of occurrence (%F= number of stomachs containing the prey *i* /total number of filled stomachs × 100), relative numerical abundance (%N = number of the prey *i* /total number of preys × 100) and relative gravimetric composition (%W= weight of the prey *i* /total weight of all preys × 100). The index of relative importance (IRI) of Pinkas et al. (1971) [81], as modified by Hacunda (1981) [82]: IRI = %F × (%N + %W). This index, that integrates the three previous percentages, allows an interpretation much more real for food by minimizing the skews caused by each one of these percentages [83].
IRI was expressed also as:

$$\%IRI = 100\frac{IRI}{\sum IRI}$$

The hypothesis that the diet changed in relation to body size and sex was evaluated with a generalized linear model (GLM). The numbers of prey items from the most important prey groups (fishes, crabs, stomatopods, amphipoda, cephalopoda, shrimps and batoids) were used as dependent variables. These appeared to be count data: in such data the errors may be distributed non-normally and the variance usually increases with the mean values, hence we specified a "Poisson" error distribution [84]. The categorical independent variables included in the GLMs were size classes and sex.

We calculated also the Shannon- Wiener diversity index *H*' [85] to evaluate if there was more diversity between males/females and between size groups.

$$H' = -\sum_{i=1}^{S} pi \ln pi$$

where pi is the proportion of individuals belonging to the i^{th} species. The value of *H*' increases with species diversity.

We also made a *t*-test to see the differences between males and females in their feeding habits taking account of the prey categories.

Regarding Rajidae we also carried out a Pearson's correlation between the L_T (total length) and the distance from coast where they have been caught, and L_T and depth, then a linear regression between length and weight of stomach contents and total weight and total length.

Table 3.1- Prey categories used to calculate standardized diet compositions and trophic levels – compiled from Cortés and Ebert & Bizzarro. [67], [80].

Prey Category	Inclusion	Trophic Level (T_L)
MOLL	Molluscs (excluding Cephalopoda)	2.1
AMPH	Amphipoda	3.18
BRACH	Brachyura	2.52
CRUST	Crustaceans (other than elsewhere specified), including Stomatopoda	2.4
FISH	Fishes	3.24
CEPH	Cephalopoda (cuttlefishes, squids, octopuses	3.2
ELASM	Sharks, skates, rays	3.65

Seven food categories were considered to calculate standardized diet compositions and trophic levels of batoids (see Table 3.1). The formula to calculate the proportion that each prey category P_j makes up of the diet is:

$$\boldsymbol{P_j = \frac{\sum_{i=1}^{n} P_{ij}\ N_i}{\sum_{j=1}^{7}\left(\sum_{i=1}^{n} P_{ij}\ N_i\right)}}$$

P_{ij} values were calculated using quantitative method such as the %N, N_i is the number of samples of that species, j is the number of prey categories (total of 7).

Trophic levels ($T_{L(K)}$) were then calculated for *Raja asterias*, *Raja miraletus, Aetomylaeus bovinus*, *Pteroplatytrygon violacea* as:

$$T_{L(k)} = 1 + \left(\sum_{j=1}^{7} P_j * T_{L(j)}\right)$$

Where $T_{L(k)}$ stands for each species taken into account, $T_{L(j)}$ is the trophic level of each prey category *j*. Trophic level (T_L) of prey categories was taken from [67], [80].

We made also a PERMANOVA analysis with sex as factor and length as covariable to confirm our results.

Principal Coordinates Analysis (PCO) of data was carried out in order to examine the multivariate relationships between the samples in relation to the sex, length and number of preys in each stomach.

All statistical analysis were made using R 3.6.1 GUI 1.70 El Capitan build [86], and PRIMER v6 [87].

3.3. BAITED REMOTE UNDERWATER VIDEO

We introduce a non-invasive sampling method for the assessment of rays and skate population: baited remote underwater video.

Baited remote underwater stereo-video systems (stereo-BRUVs) are a popular tool to sample demersal fish assemblages and gather data on their relative abundance and body size structure in a robust, cost-effective and non-invasive manner [88].

Stereo-BRUV application enable cost-effective measurements of body size, which surpass estimates made by divers [89].

As a non-extractive technique, they have little impact on the assessed ecosystem and overcomes some of the biases associated with Underwater Visual Census (UVC) techniques [90]. Remote video eliminates the need for scuba diving, providing a strong safety advantage, while reducing the risk of incorrect fish identifications and inter-observer variability through recording a permanent and reviewable record. Furthermore, video techniques can access depths that are off-limits to divers. Multiple stereo-BRUVs can be deployed in the field consecutively, making even more efficient use of researcher and boat time [91][92].

The extent of the limitations and possible biases of stereo-BRUVs have been discussed in various studies [91][93] like the overestimations of abundance. This problem can occur when same individuals are seen at different time points in deployment: to overcome the problem, usually the MaxN is used, consisting in the count of the maximum number of individuals of any one species seen over the recording period [92].

There are 2 approaches to calibrating stereo-video cameras; both require the filming of an object with known dimensions or distance between landmarks. The 2 objects are either (1) a 3D open cube structure with markers placed on the camera-facing side of both the front and rear parts of the cube or (2) a 2D checkerboard object. Recently, 2 studies have assessed the accuracy of these calibration approaches. Wehrkamp & Fischer [94] showed that the 2D calibration approach was able to yield errors of less than 5% [95].

This BRUV in particular was entirely designed by using 3D s/w SolidWorks ™ (www.solidworks.com) taking into account of literature measurements and design [96]. Then a prototype has been built in a referenced workshop.

Videos for stereo-videography were made with two GoPro Hero 8 black and the calibration of the system with R package- "*StereoMorph*" ([97]; https://cran.r-project.org/ package =StereoMorph).

Due to COVID restriction and weather conditions, we were not able to follow the schedule as planned in sampling design.

However we calibrated the system both on land and in a pool, we also did pilot deployments in open sea: two for the calibration and one with the use of the bait trap, 6 miles away from the coast and we tried to measure objects of known length with the minimum possible error.

Further deployments are currently programmed as following the sampling design.

3.3.1. *DESIGN*

This BRUV in particular was entirely designed by using 3D s/w SolidWorks™ taking into account of literature measurements and design [96]. Then a prototype has been built in a referenced workshop. The BRUV is made of stainless-steel with a height of 70cm, weight 18kg. It is made of four pipes which provide strength, four legs angled 45° degrees to improve stability and balance. We added two bars screwed at the structure to prevent the sinking in the sediment (Fig. 3.6).

Two cameras can be mounted 700 mm distant one another (for stereo-videography) or a single camera in the center of the base bar.

In the middle of the base bar it is possible to mount another pipe with the bait canister at the end.

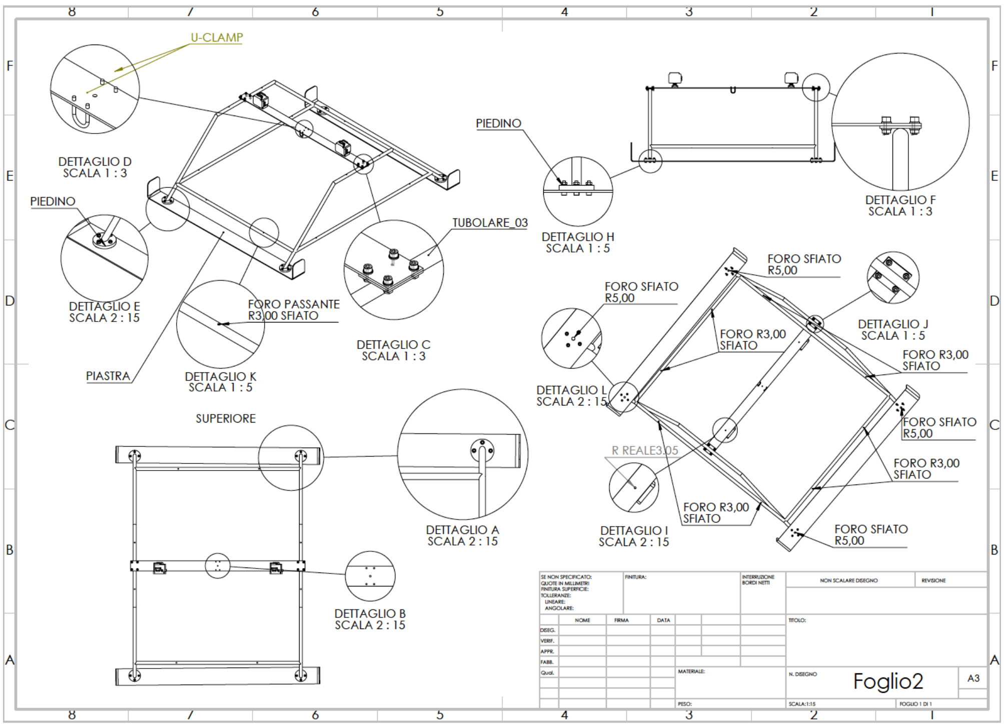


Figure 3.6- Preliminar SolidWorks table of our BRUV design

The 2 cameras are mobile, to choose the right angle for the stereo-videography (usually 6-8°degees).

If a single camera is used, results can be applied to make a population census. With 2 stereo-cameras (after calibration) it is possible to estimate organisms' length.

Our final system comprised 2 GoPro Hero 8 black in standard underwater housings. In order to prevent an incidental movement of cameras and a loss of the calibration alignment we used also two thick PVC pipes to protect them (Fig. 3.7).

GoPros were positioned 700mm apart with converged angle inwards at ~7°. These parameters provided accurate results for fish and megafauna body length estimates in previous studies [98].

Figure 3.7- Final design of the Baited-remote system

Cameras' resolution is set at 1080p to reduce battery loss and obtain at least 1h videos or time lapses with a single battery. Both cameras are set at the wide (16-34mm) field of view

(FOV) setting, and are synced by turning a dive light on and off while pointed at the cameras at the start of each filming trial. We used the resulting visual cue to set a common start time. Photogrammetric length measurements are made with some degree of error, which can be minimized by measuring only individuals when they are as close to cameras as possible [99].

3.3.2. *SAMPLING DESIGN*

Due to COVID-19 restriction it was not possible to carry out the whole sampling design we arranged. The original idea was to cover 3 main areas of deployments in the North-Western Adriatic Sea:

- Ancona (lat 43.5942, lon 13.50337)
- Fano (lat 43.84052, lon 13.01665)
- Chioggia (lat 45.21857, lon 12.27774)

Each site had different bottom structure, but they are all subjected to massive fishing.

Ancona and Conero area are mainly structured with rocky surfacing near coast and sandy substrate, while in Fano there are artificial substrates that probably could work as FAD (Fish Aggregating Devices). Finally, Chioggia (besides the usual sandy substrate) has some rocky surfacing offshore and it is also subjected to freshwater input from the Po river (Fig. 3.8).

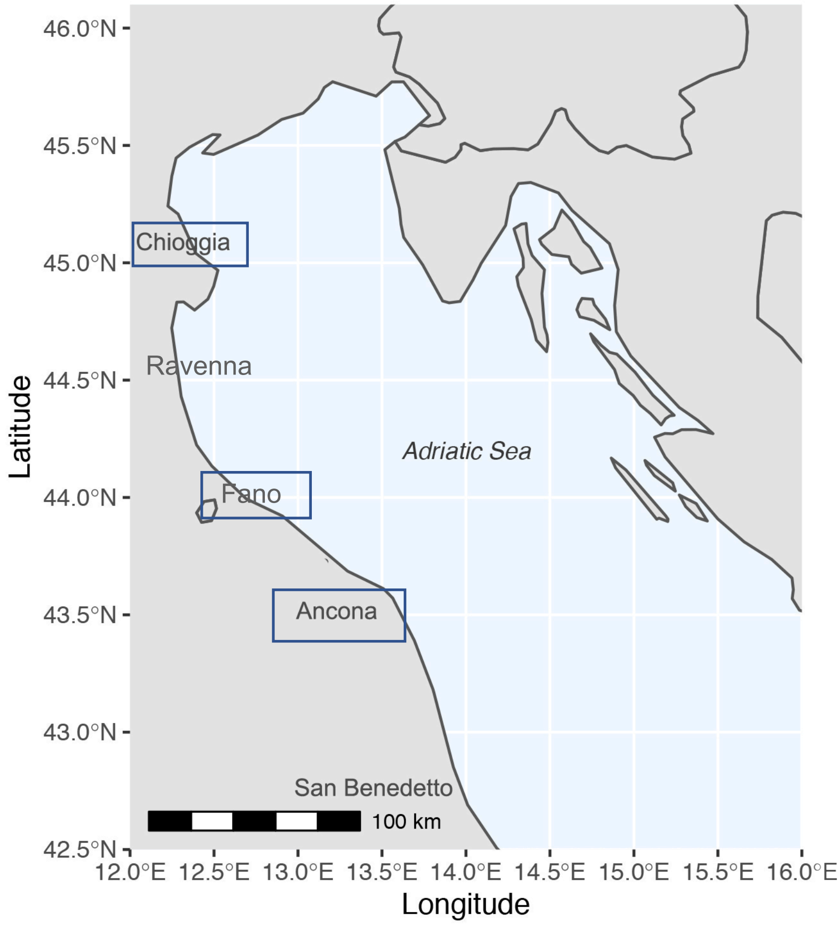


Figure 3.8- Map of the area for the sampling design, with the three main station for deployments

The analysis we would have conducted in the 3 locations could have allowed us to understand if these points work as nursery areas or if juveniles approach their shores, taking into account the huge exploitation due to fisheries.

According to boat availability and weather, we planned to do at least 36 deployments in total. We would have chosen 3 places per area and in every point, we would do 3 drops. Each BRUV drop would work as a replicate per site.

Separation distance between replicates depends on the mobility of the species and the habitat being studied: for typical demersal fish assemblages, a minimum of 500 m for 1-hr deployments is recommended [100] or 250 m for 30-min deployments [101], to avoid bait plume overlap and animals moving between cameras.

Our goal was to move at least 300m for each drop within sites randomly chosen in the area.

There is no optimal soak-time for deployments, different studies used soak times from 30 to 90 min. Our aim was to do 50/60min of soak time, with the first 10 minutes used to settle the structure on the substrate and time of plume dispersion and at least ~40min of footage available for measurements.

Distance from coast will be subjected to the boat license and will probably be 6-8miles from coast to have higher probability to find batoids not disturbed by top predators or fishermen.

The equipment needed for the field work is:

1. Ropes for easily soak and retrieval
2. Marker buoy (Fig. 3.9)
3. BRUV support
4. 2 GoPros and additional batteries
5. Bait traps: anchovies, crustaceans, sardine

Regarding bait type there are many studies that conclude the benefits of using bait in marine environments: it appears to outweigh any perceived costs.

Bait choice is a discussed issue: Dorman *et al.* (2012) [80] and Wraith *et al.* (2013) [81] each investigated different bait type. Dorman *et al.* compared sardines, cat food, and a vegetable mix with unbaited controls and found similar assemblages between these three bait types but still many authors recommended using oily fish such as sardines [91].

Figure 3.9- BRUV deployment and marker buoy.

Figure 3.10-Trial deployment.

Considering our soaking time and distance between replicates, one kilo or one kilo and half of chopped sardines has been ideal as bait trap. This was placed in the canister and settled on the pipe in the middle of the structure. Distance from the base bar is 110cm.

Cameras and trap are raised 70cm from the ground.

Deployments are planned in the morning (Fig. 3.10), at least half an hour after the sunrise to improve visibility. Depths range from 20m to 50m.

It is important to take note of place, coordinates, depth, data, site and timing for every BRUV drop, hence we created a sheet for all the metadata and comments.

3.3.3. *CALIBRATION*

The system was calibrated by filming a checkerboard 9x11 (measures: total dimension= 840x700 mm, square size 70x70mm) (Fig. 3.11) both outside and inside water. Firstly, we tried some calibration outside the water because we wanted to test the 2D calibration method and minimize errors as much as possible.

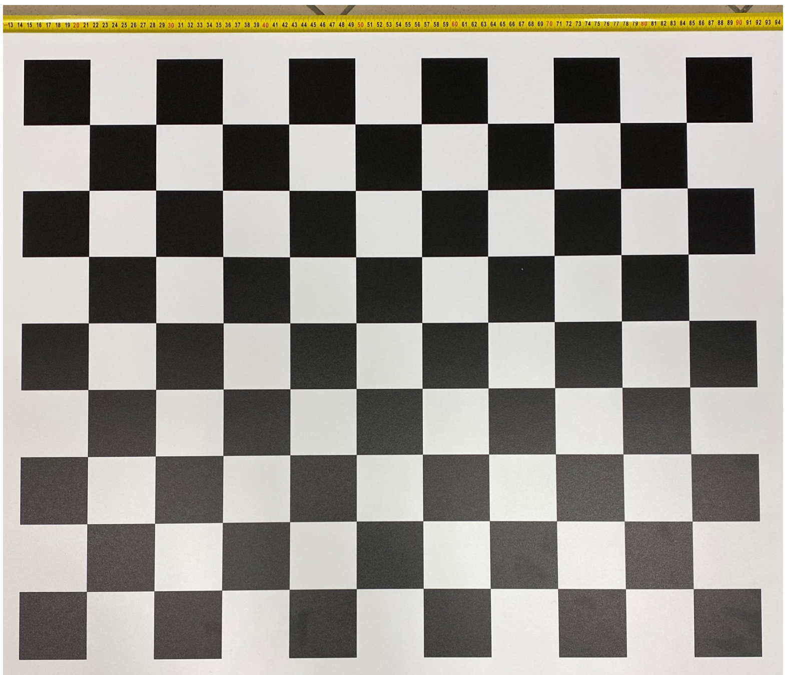


Figure 3.11- Checkerboard 9x11 used for calibration.

The cameras were immobilized on the BRUV structure both in dry and wet environment and the checkerboard had to be moved throughout the visual field of both cameras up to a distance of 5 m from the cameras. The board was also held at different angles to ensure a calibration for every point along the dimension (any frames in which the checkerboard was too severely angled for corner detection were ignored) (Fig. 3.12).

Figure 3.12- Examples of calibration in pool and on land

Calibration videos usually lasted from 3 to 5 minutes, then the still frames were extracted using the GoPro Quik app and used as images. *Stereo-Morph* package advise to use at least 5 to 30 frames per view [97].

Every frame has to be named in numerical order and both views need to have same name and same number of images in each folder.

The image sets and the *Stereo-Morph* function "`calibrateCameras`" were then used to calibrate the camera system. This function provides a completely automated workflow for stereo camera calibration, performing 3 main steps: checkerboard corner detection, undistortion coefficient estimation, and calibration coefficient estimation.

All the procedures were made following the *Stereo-Morph* user guide (available at https://aaronolsen.github.io/software/stereomorph.html).

The shape.file created from the "`calibrateCameras`" function is used for the measurements.

A total of about ~10 hours of footage has been processed for calibration and measurements. Dry calibration was achieved almost 85% of the trials, with a range of 5 to 15 images per view, obtaining calibration files more and more accurate every time: we tried with different size of checkerboards in order to calibrate more volume [97] (Fig. 3.13).

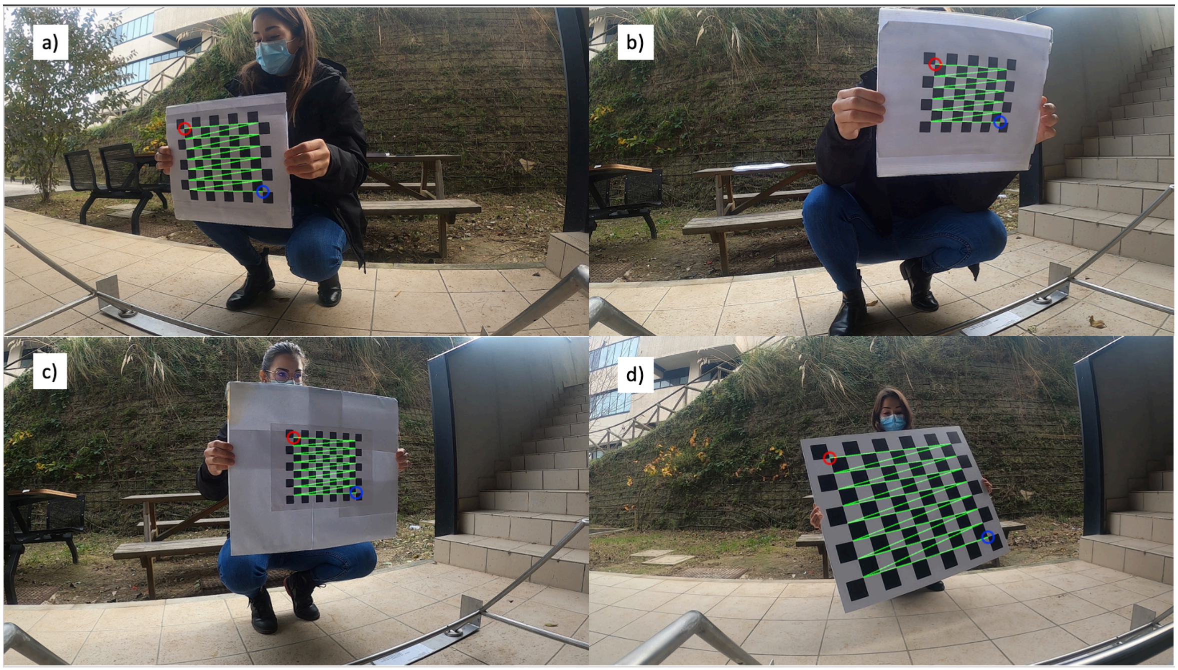


Figure 3.13- Examples of different size of the checkerboard: a)-b)A4,c)A3, d)A1.

All corners in both views were detected and measurements of objects with different landmarks also was successful.

We used objects measured with tape and assumed this measure was nearer to the real size: at the beginning the error of the "`distancePointtoPoint`" measure was higher because of the small size of the checkerboard in relation to the volume calibrated, but then we managed to create a shape.file useful for accurate measurements.

A checkerboard of A1 size (84x70 cm) is capable of measuring objects in a volume of 2 m x 3 m x 4 m and we had enough space to move the checkerboard around the view maintaining it in the frame of the video [97].

We did two trial deployment in sea for calibration (Fig. 3.14).

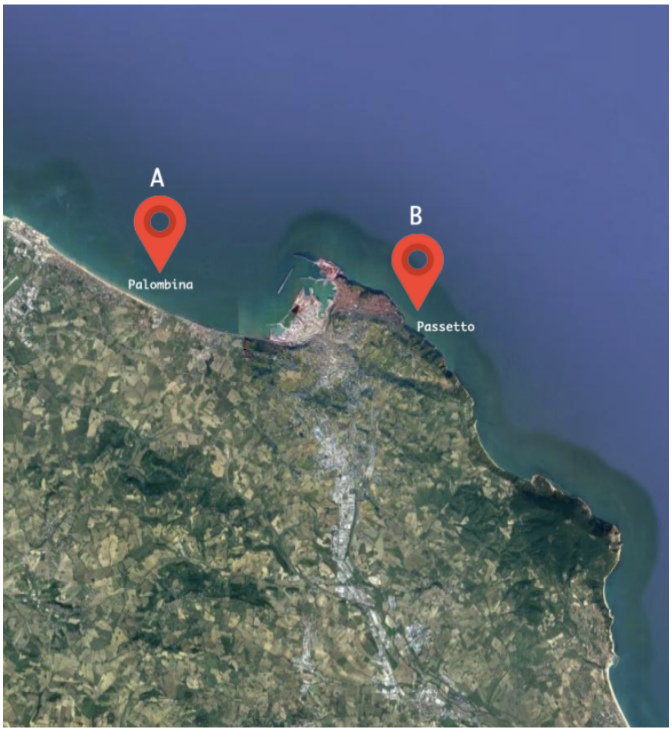


Figure 3.14- Sites of trial deployments: A- Palombina, B-Passetto.

First deployment was in Palombina (Fig. 3.14: Point A, near Ancona), but sea conditions were not optimal for the calibration due to waves and high turbidity on the water, anyway we tried to realize the calibration at 3m depth. The whole protocol worked for the deployment and retrieval of the BRUV (Fig. 3.15 and 3.16), but then videos were not suitable for the *Stereo-Morph* application.

Figure 3.15- Example procedures for BRUV deployment.

Figure 3.16- BRUV retrieval.

Second trial deployment was near the coast, in Passetto, Ancona (Fig. 3.14 B). Water visibility and weather were better, so we tried a deployment at 10m depth. The visibility was quite good but there was a strong bottom current which resulted in high disturbance of the pictures for the calibration.

After the extraction of frames with Quik app, we tried the optimalization of images with CLAHE enhancement, but even with this adjustment only corners in 4 images in both views were detected, hence we could not calculate any calibration coefficients.

With the aim to realize a successful calibration in a wet environment we end up in public pool in Ancona: the footage was suitable for the calculation of the calibration file. We extracted 30 views and corners were detected in 28/30 of pictures, then the calibration file was produced and applied to make all the measurements (Fig. 3.17).

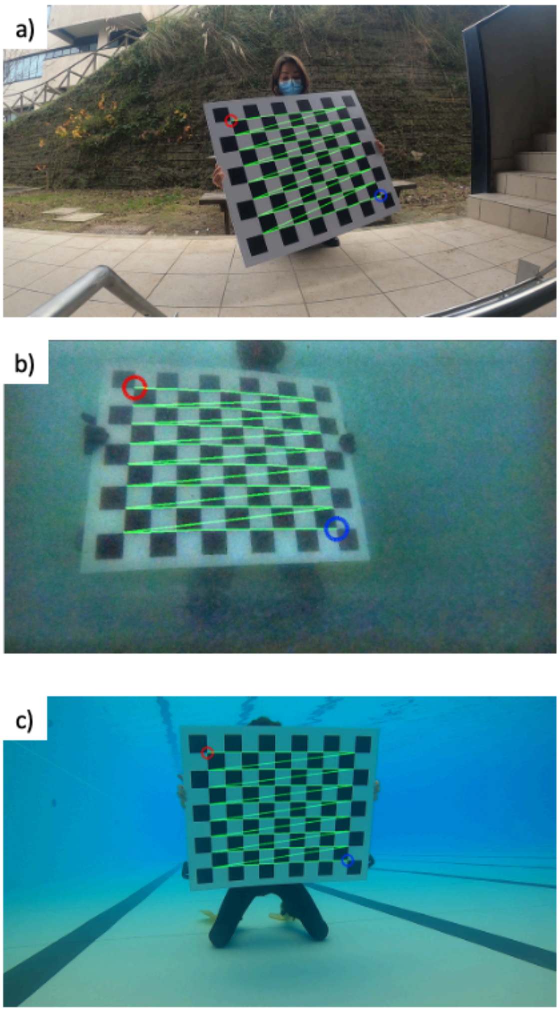
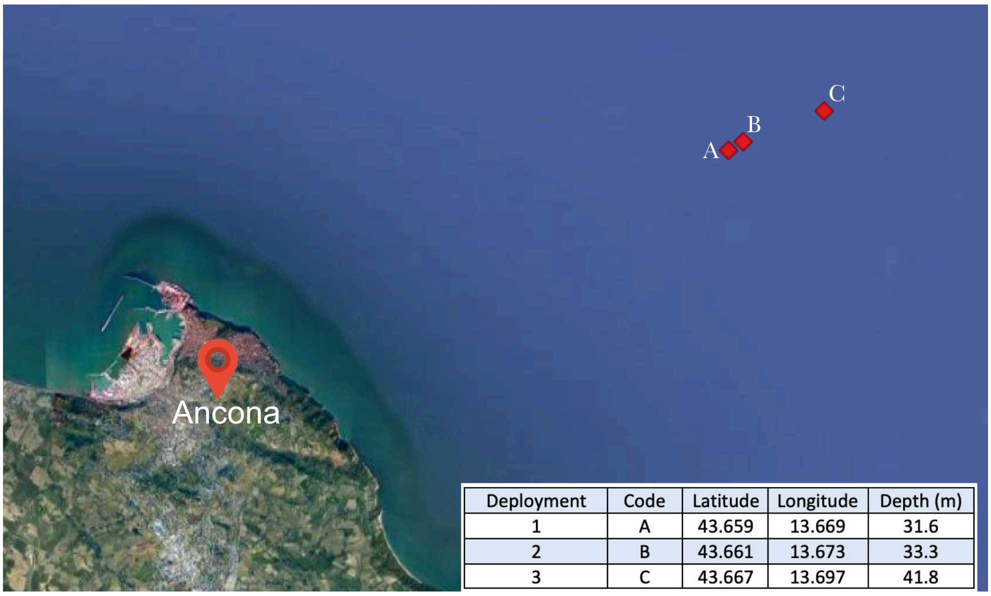


Figure 3.17- Corner detection in a) dry, b) sea, c) pool

Finally, despite COVID constrains, a first BRUV deployment was carried out with the aim to test the method on the field and make the first pool of measurements (Fig.3.18).



Deployment	Code	Latitude	Longitude	Depth (m)
1	A	43.659	13.669	31.6
2	B	43.661	13.673	33.3
3	C	43.667	13.697	41.8

Figure 3.18- Map of the 3 deployments with table showing latitude, longitude and depth of each replicate.

We used 1.5 kg of smashed sardines as a bait (Fig. 3.19) and realized 3 deployments at 300/400m of distance between each other. Three places were chosen randomly with depths from 30 to 40m. Video length varied between 42-55 minutes.

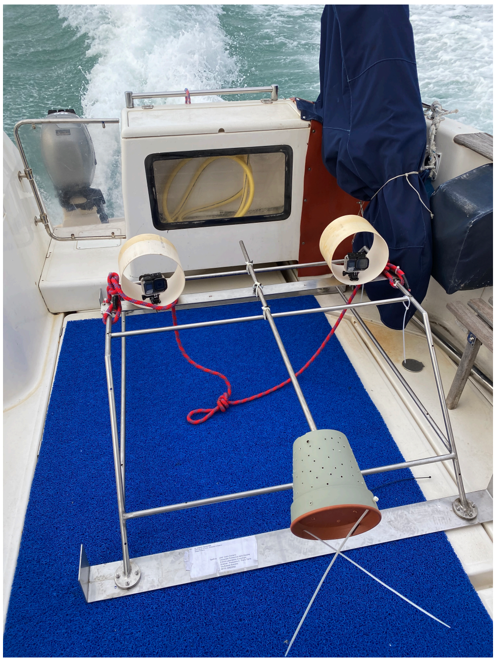


Figure 3.19- BRUV with the bait canister mounted.

3.3.4. *DATA ANALYSIS*

Footage analysis were done manually and, for both calibration and measurements, frames were extracted using Gopro Quik app.

For each replicate we would have record the percentage of BRUV drops on which a batoid occurred (frequency of occurrence or FO), and the maximum number of individuals observed together for one species over a set of time (MaxN, e.g. 5 minutes) [91], [104]. MaxN is a commonly used conservative measure of species' relative abundance in BRUV analyses because it avoids double-counting [105], [106].

Under low visibility conditions objects in the underwater images are not clearly visible due to low contrast and scattering of light and the large noise present in the environment, hence it is difficult to segmentation in such environment without losing the details of the objects. Eventual adjustments on frame extracted will be done using CLAHE (contrast limited adaptive histogram equalization) enhancement within the R package "*EBImage*". [107]

Length measurements for the trials were made by manually digitizing the relevant markers in both camera views using the Stereo-Morph digitizing application The function used is "`digitalizeImages`" and the list of the names of the landmarks to be digitized are first saved in a .txt file. The digitized markers were then reconstructed into 3D using the function "`reconstructStereoSets`": this allows the reconstruction of all the landmarks digitized in at least two views and save these into the "shapes.3d" folder using the same name as the corresponding 2D shape files. Using the function "`readShapes()`"we read the file in which the marks are in and trough "`distancePointToPoint()`" we find the length in mm of any two landmarks in each frame.

The reconstructed landmarks will be in the same units used in the calibration step (usually in mm) and lengths calculated by measuring the distance between the resulting 3D marker positions.

Eventual 3D visualization of landmarks is possible through the function "`plot3D`" within the R package "*rgl*".

Landmarks in our study were purposely chosen to gain a "profile" of the object measured. The function launches an in-built view from the XQuartz 2.7.11 support application. (https://www.xquartz.org)

4. RESULTS

4.1. LEK AND FISHERY DATA

A total of 15 interviews were conducted (to 10 fishermen from Ancona, 2 in Fano and 3 in Senigallia) (Fig. 4.1). The age of interviewees ranged from 27 to 80 years old, with most of them working for more than 30 years.

LEK could provide a reliable and alternative source of information to study the spatial distribution of species [10], [57], [60], [108] and contributes as a tool for fishery management.

Figure 4.1- Interview carried with a fisherman in Ancona.

Because of the qualitative information gathered with interviews, we couldn't provide an exact model of pattern of declines, nevertheless we could interpret and compare the qualitative background with fishery markets' data.

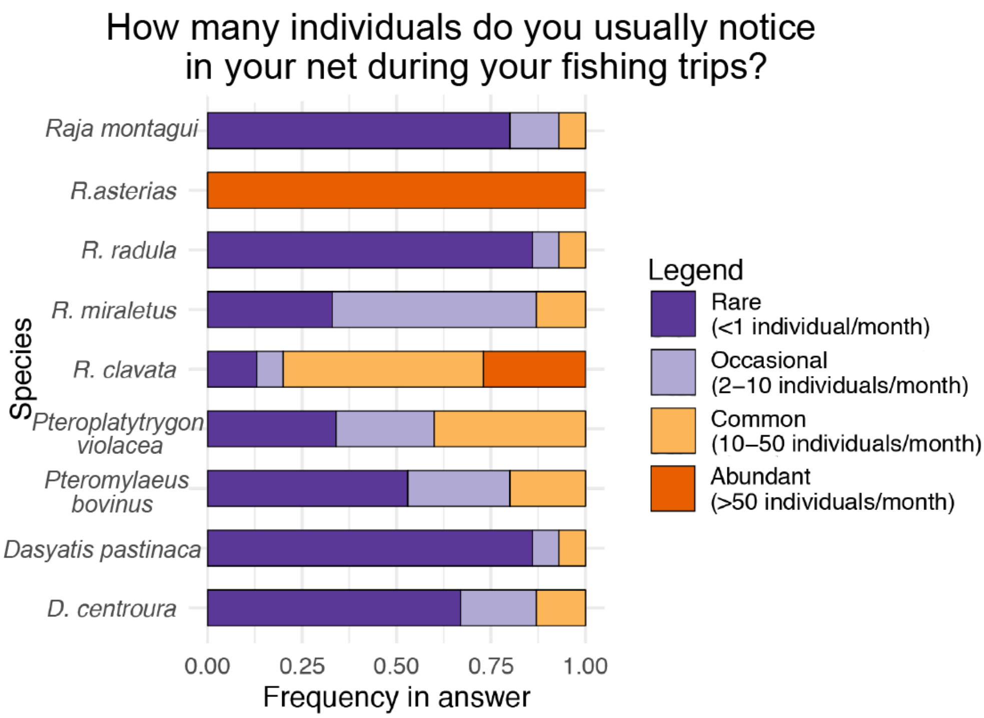


Figure 4.2- Frequency in answer to the specific question and legend with the 4 possible answers regarding abundance.

It resulted that the starry ray is surely one of the most abundant species of these years, and the most common in catches, followed by the thornback ray (Fig.4.2).

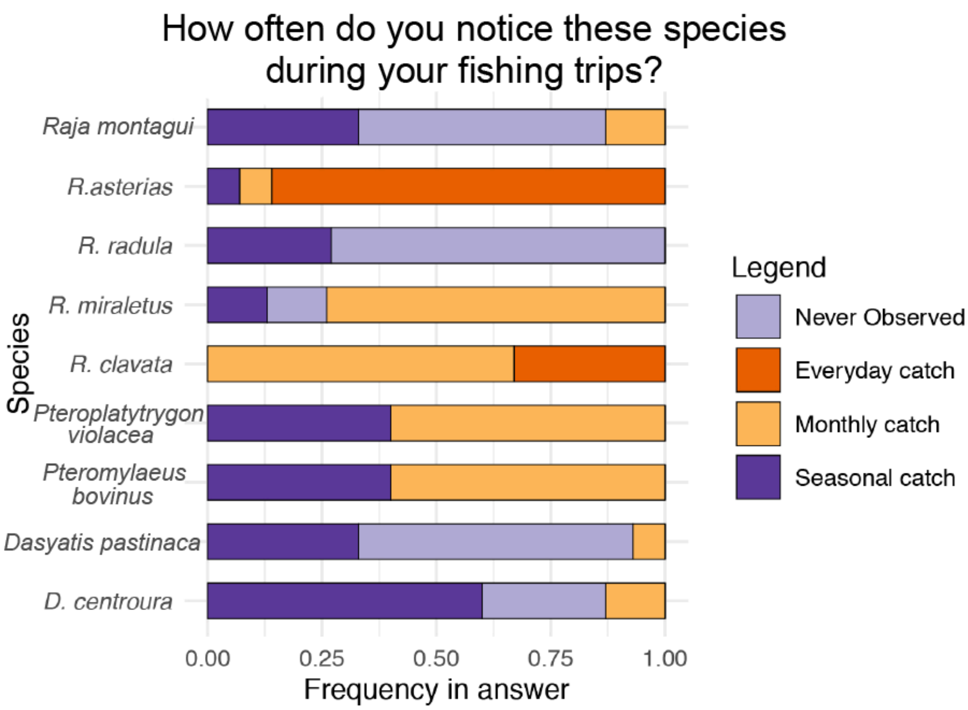


Figure 4.3- Frequency in answer to the specific question and legend with the 4 possible answers regarding frequency.

In addition, *P. violacea* appears to be quite common in catches as well as *A. bovinus* that appears to be quite common among trawlers' catches. On the other hand, *R. miraletus* is an occasional catch and only seasonal, consistently with its poor economic value. *A. bovinus* appears to be quite common among trawlers' catches (Fig. 4.3).

The question about the actual increasing of rays had a positive answer in the 75% of interviewers. Comparing the same mating period (Oct-Dec according to Serena *et al.* [109]) in past few years and 10 years ago, it appears to be more common to catch batoids nowadays.

The analysis of long-term and short-term data series of elasmobranch in the whole Mediterranean Sea, as we said, showed a drastic reduction in abundance. This is mainly related to trawl fisheries, which not only destroy seafloors but also have strong effects on demersal communities because of the use of unselective nets.

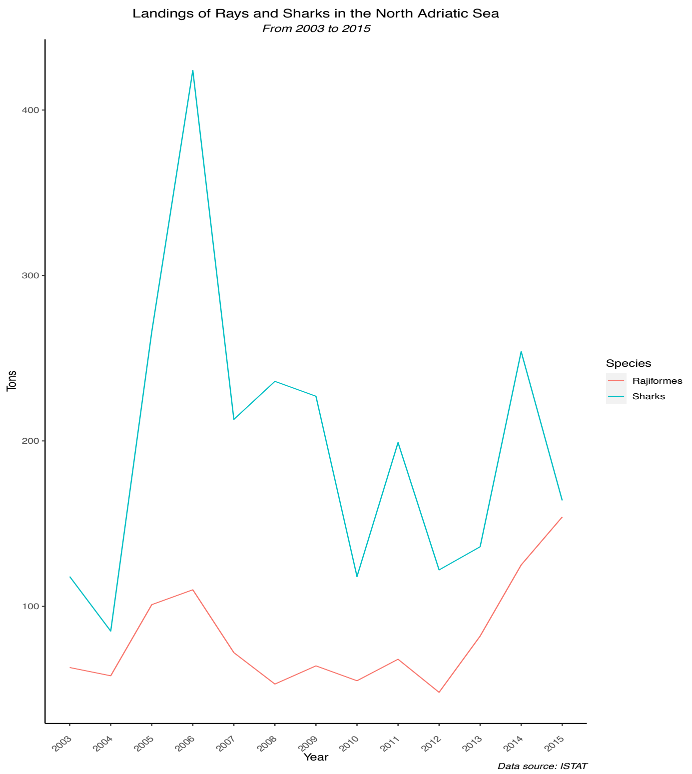


Figure 4.4- Trends in landings of Sharks and Rays in Northern Adriatic Sea between 2003 and 2016, according to ISTAT.

In Chioggia, fishermen confirmed that the decrease in landed elasmobranchs reflects a reduction in catches, rather than an increase in underreporting or a decrease in market demand, which are unsupported (C. Mazzoldi, unpublished interviews).

Elasmobranchs mainly represent bycatch of the Chioggia fleet; therefore, changes in landings are unlikely to reflect only changes in fishing gear. The high fishing pressure exerted in a small, landlocked area, such as the northern Adriatic Sea, makes it implausible that such a decrease in landings can be attributed to marked changes in fishing grounds, as suggested for other Mediterranean zones [29]. The decrease in landings, therefore, likely reflects a real biomass decline, also reflected in the landings reported on ISTAT from 2003 to 2016 (Fig. 4.4).

Nevertheless, this decline in elasmobranchs is notable mainly for sharks-like species, while meso-predators like batoids appeared declined at the beginning of the 90s [22] but never with a clear trend. Then from 2010 their trend tended to increase.

In fact even if batoids are a small part of total markets, in the past 10 years shifted from being 0.05% of biomass (like in Chioggia or San Benedetto) to the 0.2% at least or even more than 1% (Ancona) of the total sold biomass, as seen in Table 4.1.

Table 4.1- Percentage of batoid's biomass on total biomass sold for fishery markets of Ancona, Chioggia and San Benedetto del Tronto.

	Markets		
Year	Chioggia	Ancona	SBT
2011	0.04	0.7	0.05
2012	0.05	0.8	0.07
2013	0.05	1.4	0.11
2014	0.09	2.1	0.3
2015	0.08	2.1	0.5
2016	0.10	2.7	0.4
2017	0.12	2.1	0.4
2018	0.13	1.6	0.2
2019	0.14	1.6	0.22

The trend of the total rajiformes' biomass sold in the 3 different markets showed an increase in the demand. In particular during past 3 years after a common decline between 2015 and 2016. Since 2017 the rajiformes biomass exceed 20 tons in Ancona and SBT, and more than 10 in Chioggia (Fig. 4.5).

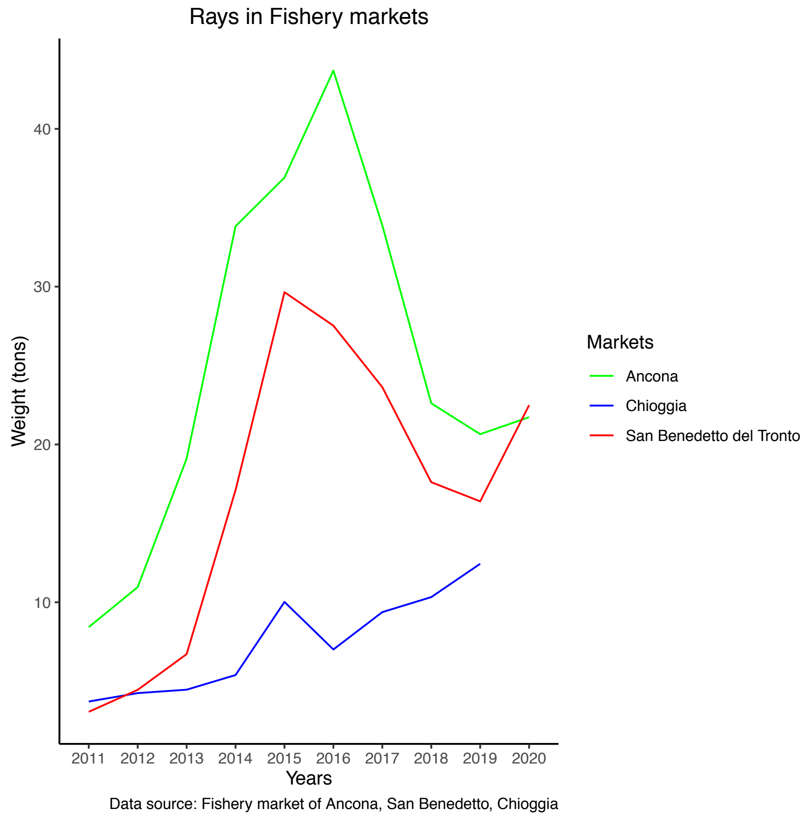


Figure 4.5- Trends of batoids sold in fishery markets of Ancona (green), Chioggia (blue) and San Benedetto (red).

The main problem of not having species-specific data is that we could not realize a shift in the composition of ray's populations. For this reason, data from Ancona and San Benedetto were also processed alone taking account of the qualitative division in starry ray, thornback ray and brown ray: therefore, we were able to notice that *R. miraletus* (brown ray) in the past 10 years remained stable in market demand while *R. clavata* and *R. asterias* shifted in composition. (Fig. 4.6)

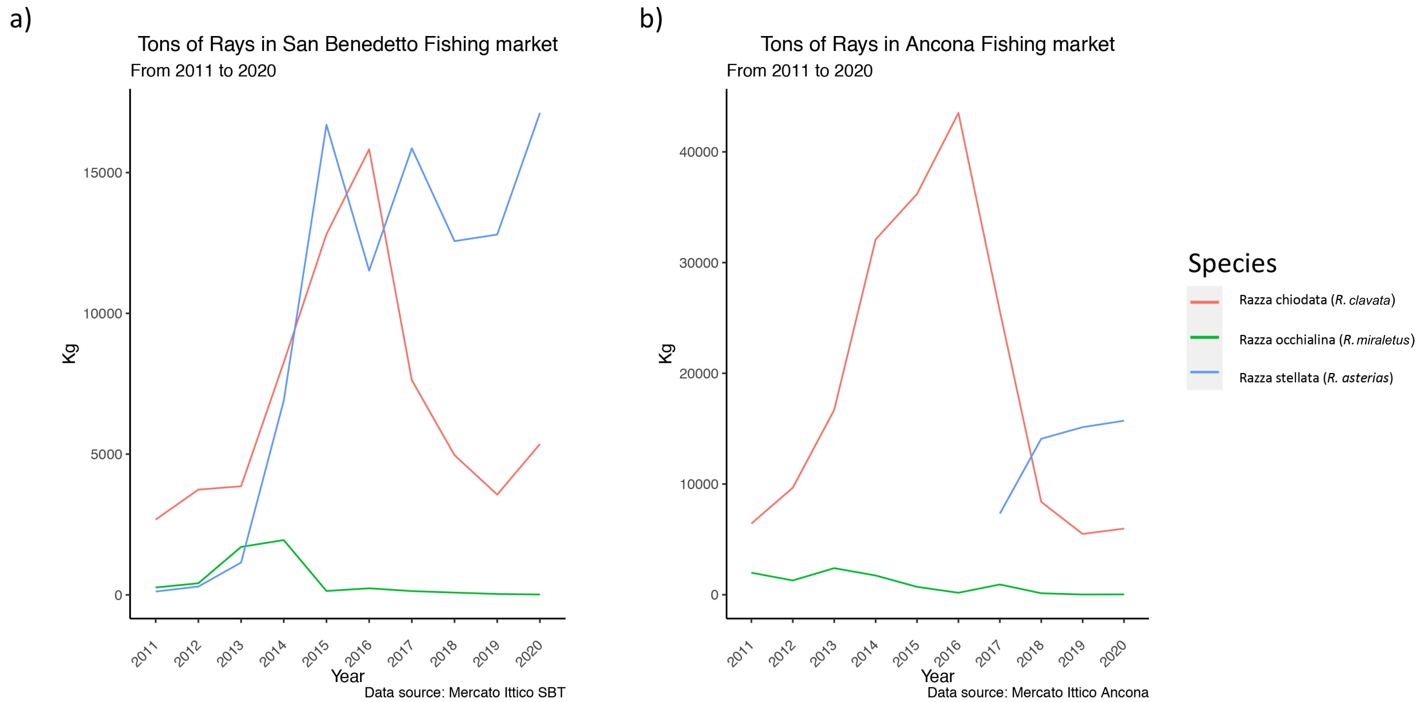


Figure 4.6- Trends of batoids sold in Ancona and San Benedetto divided in 3 main species: R.clavata *(red),* R. miraletus *(green) and* R.asterias *(blue)*

The thornback ray appeared to be the most common species in the Northern Adriatic Sea for many years (also supported by Barausse *et al*. [61] and Mazzoldi *et al.* [62]) but recorded a big decline between 2018 and 2019, while the starry ray began to increase.

In Ancona's Fishery the starry ray appears for the first time in 2017, but this is probably due to the fact that the partition between starry ray and thornback ray is not always reliable (they differ for the maximum total length mainly and the presence of thorns (FAO)).

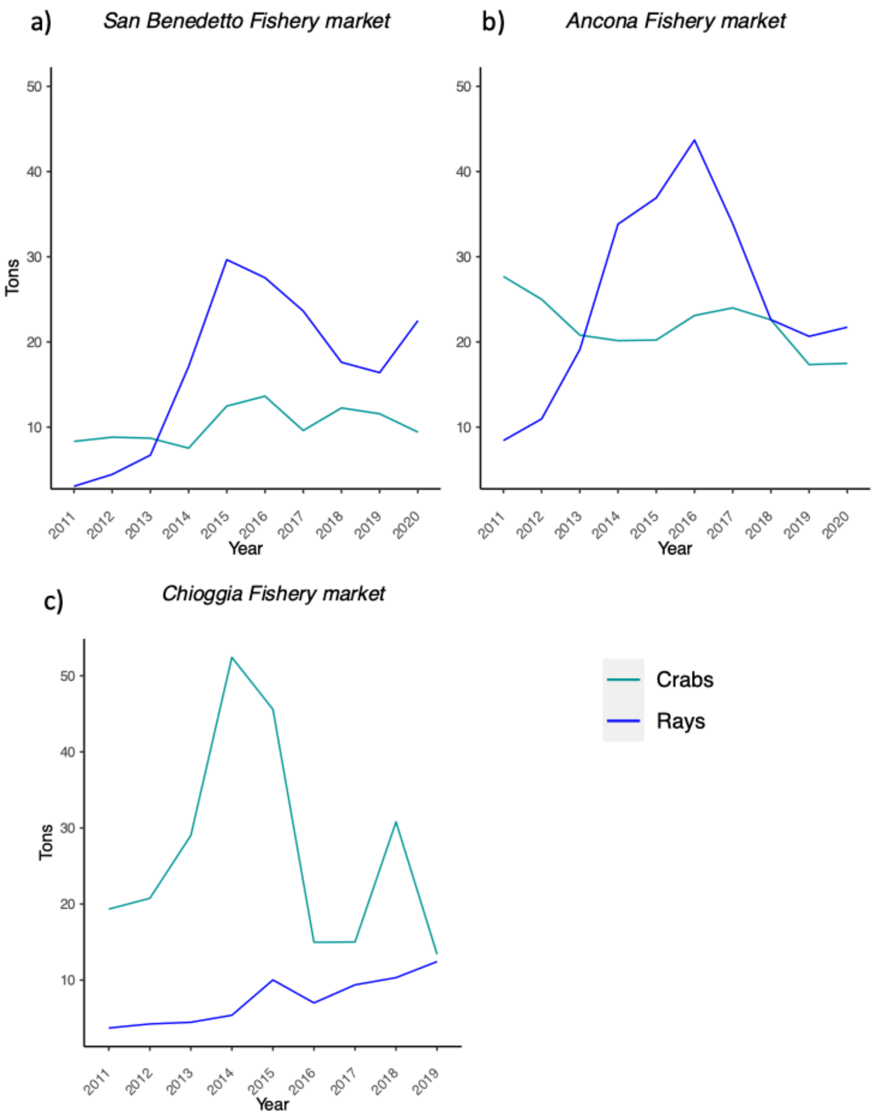


Figure 4.7- Biomass sold of crabs and rays compared to each other for the fishery markets of a) San Benedetto, b) Ancona and c) Chioggia.

Crabs' biomass appears to be almost consistent with the biomass of rays (Fig.4.7). We can notice fluctuations in landings: in Chioggia we have in 2015 a decline in crabs but an increase in rays and during 2017-2019 there is an increase in rays while Brachyura are declining.

4.2. Analysis of Stomach contents

Since we started this collaboration, it was possible to collect 75 samples (Fig. 4.8) from July 2020 to December 2020. These samples belonged to four different families:

1. *Rajidae* (65 individuals)
2. *Myliobatidae* (4 individuals)
3. *Torpedinidae* (2 individuals)
4. *Dasyatidae* (4 individuals)

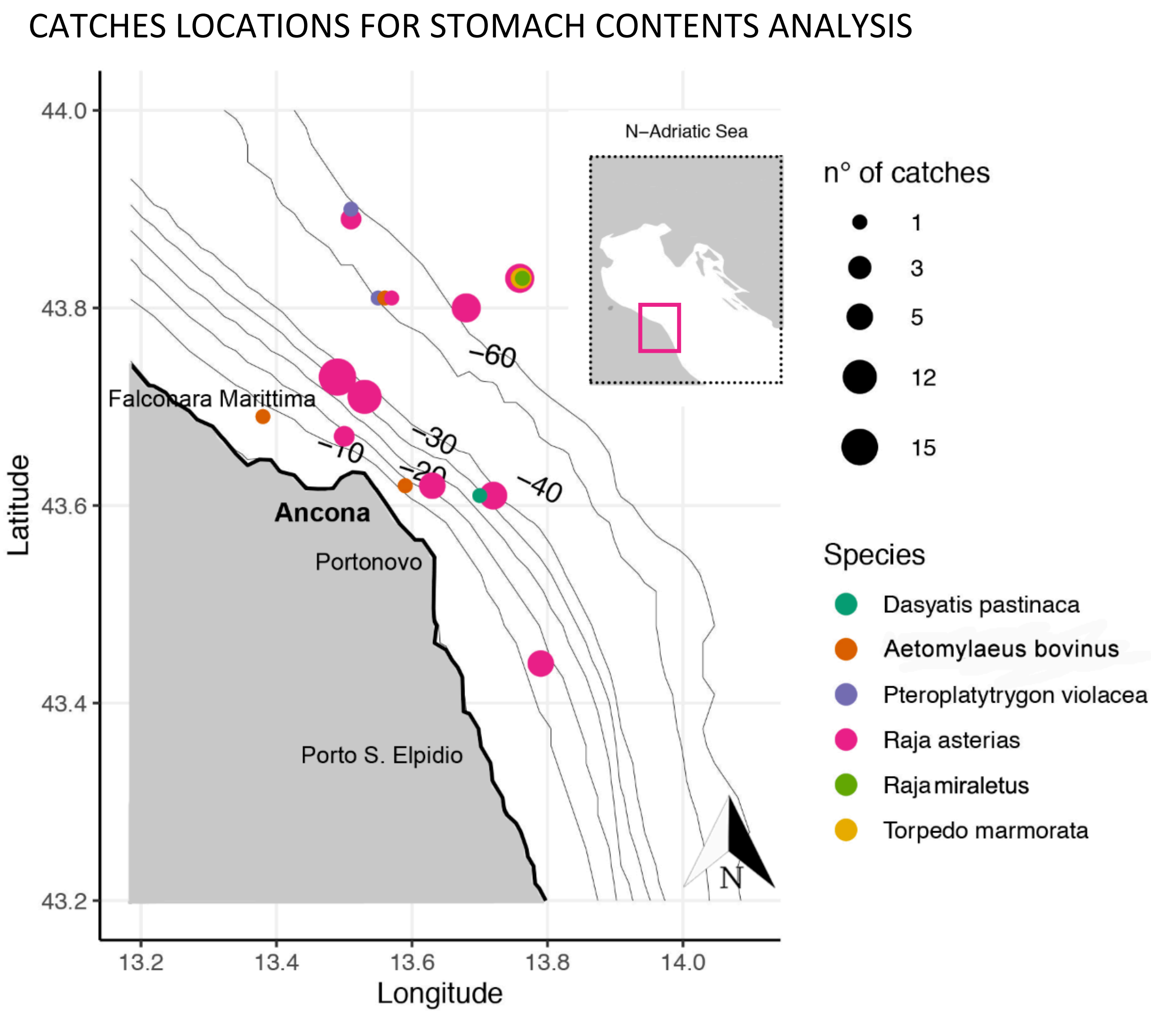


Figure 4.8- Fishing area of Ancona's trawl fishery, with bathymetry and catches of samples for this study.

The percentage of occurrence of the four families in catches was greater for Rajidae (Fig. 4.9) probably due to their strong presence in the area and their economic value. Myliobatidae, Dasyatidae and Torpedinidae are not only fewer in number but they are usually thrown back in the sea as soon as they are noticed in the net.

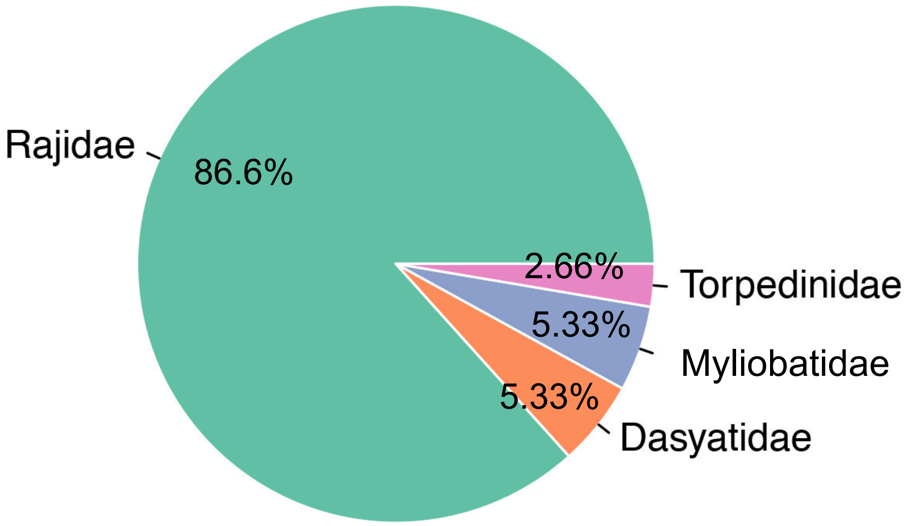


Figure 4.9- Pie chart of main occurrences in catches related to families

According to the size, we measured total length T_L (when the tail was present), body length (without the tail) and disk width (D_W). Whereas Rajidae were almost all with the tail, Myliobatidae and Dasyatidae usually had missing tail (fishermen usually cut it because of the stinged spine), for this reason we present the Disk Width to compare organisms' size. This measure varied from 39-103 cm for Myliobatidae, 28.5-63 cm for Dasyatidae, 15 cm for Torpedinidae and ranged from 16.5 to 37 cm for Rajidae.

Maximum recorded weight for Myliobatidae and Dasyatidae was 18 kg and 8.5 kg, respectively.

Weight for Torpedinidae was between 232-298 g and in Rajidae varied from 82.1 g to 1.3 kg.

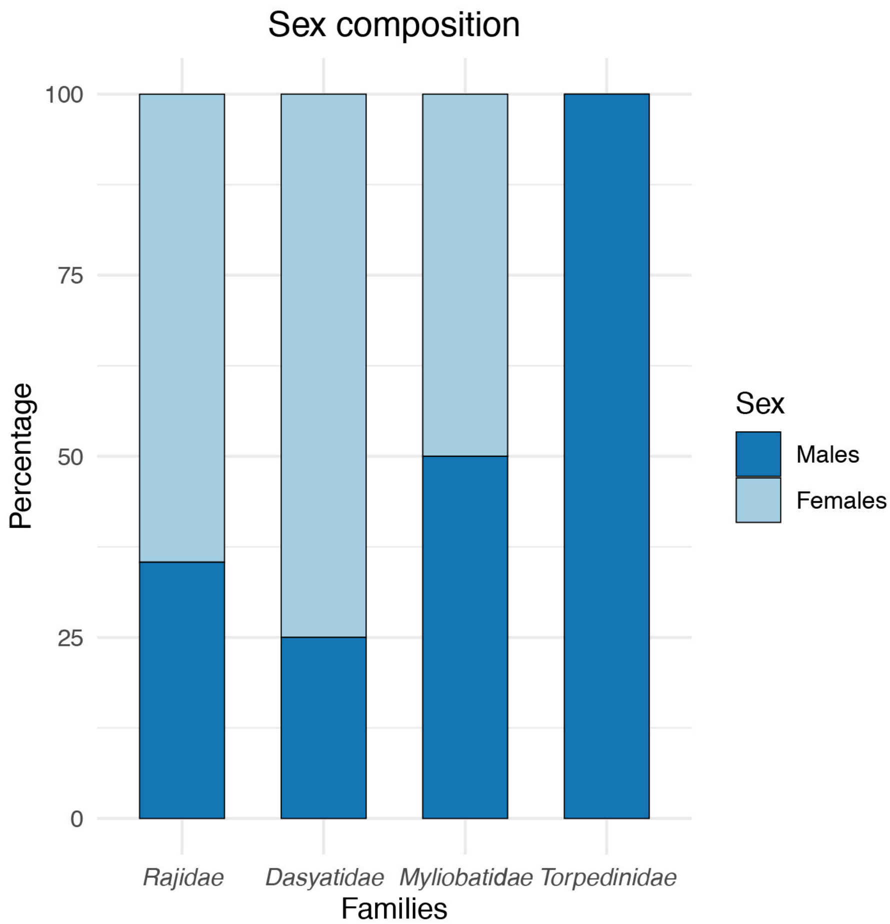


Figure 4.10- Percentage of sexes in the four considered families from Ancona's Trawl fishery.

Regarding sex composition we found more females than males, except for Torpedinidae for which we had only two males individuals. (Fig. 4.10)

Rajidae appeared to be more female individuals probably due to the fact that from October to December is considered their mating period [109].

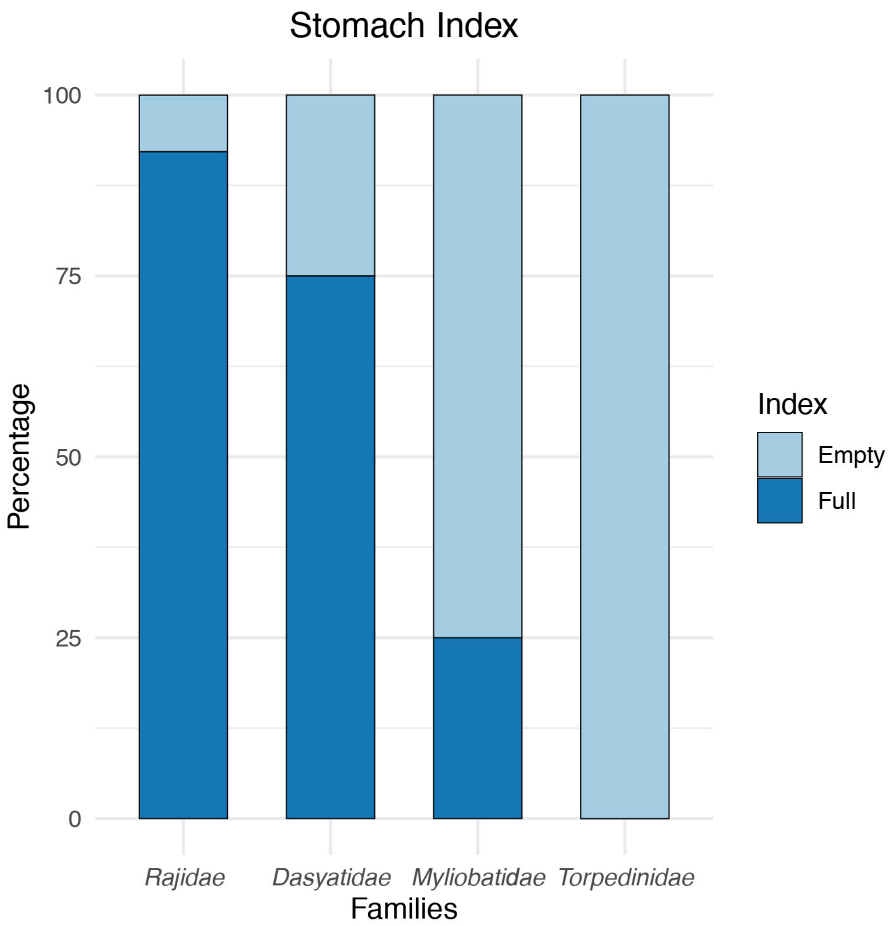


Figure 4.11- Vacuity index calculated for all the families occurred in samples.

As shown in figure (Fig. 4.11) the percentage of vacuity index was very low for Rajidae, instead concerning Torpedinidae both individuals had empty stomachs, Myliobatidae 3 out of 4 were fasting, and for the Dasyatidae the *D. pastinaca* had empty stomach while the *P. violacea* appeared to be always with full stomach.

4.2.1. *Overall Diet analysis of Rajidae*

The size of Rajidae specimens examined ranged from 23–47.5 cm L_T (Total length). In order to evaluate variation in food habits as function of size, specimens were separated into five size groups. We searched for a correlation between L_T and W (weight in grams). The

Shapiro-Wilks W test indicated that data were not normally distributed for Weight, therefore we applied nonparametric Spearman correlation and found a positive linear relationship between T_L and weight (Spearman rank order correlation r_s = 0. 952753, $p < 0.05$) (Fig. 4.12).

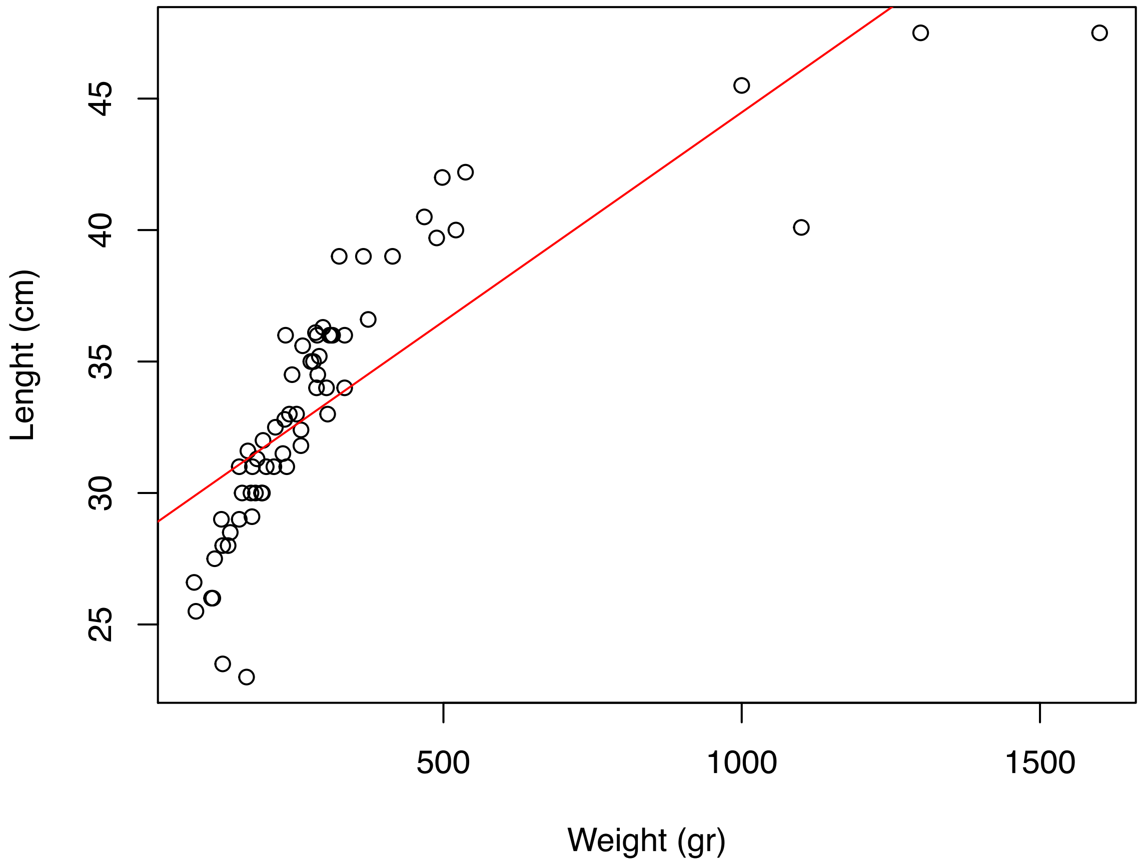


Figure 4.12- Relationship between the total length (cm) and body weight for Rajidae from the Adriatic Sea. The curve was fitted y=28.5729+0.0159x, (r_S= 0.9527536, p< 0.005)

The number of individuals in the size classes was unbalanced and the most common size class was the one ranging from 30 to 32.9 cm with 17 individuals (see Table 4.2).

Table 4.2- Size classes of Raja asterias *according to total length*

Class	Lenght (cm)	Number of individuals
I	<30	11
II	30-32.9	17
III	33-35.9	12
IV	36-39.9	13
V	>40	7

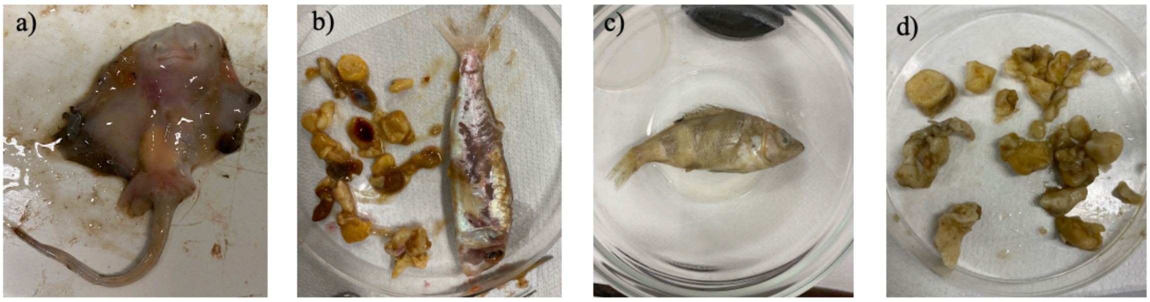


Figure 4.13- Examples of stomach contents: a)batoid, b)mullet and remains of gasteropods, c)comber, d)squid and undefined remains

We also searched for a correlation between Total Length-Fishing depth and Total Length-Fishing distance in nautical miles.

Shapiro-Wilk W test showed that data were not normally distributed (W= 0.73 and W=0.74 respectively for depth and distance) and so we proceed with a Spearman correlation test showing no correlation among our variables, with a Spearman's rank value of r_S=0.17 for depth and a and r_S= -0.28 for distance, a slightly negative correlation in the last case but still too low.

Diet composition analysis was performed for 60 non-empty stomachs, where identified prey items (Fig. 4.13) belonged to 7 major groups: Teleostea (bony fishes), Decapoda (shrimps), Brachyura (crabs), Stomatopoda (squillas), Amphipoda, Coleoidea (squids), Batoidea (little skates).

Crabs appear to be the most common group, followed by bony fishes (%IRI=48.03 and %IRI=41.51, respectively) (Table 4.3).

Table 4.3- Diet composition of 60 non-empty stomachs of R. asterias

Prey item	**%N**	**%F**	**%W**	**IRI**	**%IRI**
Teleostei	33.140	65.574	39.045	4733.428	41.514
Decapoda	18.605	27.869	12.693	872.233	7.649
Brachyura	36.628	78.689	32.975	5476.919	48.035
Stomatopoda	5.814	16.393	7.947	225.589	1.978
Amphipoda	2.326	6.557	0.386	17.782	0.155
Coleoidea	2.907	8.197	6.043	73.360	0.643
Batoidea	0.581	1.639	0.911	2.445	0.0214
Total	100.000	204.918	100.000	11401.761	100.000

We calculated the average prey weight (mean of all the prey weights), average meal size (∑total biomass of stomach content/n° of filled stomachs) and Shannon-Wiener index for each size class. (Table 4.4)

There is an increasing trend in average prey weight from I to V (0.307 g, 0.636 g, 0.828 g, 0.998 g and 1.131 g respectively). The same trend was also recorded for the mean meal size (2.463 g, 4.452 g, 6.625 g, 7.984 g, 10.185 g).

The diet diversity is broader in the size-class IV (H'= 1.578) and is more or less decreasing with the increasing size in other size-classes (I H'= 1.396; II H'= 1.306; III H'= 1.368; V H'= 0.942), showing probably a specialization in the feeding habits for bigger individuals (Table 4.4).

Table 4.4- Diet parameters for R. asterias *according to Size Classes*

Parameters	I	II	III	IV	V
Average prey weight [gr]	0.307	0.636	0.828	0.998	1.131
Average meal size [gr]	2.463	4.452	6.625	7.984	10.185
Shannon (H')	1.396	1.306	1.368	1.578	0.942

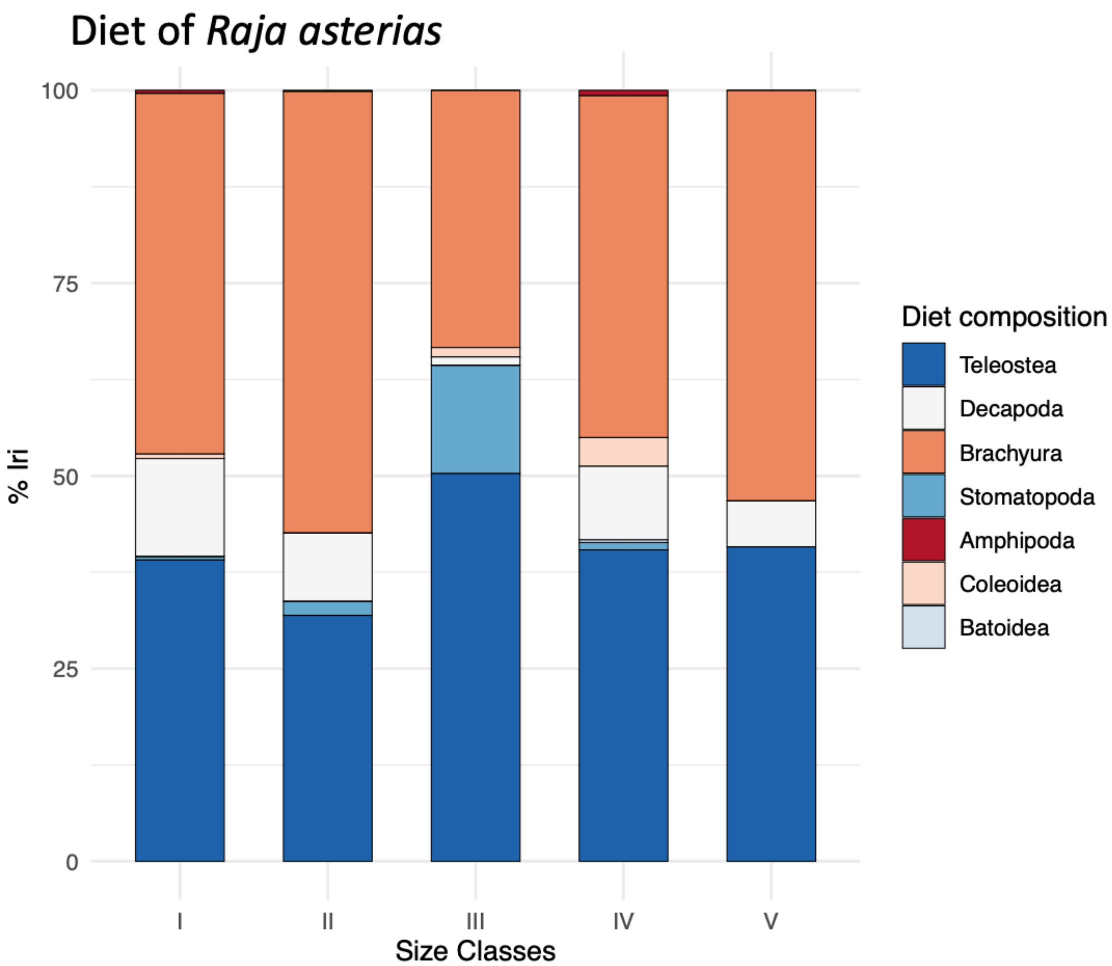


Figure 4.14- R. asterias' *diet composition in different size classes according to %IRI (Index of relative importance). Size classes: I (<30 cm), II (30-32.9 cm), III (33-35.9 cm), IV (36-39.9 cm), V (>40 cm).*

Furthermore, the stacked plot (Fig. 4.14) showing the diet composition according to %IRI confirmed bony fishes (%IRI I= 39.10, II= 31.91, III= 50.31, IV= 40.39, V= 40.79) and crabs (%IRI I= 46.79 , II= 57.26, III= 33.36, IV= 44.36, V= 53.24) as preferred food also in different size classes, apart from class III in which we find also Stomatopoda as favorite meal with %IRI= 14.01 (see also Table 4.5).

Calculating the Index of Relative Importance (%IRI) in dietary studies is important because it avoid biases between prey categories and allows an interpretation much more plausible for food habits by minimizing the skews caused by each one of these percentages.

For example in size class II we have %F relatively high for Stomatopods (%F= 17.65) but actually the biomass and number of preys (%W= 7.27 and %N= 5.77) are not reflected in the frequency. Same happens in class IV with Coleoids that have a %F= 12.43 but their %IRI shows that the importance in stomach is only the ~3% (Table 4.5).

Table 4.5- Diet composition and index of R. asterias *divided in Size Classes*

	Size groups									
	I					II				
	N° individuals 11					N° individuals 17				
Prey item	**%N**	**%F**	**%W**	**IRI**	**%IRI**	**%N**	**%F**	**%W**	**IRI**	**%IRI**
Teleostei	37.50	54.55	39.48	4199.094	39.106	25.00	64.71	37.91	4070.829	31.914
Decapoda	25.00	36.36	12.55	1365.314	12.715	26.92	29.41	11.62	1133.762	8.888
Brachyura	28.13	72.73	40.96	5024.321	46.791	40.38	88.24	42.40	7304.898	57.269
Stomatopoda	3.13	9.09	1.85	45.182	0.421	5.77	17.65	7.27	230.025	1.803
Amphipoda	3.13	9.09	1.48	41.827	0.390	1.92	5.88	0.79	15.975	0.125
Coleoidea	3.13	9.09	3.69	61.955	0.577	0.00	0.00	0.00	0.000	0.000
Batoidea	0.00	0.00	0.00	0.000	0.000	0.00	0.00	0.00	0.000	0.000
Total	100.000	190.91	100.000	10737.693	100.000	100.000	205.88	100.000	12755.489	100.000

Table 4.5- continued

	Size groups									
	III N° individuals 12					IV N° individuals 13				
Prey item	%N	%F	%W	IRI	%IRI	%N	%F	%W	IRI	%IRI
Teleostei	39.29	58.33	41.64	4720.388	50.316	32.50	76.92	32.76	5019.638	40.396
Decapoda	10.71	8.33	1.64	102.913	1.097	12.50	38.46	18.40	1188.491	9.565
Brachyura	32.14	58.33	21.51	3129.717	33.361	35.00	84.62	30.15	5513.043	44.367
Stomatopoda	14.29	33.33	25.16	1314.765	14.015	5.00	15.38	2.70	118.423	0.953
Amphipoda	0.000	0.00	0.00	0.000	0.000	5.00	15.38	0.39	82.852	0.667
Coleoidea	3.57	8.33	10.06	113.619	1.211	7.50	23.08	12.43	459.871	3.701
Batoidea	0.000	0.00	0.00	0.000	0.000	2.50	7.69	3.18	43.686	0.352
Total	100.000	166.67	100.000	9381.402	100.000	100.000	261.54	100.000	12426.004	100.000

Table 4.5- continued

Size groups

V

N° individuals
7

Prey item	**%N**	**%F**	**%W**	**IRI**	**%IRI**
Teleostei	36.84	71.42	44.88	5836.442	40.797
Decapoda	11.05	28.57	18.79	852.529	5.959
Brachyura	52.63	85.70	36.25	7617.016	53.244
Stomatopoda	0.00	0.00	0.00	0.00	0.00
Amphipoda	0.00	0.00	0.00	0.00	0.00
Coleoidea	0.00	0.00	0.00	0.00	0.00
Batoidea	0.00	0.00	0.00	0.00	0.00
Total	100.000	185.69	100.000	14305.987	100.000

We explored the relationship between size and weight of stomach contents (Fig. 4.15), but the regression was uncertain: the prediction of data decreases as the length increases. This happens because the variance is not constant. We tried with a log transformation of the data but the regression coefficient (R^2) appeared to get worse.

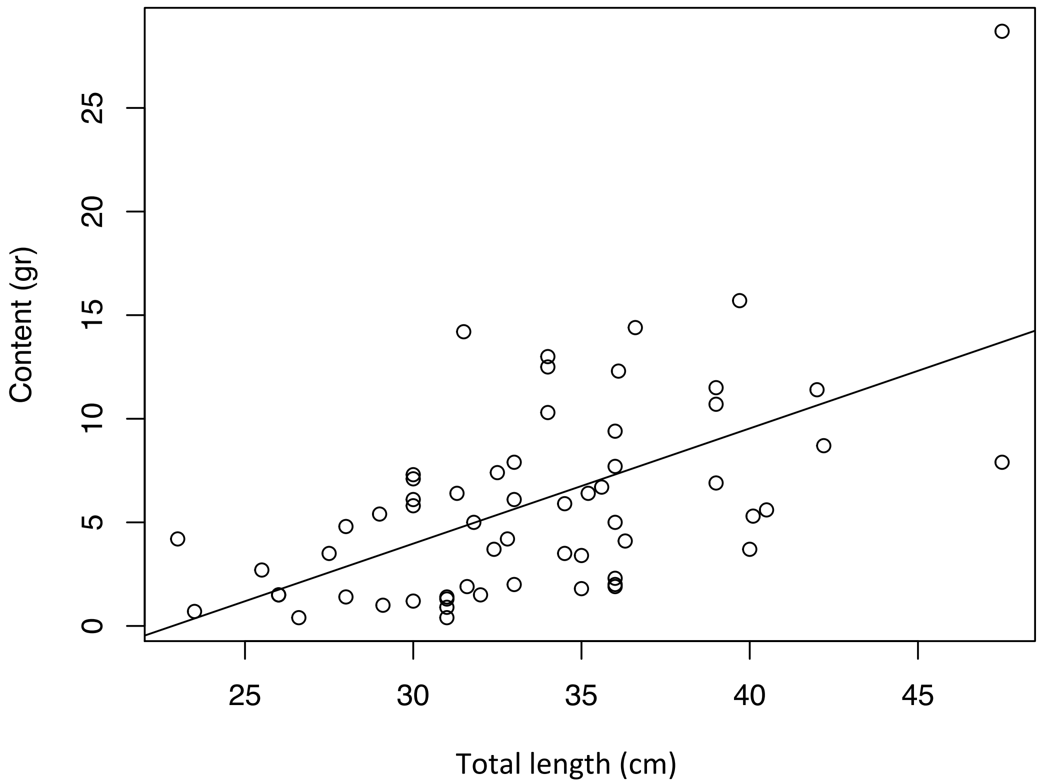


Figure 4.15- Linear regression between Total length (cm) and stomach contents (gr). (R^2=0.33)

Of 65 specimens, sex was determined for all individuals with 44 females and 21 males. Only the stomach of four females was found empty (V_i% = 10.25) while in one female was taken off by the fisherman before the sampling, hence this individual was not used as sample for the analysis of diet composition. We used a total of 60 full stomachs, from which 39 females and 21 males. Average prey weight was 0.792 gr and 0.655 gr for females and males respectively (Table 4.6), and average meal weight was higher in females (6.338 gr vs 5.247

gr). The Shannon-Wiener index showed how males usually have a more varied diet than females.

Table 4.6- Diet parameters for R. asterias according to Sex

Parameters	F	M
Average prey weight [gr]	0.792	0.655
Average meal size [gr]	6.338	5.247
Shannon (H')	1.372	1.509

This variety is also showed in the stacked plot (Fig. 4.16), based on %IRI values: males appear to feed on more Stomatopoda (%IRI= 3.77) and Coleoidea (%IRI= 1.89) (Table 4.7).

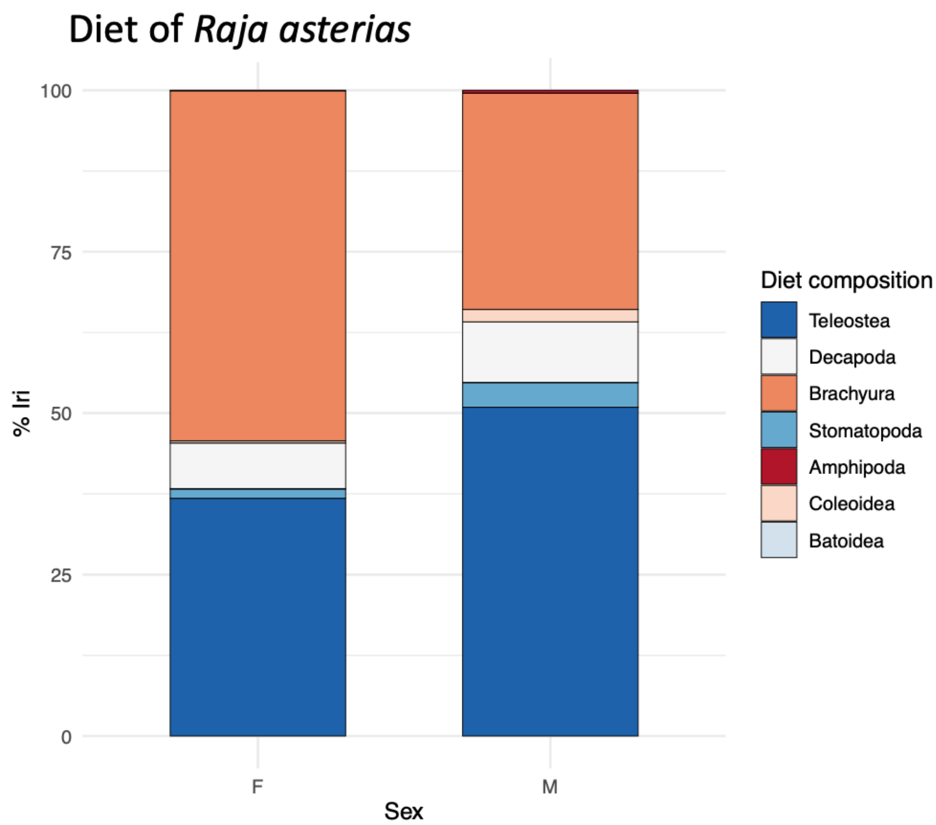


Figure 4.16- R. asterias' *diet composition between males (M) and females (F) according to %IRI (Index of relative importance).*

Table 4.7- Diet composition and index of R. asterias according to sex

Table of diet composition for *R. asterias*

	SEX									
	MALES N° individuals 20					**FEMALES** N° individuals 39				
Prey item	%N	%F	%W	IRI	%IRI	%N	%F	%W	IRI	%IRI
Teleostei	39.583	65.000	40.926	5233.080	51.586	30.645	66.667	37.783	4561.889	36.804
Decapoda	12.500	30.000	18.966	943.966	9.305	20.968	28.205	10.154	877.785	7.082
Brachyura	29.167	65.000	22.323	3346.832	32.992	39.516	87.179	37.581	6721.280	54.226
Stomatopoda	8.333	20.000	10.799	382.638	3.772	4.839	15.385	6.837	179.620	1.449
Amphipoda	4.167	10.000	0.454	46.204	0.455	1.613	5.128	0.364	10.138	0.082
Coleoidea	6.250	15.000	6.534	191.754	1.890	1.613	5.128	5.947	38.767	0.313
Batoidea	0.000	0.000	0.000	0.000	0.000	0.806	2.564	1.335	5.491	0.044
Total	100.000	205.000	100.000	10144.472	100.000	100.000	210.256	100.000	12394.969	100.000

We applied a *t*-test to compare the differences between sex and prey categories using the number of prey items per stomach. The test revealed no statistically significant differences (conf. level= 0.95 $p>0.05$ for fishes, decapods, batoids, squillas, coleoids and amphipoda) in the two diets except for crabs (conf. level=0.95 $p<0.05$).

To test the hypothesis that the diet changed in relation to both body size and sex we performed a GLM.

The model was made twice, once with the number of prey ad once with their weight: this was due to the fact that we didn't have one value containing both information in every stomach. We used a Poisson regression in both cases, taking always as the response variables the prey categories (once with numbers and once with grams) while the explanatory variable were L_T and sex.

The GLM showed that sex Female influences the diet ($p<0.05$) in relation to nº of prey and fish length also significatively influenced the diet ($p<0.001$) related to grams of prey.

Also the PERMANOVA confirmed that concerning the frequencies of preys the diet is not influenced by the size (L_T) but the sex ($p<0.005$). The PCO (Fig. 4.17) depicted a quite clear separation between males and females, also the diet appears to be different: female individuals have less variety in diet, mostly composed by crabs (H'=1.372) while males appear to have a broader feeding habits (H'= 1.509).

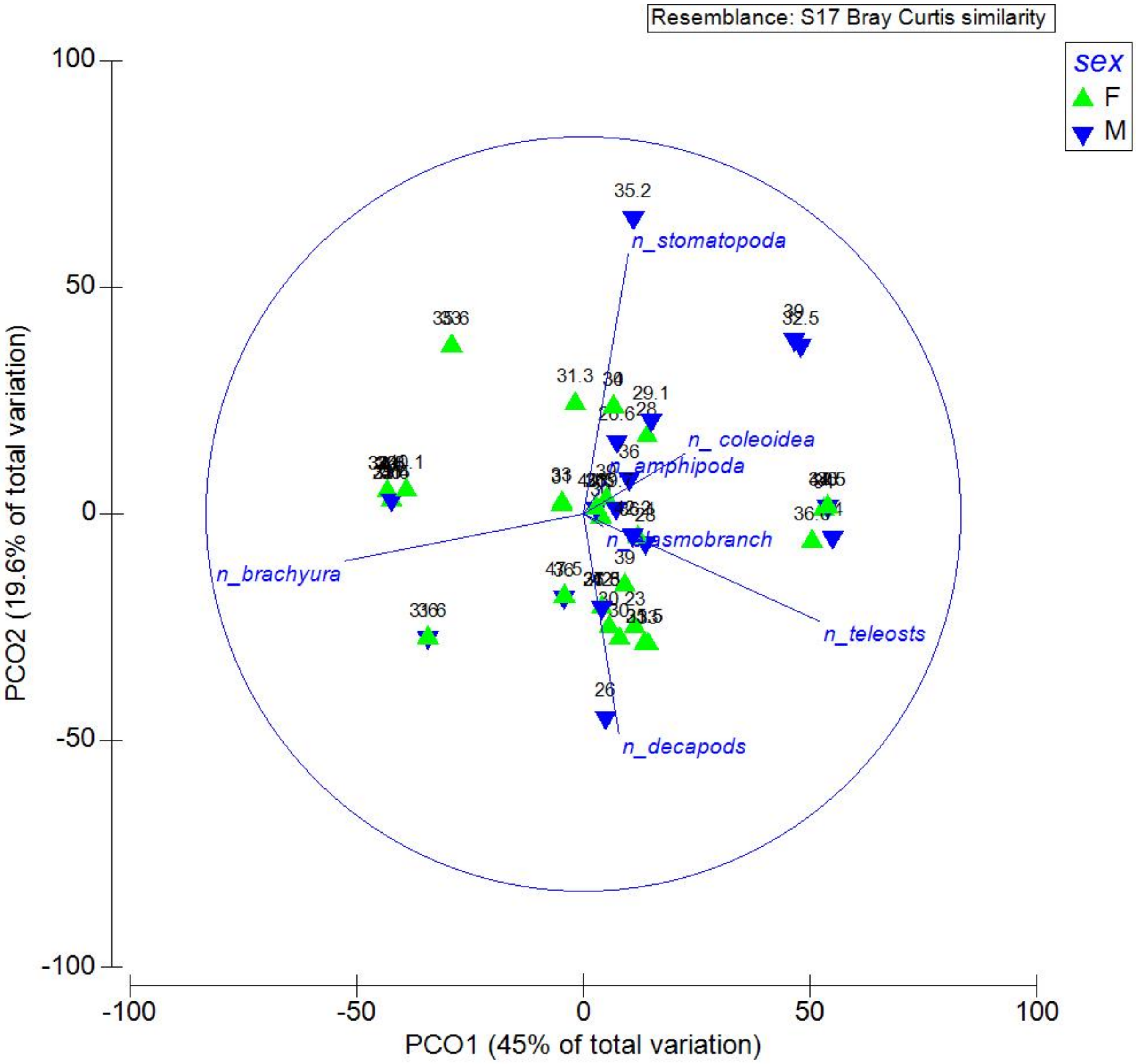


Figure 4.17- Principal coordinates analysis (PCO) plot based on Bray-Curtis similarity matrix showing differences in diet related to sex composition (males in blue and females in green)

Table 4.8- Trophic level calculated from the samples and compared to the trophic level of the same species found on FishBase, "n" corresponds to the number of full stomachs used for the estimate.

Species	Trophic Level (T_L) estimate	Trophic Level (T_L) from FishBase
Raja asterias (n=60)	3.76	3.8 ±0.6 se
Raja miraletus (n=1)	4.18	3.3 ±0.5 se
Aetomylaeus bovinus (n=1)	3.38	3.8 ±0.57 se
Pteroplatytrygon violacea (n=3)	4.08	4.4 ±0.54 se

Trophic level was calculated for *R. asterias*, *R. miraletus*, *A.bovinus* and *P.violacea* according to Cortéz, 1999 [80] (Table 4.8).

R. asterias estimation was T_L= 3.76, which is similar to the value we can find in FishBase.

Also *P. violacea* estimation had a value close to the one found in FishBase, this species seems to be higher in the trophic chain with a T_L=4.08. This suggests that *P. violacea* is an active and voracious feeder and, unlike other specimens, not only feeds on benthic communities but it also feeds on pelagic resources. This behavior can be reflected on its teeth structure, different from other batoid species, presenting a morphological adaptation on mouth and teeth that allows them to be efficient consumers on demersal resources.

Concerning *A. bovinus* and *R. miraletus* the estimation was not taken into account for the discussion since we only had one full stomach.

4.3. BAITED REMOTE UNDERWATER VIDEO

4.3.1. *MEASUREMENTS*

Measures were calculated using digitalized landmarks: we used 18 landmarks in total for different kind of objects. We used both shark-like figures and different-sized bars on land and in the pool with each calibration file (Fig. 4.18).

Figure 4.18- Examples of digitalization and manual detection of landmarks in both views.

On land the calibration file was pretty accurate with less than 1 cm of error for objects, but we needed to know how the system worked underwater to manage eventual distortion.

Using the pool calibration file, we did a total of 45 measurements. The errors between the tape-measured and stereovideo-measured lengths of objects in the pool ranged from -0.09 to 2.73 cm, with a standard error of 1.4 cm. Measurements were taken at the pool from different distances, consistent with the methodology applied by previous studies [110]. Length errors showed a tendency to increase as the angle of the bar relative to the camera plane increases. Paired tape and stereo-video length measurements were highly correlated (R^2=0.99) (Fig. 4.19).

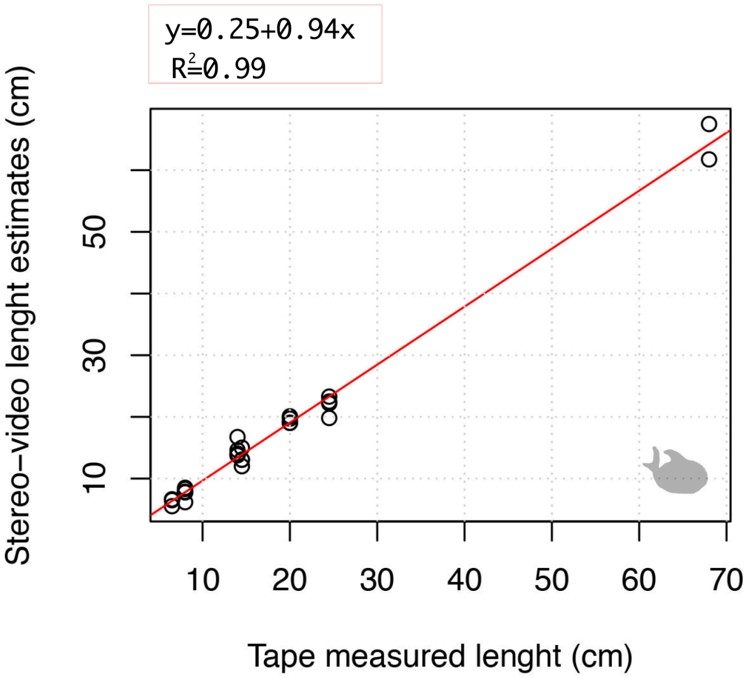


Figure 4.19- Linear regression between tape measured object and stere-video estimates.

3D visualization of landmarks trough the function "`plot3D`" resulted in a plotting of the correspondent measured objects and landmarks in the 3D space (Fig. 4.20).

Landmarks were set on purpose to gain a "profile" of the object measured, which is then added in transparency. "`plot3D`" function launches an in-built view from the XQuartz support application, environment where the plot can be moved in three dimensions.

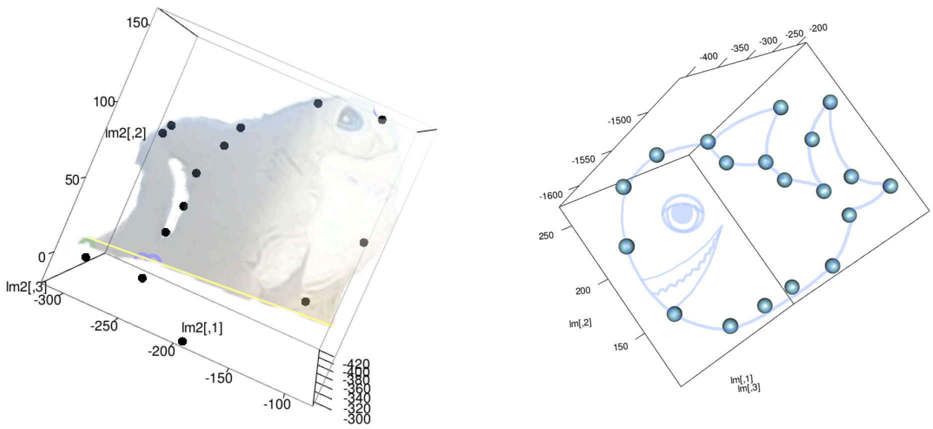


Figure 4.20- Example of 3D visualization of landmarks.

Unfortunately, during the analysis of the footage in Ancona (~ 3 hours of video) only 3 or 4 bogues (*Boops boops*, Linnaeus, 1758) were noticed.

We proceeded with the enhancement of the frames with CLAHE (R-package "*EBImage*"), which eventually helps defining edges of objects in frames, and measures were taken applying the shape.file obtained with the pool calibration (Fig. 4.21).

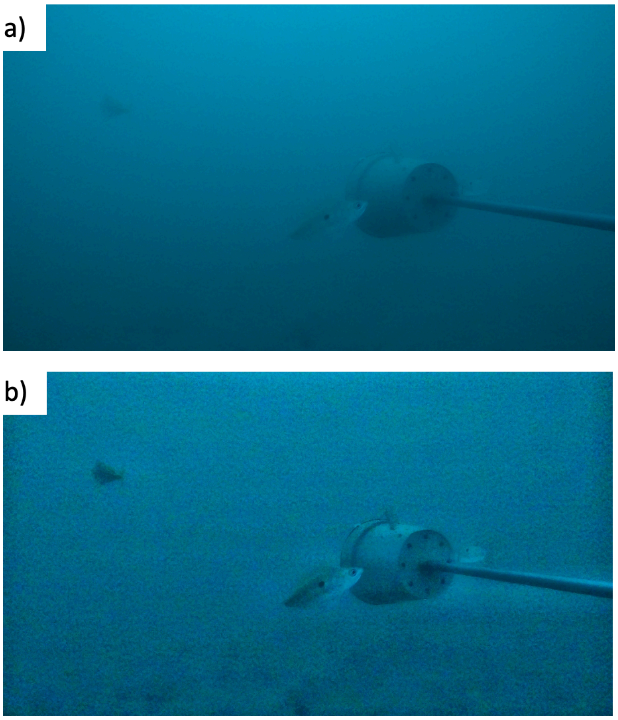


Figure 4.21- Clahe enhancement on a frame of the footage: a) before and b) after

Results of the "`distancePointtoPoint`" function indicated that these organisms ranged from 8.5 to 10 cm, which is consistent with an eye-observation taking account of the known length of the bait canister (Fig. 4.22).

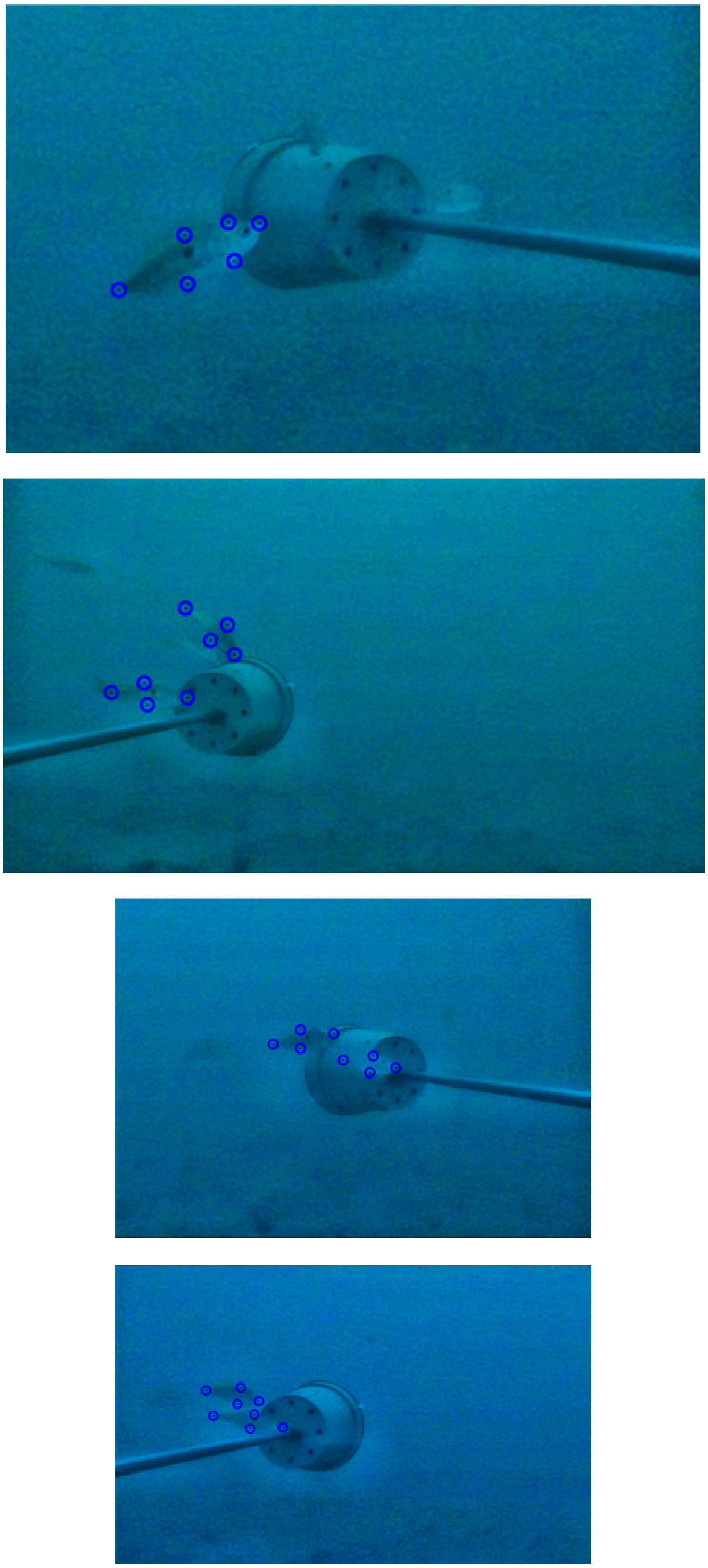


Figure 4.22- Examples of digitalization of landmarks on Boops boops *from both views*.

5. Discussion

Results obtained from the three different approaches are complementary and helped us to better understand the trophic ecology and abundance of batoids in the Northern Adriatic Sea. Fishing activity, interviews and markets' data confirmed the high presence of rays and skates in the area, in particular of *R. asterias*, and trough the analysis of stomach contents we assessed their feeding habits and trophic level.

Fishery data and LEK were essential because most of batoids occupy high trophic level, as we proved with stomach contents, and consequently, changes in their abundance can arise alterations in predator-prey relationships, which in long term views means also trophic cascades.

As the Northern Adriatic is an overexploited basin since beginning of XX Century, the occurrence of a meso-predator release probably happened after the World War I, as evidenced by D'ancona [22]. At present time we are facing the settling of some generalists' species with some shift in population and an increase in abundances due to normal fluctuations.

Markets' data of Ancona and San Benedetto depicted a clear trend in specie-specific batoids: during 2017-2020 there is a slight increase in the presence of the starry ray in markets. This doesn't mean that the species is new in the area, but only is growing in abundance while the other common species (*Raja clavata*) is decreasing after an overexploitation during 2014/2015.

When comparing Ancona, Chioggia and San Benedetto also is clear that in all three places there is an increase of rays' stocks.

Fishery markets' data alone could not help to give a greater image of the distribution and presence of all batoid's specimen: for this reason the historical memory and present perception of fishermen was essential to create a full picture of the situation.

Thanks to the interviews we can assume that the *Raja asterias* is more likely to approach the shores, as many fishermen catch these specimens also 3 or 4 miles away, hence it has a generalist feeding habit and could cover a wider space in his home range. On the other hand, *R. clavata* is found at 10-15 miles away from the coast, in the surroundings of oil platforms. We evidenced an higher frequency of females belonging to Rajidae, sampled from October to December confirming the reproduction period [109].

Instead, for *Myliobatidae,* 3 out of 4 individuals had an empty stomach (V_i= 75%) and considering the length at first maturity of 90 cm (from *Fishbase*), compared to the size of our samples we classify these specimens as juveniles accidentally caught in trawlers' net.

All individuals belonging to the family *Myliobatidae* were recognized as *Atomylaeus bovinus,* formerly considered as rare in the Northern Adriatic [111]. Dulčić *et al.* [112] hypothesized that the rarity of *A. bovinus* captures in the area could be explained by their scarce economical value, since they are generally discarded at sea such as other elasmobranch species; even though bull rays inhabit the area regularly. Furthermore *A.bovinus* is similar to the *Myliobatis aquila* with which is usually misidentified, but they differs in the shape of the head, position of caudal fin and chromatic pattern of the upper part, evidencing brown/yellow stripes.

Feeding habits for *P. violacea* and *A. bovinus* could not be fully assessed because of the few individuals sampled with full stomach. The pelagic sting ray is a voracious feeder which also feed on pelagic resources and it is becoming more common near the shores according to fishermen. Unfortunately, even if there is a lack data on the stocks of no commercial species that are usually discarded, it seems to have an important impact to fishermen, which, if caught, ruins the net and eats the fishes.

Instead, the bull ray is quite common in the area, even if not noticed before because of its similarity with the eagle ray. This species is a top predator, but it is possible that uses the Western Northern Adriatic Sea only as nursery ground, since we only sampled empty stomachs juveniles.

We could analyze the feeding habits only of Rajidae family because of the high frequency of samples collected (86.6%). Individuals were sorted in five different size classes and then in sexes. We calculated all the qualitative indexes such as %F, %N, %W and %IRI.

The results showed that their diet is mainly composed by species inhabiting sandy-muddy bottoms, in accordance with Rajidae's habitat preferences [113]. Vacuity index, on the other hand, confirmed them as voracious feeders which probably use the Northern Adriatic Sea as nursery area [114].

Indexes showed that rays' main source of food are crabs, followed by bony fishes. Seen the poor status of stomach contents we couldn't identify a single preferred prey.

When divided in size classes we did not notice a significative difference in the diet preferences, but regarding diversity in the diet, the Shannon diversity index (H') showed a decrease with an increase in size, probably because older individuals specialize the diet also with a better hunting experience.

Regarding the division in males and females, we noticed a difference in the diet. Female individuals appear to have a preference to feed on crustaceans, crabs in particular, with %IRI values of females=54.22% versus males=32.99%.

At the beginning we supposed that this difference was a result of both size and sex affecting the preferences in food habits. Statistical analysis showed that size was not affecting the selection of prey, but sex was. We then proceed with the PERMANOVA analysis and the PCO (Principal coordinates analysis, using Bryan-Curtis distances and showing the dissimilarities) which confirmed a significant difference in terms of %IRI.

Shannon-Wiener Index appear to be higher in males than females. This could be attributed to a higher specialization of females in food choice.

Brachyura, which are in general palatable for rays, are probably chosen in a higher frequency by females because of their lipidic profile. We supposed that there could be a relation between the sampling period, mating season, and the feeding habits. This relation has never been further explored by other studies on same species [115]. However, it is still unclear if these crabs in the diet of starry ray reflects an active selection of prey or rather the natural pattern due to prey abundance. In order to validate these assumptions, it would be necessary to go into further analysis of lipid profile of crabs and how their intake can influence the reproduction.

Estimate on Trophic Level for Rajidae resulted to be 3.76, meaning that they are top predators in their environment and their presence could potentially impact the whole food web. Even if it is a common endemic species, little it is known about their abundance and patterns of exploitation.

Analysis of its diet has already been studied in different parts of the Mediterranean Sea and they all show same results. Our study appears to be consistent with them almost in everything except for the difference we found in diet between males and females.

Concerning the high occurrence of crabs in rays' diet, Navarro *et al.* (2013) [115], studying the starry ray in the NW Mediterranean, supposed that given the importance of those individuals, eventually the decline that rays experienced during 2007-2009 could have been caused by: i) direct impact of trawlings, or ii) trawls impact on crustaceans, and so on the main prey of skates. This made us hypothesized that if the starry ray is currently increasing in the Northern Adriatic Sea, it could be a result of an increase on their preys.

Temporal variations in abundance and distribution of preys usually explains spatial and temporal patterns in the diet and trophic levels of predators. Hence, in this case, we explored also the biomass sold of crabs comparing it with rays' one without noticing a big temporal variation.

It is important to highlight that biomass sold in San Benedetto, Ancona and Chioggia is generally increasing, nevertheless, Chioggia appears to be the fishery with less rays' biomass sold. In addition, when comparing rays and crabs: while in Ancona and San Benedetto crabs are always in a smaller amount, in Chioggia the biomass of crabs in past 10 years has been higher than rays' one.

It is known that the area surrounding Chioggia acts as a nursery ground for the sandbar shark (*Charcharinus plumbeus*) [116], [117],[118], becoming probably the only area in which rays appear to have a higher predator keeping the population stable.

Thanks to the BRUV, it would have been possible to better assess the size and the distribution of these species, but with current COVID-19 restriction we could only made trials and do the background work in order to obtain an accurate calibration file that will certainly help on the characterization of population of batoids.

Nevertheless, the system appears to be useful in field estimates of marine organisms, even if it is made of low-cost cameras, a simple checkerboard pattern and an open-source calibration software (R and "*Stereo-Morph*").

This result is supported by Delacy *et al.* (2017)[95] but as a whole, in contrast with other recent studies (e.g. Wehkamp & Fischer 2014 [94], Boutros et al. 2015 [119]) that have suggested that 2D calibration approaches are less accurate than 3D calibration approaches when employed under similar conditions.

For marine megafauna studies in particular, the stereo-video system is highly suited, as there is less concern with the speed, angle, and distance to the camera compared to studies on reef fishes.

This system, with affordable feasibility, is a lowrisk, cost-efficient option for researchers, requiring no more than the cost of the cameras, the mounting system and a waterproof checkerboard. This reduction in cost should allow for more researchers to use stereo-video systems to improve length estimate data. Marine megafauna would benefit from demographic studies using accurate length data to guide conservation and management efforts [95].

6. CONCLUSIONS

The full understanding of distribution and possible impact of batoids in the northern Adriatic Sea is still to be completed. It is possible to affirm that any increase of generalist species at high trophic levels have the potential to affect the whole trophic web, implicating economic consequences in terms of ecosystem functioning and exploitation of other marine resources, as already happened in other regions.

Here in the North Adriatic, we can assume that rays and skates will affect crustacean stocks, crabs in particular, as they appear to be batoids' preferred food. We could not notice a high negative correlation between these two populations, but long-term speaking, the bad management of meso-predator resources could bring consequences also on the fishing industry.

Further research is recommended on the analysis of stomach contents, where a higher number of individuals would end up in a better representation of the feeding habits, as well as an increase in the number of interviewed fishermen could help to gain crucial information on presence and distribution of targeted species. However, greater importance has to be given to estimation of fishing pressure, biomass distribution and ecosystem dynamics of Rajidae and hopefully also of Myliobatidae, through the use of BRUV deployments, an innovative non-invasive methodology applied for the first time here in the northwestern Adriatic on megafauna.

We already worked on the background procedures: machine learning of "*Stereo-Morph*" package and BRUV trials in the field with bait traps. Further research on this aspect will be conducted to offer a complete analysis on the presence and distribution of species, in order to avoid risks for the ecosystem and help a better fishery management.

7. ACKNOWLEDGEMENTS

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APPENDIX

Supplementary Material- Interview

Legend

N° Species:
1. Rare: < 1 species/ month
2. Occasional: 2-10 species / month
3. Common: 10-50 species / month
4. Very abundant: >50 species /month

Catches:
- Everyday catch
- Monthly catch
- Seasonal catch (and which season)

Interview

N° interview	**Date**
Place	

Main gear and habitual fishing trip

Years of activity	
Type	
Fishing days x week	
Did you notice an increase in rays and skates?	
In which months do you notice an increase/decrease?	
Are rays targeted fishing or bycatch?	

What was the biggest catch of these species?	

Rajiformes

(with the use of the photographic guide and of the map of the study area)

Rajidae		
Raja asterias	**N° species**	**Catches**
	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)
Raja clavata	**N° species**	**Catches**
	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)
Raja radula	**N° species**	**Catches**
	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly

	☐ Abundant	☐ Seasonal (specify season)
Raja montagui	**N° species**	**Catches**
	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)
Raja miraletus	**N° species**	**Catches**
	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)
Dasyatidae		
Dasyatis centroura	**N° species**	**Catches**
	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)

	N° species	**Catches**
Dasyatis pastinaca	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)
	N° species	**Catches**
Pteroplatrygon violacea	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)

Myliobatidae		
	N° species	**Catches**
Myliobatis aquila	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)
	N° species	**Catches**

Pteromylaeus bovinnus	☐ Rare	☐ Never observed
	☐ Occasional	☐ Everyday
	☐ Common	☐ Monthly
	☐ Abundant	☐ Seasonal (specify season)