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Influenza delle diverse variabili ambientali e della diversa pressione di pesca sulla crescita della sogliola (*Solea solea*) nell'Adriatico settentrionale

Influence of different environmental variables and different fishing pressure in the growth of common sole (*Solea solea*) in the northern Adriatic Sea

Tesi di Laurea Magistrale di:

Giulio Pellini

Relatore: Chiar.mo Prof.

Emanuela Fanelli

Correlatori:

Dott. Giuseppe Scarcella

Dott.ssa Fortunella Donato

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Riassunto

La sogliola comune in Adriatico riveste un ruolo molto importante a livello commerciale (Scarcella *et al.*, 2014) arrivando ad un ammontare di catture che si attesta sulle circa 2000 tonnellate all'anno (Fishstat), le quali tra l'80 e l'85% provengono dalle marinerie italiane. Studi effettuati in passato, hanno evidenziato, inoltre, come nel bacino centro nord dell'Adriatico ci siano alti livelli di sforzo di pesca (Scarcella *et al.*, 2012, Scarcella *et al.*, 2014, Ferrà *et al.*, 2018, Ferrà *et al.*, 2020). Diventa quindi necessario avere una valutazione ed una gestione di questa risorsa ittica precisa e puntuale. Per far questo, dati sulla determinazione dell'età e gli studi sulla crescita delle specie (Pauly, 1987; Khan and Khan, 2014), così come studi sulle possibili aree in cui le specie ittiche si possano rifugiare, risultano essere di vitale importanza. Per effettuare la determinazione dell'età le strutture ossee più utilizzate sono gli otoliti (Ricker, 1975). In quest'ottica, la presente tesi ha confrontato i dati sull'età, provenienti dall'analisi delle sezioni di otoliti, e i dati sulla crescita ottenuti, durante i campionamenti della campagna scientifica SoleMon, nell'area del "santuario della sogliola" e nelle zone limitrofe, dimostrando l'importanza del santuario come zona rifugio. Infatti i dati ottenuti in questa tesi dimostrano come all'interno di quest'area le sogliole crescano più lentamente, possono arrivare a taglie maggiori e che la maggior parte delle catture all'interno del

“santuario della sogliola” risulta essere composto da femmine che arrivano fino ai 15 anni.

Chapter one

INTRODUCTION

1.1 Ecosystem Approach to Fisheries Management.

It is well recognized that understanding the whole ecosystem requires studying all components of biodiversity, from the genetic structure of populations, to species, habitats and ecosystem integrity, including food webs and complex biophysical interrelationships (Borja, 2014).

The management of marine systems, including the assessment of their general state of health, is, in fact, increasingly carried out by applying ecosystem approaches (Borja *et al.*, 2008). After all, the protection and conservation of marine ecosystems, along with the sustainable use of the services they provide, are critically important for maintaining global marine health (Tett *et al.*, 2013).

As far as the human impact on the ecosystem is concerned, certainly one of the greatest is fishing and this impact concerns both the target stocks (e.g. by changing abundance, productivity, size and composition of stocks), non-target species (such as endangered species, by-catches, discards) and habitats (Murawski, 2000).

For this reason, the European Common Fisheries Policy (CFP, 2016) aims to apply Ecosystem Approach to Fisheries (EAF), to preserve the reproductive

capacity of the target resources, having as one of its objectives safeguarding the stock of spawners.

In the case of common sole of the northern and central Adriatic, several studies (Grati *et al.*, 2013, Scarcella *et al.*, 2014, Bastardie *et al.*, 2017) have shown how, every year, a certain number of older spawner find themselves in a particular area that Scarcella *et al* (2014) named the "Sole sanctuary".

This area, thanks to its seabed features (see: Santelli *et al.*, 2014), could serve as a refuge for the breeding stock of soles. Therefore, it is important to carry out focus studies on this area to improve the understanding of the dynamic of target resources as common sole.

1.2 Biology of common sole in the Adriatic Sea

The common sole, *Solea solea* (Linnaeus, 1758), belongs to the class of Actinopterygii, order Pleuronectiformes and family of Soleidae. This fish is characterized by an oval, flat and asymmetric body . The eyes and snout are located on the right side of the head, with the upper eye less than its own diameter from the dorsal profile of the head. The preopercle is covered by skin and the opercle is entirely scaled. The sole has two well developed pectoral fins and the dorsal and anal fins, lacking spinous rays, are united to caudal fin by

well-developed membrane, but with the caudal peduncle distinct. The body of this fish is covered with rectangular ctenoid scales.

Depending on the substratum, the color of the sole can vary between gray, reddish brown and gray-brown with dark blotches.

The adults occur at a temperature range of 8.0-24.0°C (Moreira *et al.*, 1992).

S. solea is usually found on sandy and muddy bottoms and in estuarine habitats.

The common sole is present from 1 to around 70 m depths, but in some cases, especially in winter, it occurs up to 120 m deep (Frimodt, 1995).

As for the Adriatic, its presence has been found up to 90-100 m depth (Vrgoč, 2000, Fabi *et al.*, 2009). In this basin, *S. solea* is widespread, especially in the central and northern part, where the recruits are concentrated mainly in the western portion of the basin, characterized by soft bottoms and shallow waters with high concentration of nutrients. Migration to deeper waters occurs with growth. This migration pattern was postulated for the first time by Piccinetti and Giovanardi (1984), and it was confirmed by results obtained during scientific surveys carried out since 2005 using a modified beam trawl (SoleMon project; Bolognini *et al.*, 2009; Fabi *et al.*, 2009; Grati *et al.*, 2013). Furthermore, the experiments conducted by Pagotto *et al.* (1979) showed that the majority of the Adriatic population moves from North to South along the

western Adriatic coast and, probably, from South to North along the eastern coast.

In the Adriatic Sea, spawners are concentrated more outside the western coast of the Istrian Peninsula (Fabi *et al.*, 2009), with the core of recruits' distribution for the GSA 17, located around the Po river mouth. In the central and northern Adriatic Sea reproduction takes place from November to March (Fabi *et al.*, 2009). At the end of winter, the movement of eggs/larvae off the Istrian peninsula seems to follow the general circulation of the basin, bringing this portion of the population into the coastal lagoons of the North Adriatic Sea, where the young of the year start the benthic phase and remain until late summer, then leave and move southward (Fig. 1, Grati *et al.*, 2013).

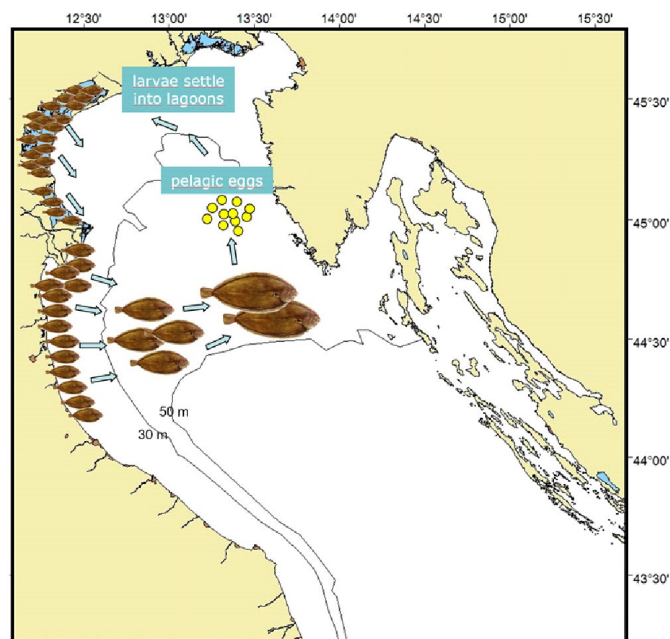


Figure 1 Distribution of *S.Solea* within the north-central basin of the Adriatic Sea (personal concession of F. Grati)

Growth rates for the Adriatic, obtained through otolith analysis, labeling experiments and size distribution analysis, showed a wide variety: from 2cm / month to 2cm / year (Piccinetti and Giovanardi, 1984) and the weight-length relationship always showed an isometric or positive allometric relationship (Tab. 1) (Piccinetti and Giovanardi, 1984; Froglia and Giannetti, 1986; Santojanni *et al.*, 2012).

Table 1 Length-weight relationship parameters for *S. solea* for the different Italian GSA (source: Scarcella *et al.*, 2017)

GSA/Area	Sex	a	b	Units	Reference
GSA 9 Ligurian & Northern Tyrrhenian Sea	M	0.0075	3.03	cm, g	Ligas <i>et al.</i> , 2012
	F	0.0022	3.4		
	C	0.0043	3.2		
GSA 10 South Tyrrhenian Sea	M	0.0256	2.66	cm, g	Spedicato <i>et al.</i> , 2012
	F	0.0163	2.83		
	C	0.0178	2.78		
GSA 11 Sardinia	C	0.0058	3.02	cm, g	Paci <i>et al.</i> , 1989
GSA 17 Northern Adriatic Sea	M	0.0042	3.24	cm, g	Santojanni <i>et al.</i> , 2012
	F	0.0025	3.4		
	C	0.0028	3.36		
	M	0.0086	3.01	cm, g	Piccinetti & Giovanardi, 1984
	F	0.0057	3.14		
	C	0.0069	3.08		
	C	0.0043	3.24		
	C	0.007	3.04		
C	0.007	3.057	cm, g	Bolognini <i>et al.</i> , 2013	
GSA 18 Southern Adriatic Sea	C	0.003	3.36	cm, g	Lembo <i>et al.</i> , 2012

The parameters of the von Bertalanffy growth function, estimated by several studies, also showed a L_{∞} between 23.20 cm and 40.10 cm of Total Length (TL) (Tab. 2)

Table 2 von Bertalanffy growth function parameters for *S. solea* from the GSA 11 and 17 (source: Scarcella *et al.*, 2017)

GSA/Area	Method	Sex	L_{∞} (TL, cm)	k	t_0	Reference
GSA 11 Sardinia	LFD	J	33.80	0.065	-0.61	Paci <i>et al.</i> , 1989
GSA 17 Northern Adriatic Sea	T/R	C	40.10	0.680*	-	Piccinetti & Giovanardi, 1984
	OR	C	38.25	0.041	-3.57	Froggia & Giannetti, 1985
		M	23.20	0.069	-1.66	Froggia & Giannetti, 1986
		F	37.87	0.042	-5.36	
		C	38.25	0.041	-3.57	
	LFD	C	39.60	0.440*	-0.46*	Fabi <i>et al.</i> , 2009

The length of first sexual maturity of common sole reported by Fischer *et al.* (1987) and Vallisneri *et al.* (2000) is 25 cm TL. Similarly, data collected during the SoleMon project (Fabi *et al.*, 2009) reported a value of about 26 cm TL. Hatching occurs after 8 days and the larvae measure 3-4 mm TL (Tortonese, 1975). Eye migration starts at 7 mm TL and ends at 10-11 mm TL and the benthic life begins after 7 or 8 weeks (15 mm TL) in coastal and brackish waters (Fabi *et al.*, 2009).

1.2 Fishery and stock assessment of common sole in the Adriatic Sea

The common sole in the Adriatic Sea is, certainly, one of the most commercially important species of the Mediterranean and Black Sea (FAO major fishing area 37), contributing for 23% to the overall sole catch of the FAO-GFCM area (Food and Agriculture Organization-General Fisheries Council for the Mediterranean) (Scarcella *et al.*, 2014). In the last decade the average capture

production of common sole in the Adriatic Sea was around 2000 tonnes per year (Fishstat), of which 80-85% landed by Italian fleets, in particular from the fleet using the “*rapido*” trawl. This gear is a modified beam trawl with a rigid mouth fitted with iron teeth (7-10 cm long) along the lower part and a nylon net bag which is tied to the frame. The gear is named *rapido* due to the relatively high towing speeds (6-7 knots), which thanks to a wooden plate acting as a depressor, pushes the gear in close contact with the sea bottom, making it ideal for targeting flatfish and other benthic organisms, inhabiting muddy bottoms of the continental shelf.

In addition, common sole appears in catches of other types of gears (for example bottom otter trawls, gillnets or trammel nets).

The catches, in terms of total length, differ according to the type of gear used. As for the *rapido* trawl (TBB) for the Italian fleet, Ferretti and Froggia (1975) reported a higher percentage of catches for a TL (Total Length) between ca. 15 and 19 cm, while Grati and Fabi (2008) reported that the specimens caught had an average TL of ca. 20.64 ± 0.05 cm.

The Croatian fleet uses a modified beam trawl similar to the *rapido* which is called *rapon* (DRB), for which an average length of the catches of about 28.6 cm is reported (Ezgeta-Balic *et al.*, 2021). According to the official data from

the DCF (Data Collection Framework), the Italian bottom otter trawls (OTB) collected specimens ranging from ca. 16 cm to 35 cm TL in the last decade. Mean TL was reported to vary between 21.7 cm and 23 cm, according to the mesh size, in gillnet (GNS) catches by the Italian fleet and between 20 and 40 cm TL, in Croatian trammel net (GTR) catches (Fabi and Grati, 2008). Generally, Italian catches are dominated by smaller individuals in TBB and OTB catches, a smaller proportion of individuals is caught by GNS. On the contrary, Croatian catches are dominated by bigger individuals caught by GTR (Fig. 2 and 3). This agrees with the spatial distribution of common sole in the Adriatic Sea.

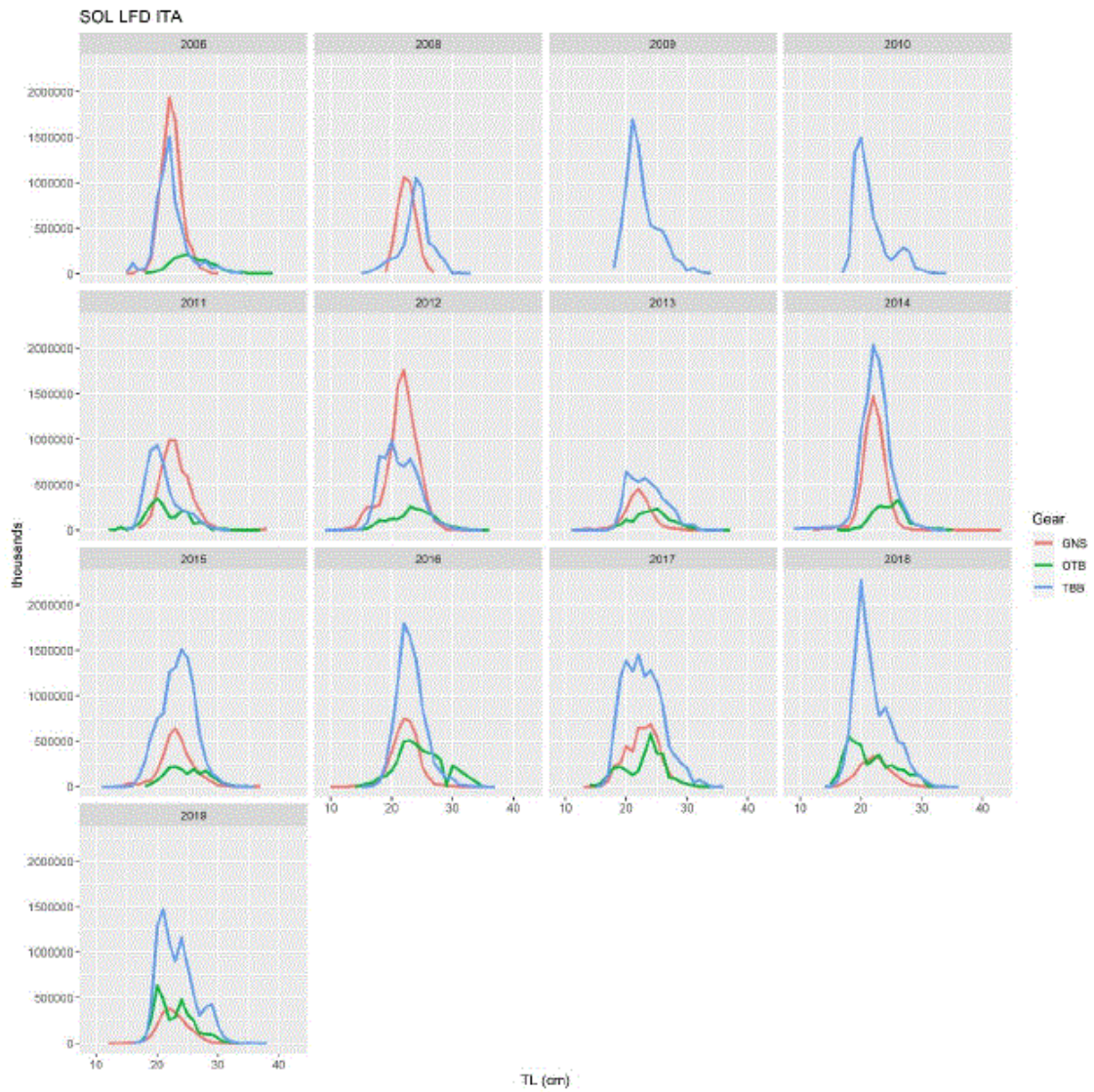


Figure 2 Length frequency distribution of Italian catches from 2006 to 2019 by fleet (modified from GFCM 2021)

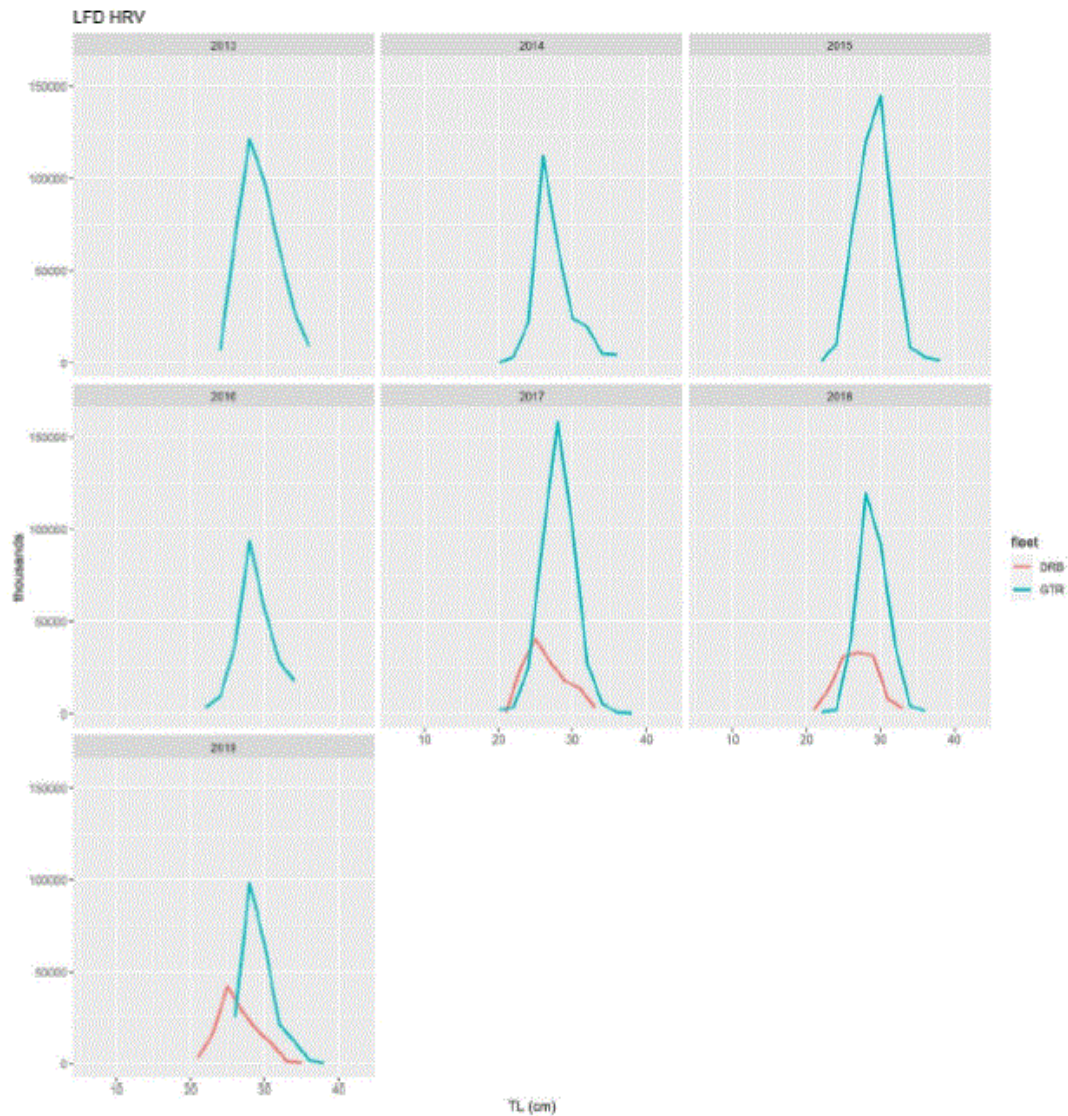


Figure. 3 Length frequency distribution of Croatian netters and “rampon” (a modified beam trawl similar to the “rapido”) catches from 2013 to 2019 (modified from GFCM, 2021)

Based on the data obtained from commercial fishing and those independent of commercial fishing (from surveys, such as SoleMon), the latest stock assessment presented by the GFCM (General Fisheries Commission for the Mediterranean, 2021), relating to the common sole in the GSA 17, showed a spawning biomass following, in absolute terms, a decreasing trend, a fishing

mortality which, after an increase until 2010, is following a decreasing trend, and a recruitment following an increasing trend. The results, due to the low spawning biomass of the stock, have led to suggestions to reduce fishing mortality.

1.3 Age and growth

For responsible fisheries management, studies relating to longevity, mortality, productivity yield and population dynamics are very important (Pauly, 1987; Khan and Khan, 2014). For the collection of these data, the study on age and growth is a fundamental prerequisite (Pauly, 1987; Khan and Khan, 2014). Therefore, the assessment of individual age has been proven to be very useful in assessing the status of any fish stock. According to Panfili *et al.* (2002), data on age and growth are essential for understanding vital traits of species and populations (e.g. lifespan, age at recruitment, age at sexual maturity, reproduction periods, migrations, mortality) and the study of population demographic structure and its dynamics (e.g. age-based stock assessment).

In the early life stages, information on age structure can be used to clarify the effects of changes in the environment on growth and survival, and can result in an improved understanding of factors affecting recruitment success. In adults, knowledge of age and growth is used to determine the effect of fishing on the

stocks, the efficacy of management policies, to understand life history events, and to maximize yield while still ensuring the future of the resource (Holden and Raitt, 1974).

In order to make the age determination of fishes, over the years, several methods of determining the age have been developed: at first the vertebrae were used (Henderstrom, 1959), then the scales (Carlander, 1987) and finally the otoliths (Ricker, 1975).

Otoliths (from the greek *oto*= ear and *lithos*= stone), formed from one or more primordial cells exocytosed by the epithelial cells of the inner ear (Mann *et al.*, 1983), are concretions of calcium oxalate which are deposited at the level of the vestibular apparatus.

The inner ear, which is found in all jawed vertebrates, functions both as an auditory system that detects sound waves and a vestibular system which detects linear and angular accelerations, enabling the organisms to maintain balance.

In fish, the inner ear is a paired structure embedded in the cranium on either side of the head close to the midbrain. Each ear is a complicated structure of canals, sacs and ducts filled with endolymph, a fluid with special viscous properties (Fig. 4).

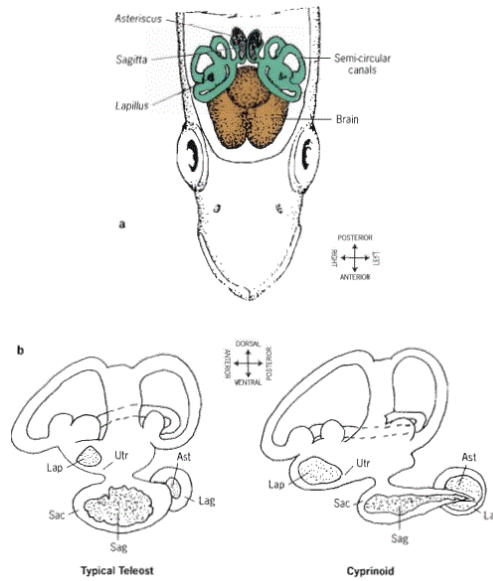


Figure 4 Position of the otoliths within the inner ear of teleost fish (modified from Secor *et al.* 1992). a) Dorsal view of the vestibular apparatus in a typical teleost species. The top of the cranium in cut away (frontal section). b) Otoliths within the labyrinth system of typical teleost and ostariophysean fishes. Ast = asteriscus; Lag = lagena (vestibule); Lap = lapillus; Sac = sacculus; Sag = sagitta; Utr = utriculus (vestibule)

The canals open into a series of expanded interconnected chambers or otic sacs that contain the macula (a sensory tissue that senses sound waves and acceleration). The three otic sacs are the sacculus, utriculus and lagena, which contain the *sagitta*, *lapillus* and *asteriscus* otoliths, respectively (Fig. 4; Campana, 1999; Campana and Thorrold, 2001).

Typically, sagitta represents the largest (and therefore most easily handled) otolith and it can be assumed that it will contain the widest increments for clearer resolution of microstructural features (Stevenson and Campana, 1992).

For this reason, sagitta is the most used otolith for the age estimation.

The growth of the otolith occurs by material in concentric layers around the core, latter, which consists of one or more primordia. This deposition is cyclical and depends on the internal rhythms of calcium metabolism and protein synthesis and continues even after a fish stops growing in length (Campana and Thorrold, 2001). Growth of otolith is not uniform in all directions but occurs according to directional gradients and following privileged axes: in fact, the greatest growth occurs along the antero-posterior axis and the least along the proximal-distal axis (Panfili, 1992).

The accreted material is composed of alternating zone (Fig. 5) that differ in density and optical properties and their deposition is partially caused by seasonal changes in the environment, both in terms of abiotic variables (first of all the temperature) and biotic variables, such as the availability of food (Morales-Nin, 1992).

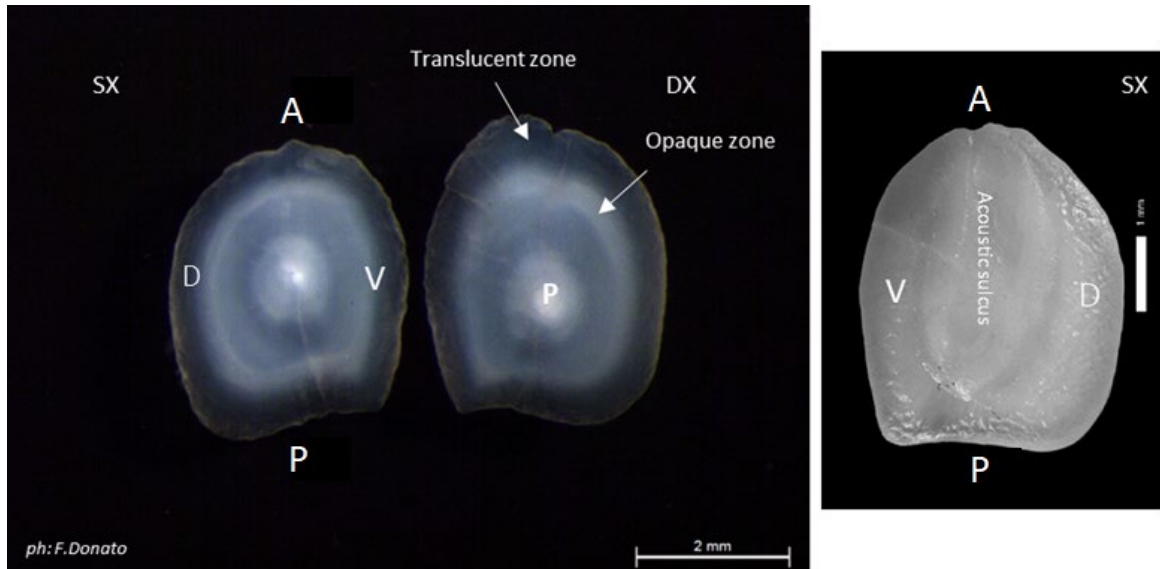


Figure.5 Whole otolith of *Solea solea*. The image identifies the left (SX) and right (DX) otolith, translucent and opaque zone, primordium or core (P), ventral (V), dorsal (D), anterior (A) and posterior (P) portions

Usually, in fish of temperate and cold waters, the translucent zone of the otolith corresponds to reduced growth in winter and the opaque zone is associated with intense fish growth in summer, but in sole and other flatfish the pattern is opposite, with the deposition of an opaque zone in winter and a translucent zone in summer (Frogliola and Giannetti, 1985, 1986, FAO, 2019).

In general, the combination of an opaque and a translucent zone is considered an annual growth (*annulus*).

In some cases, irregular translucent growth zones can be present in otoliths, defined “*checks or false zones*”. These are not annual zones, and often occur as the result of physiological or environmental stresses experienced by the fish during life. Checks may correspond with life history events such as settlement,

migration, maturation, or spawning (Penttila and Dery, 1988). Checks can be distinguished from true annual zones by their irregular spacing, relatively faint appearance, and lack of continuity throughout the otolith.

“Splitting” is a special case of false deposition, where two or more closely spaced translucent growth zones are deposited in a single year (Matta & Kimura, 2012).

Different preparation methods can be used for age determination of otoliths, both whole and sectioned otoliths can be used (ICES 2010c).

Given, however, the difficulty in using whole otoliths for the ageing of slow-growing fish, or fish with thick otoliths (Hilborn and Walters 2013), which could cause an underestimation of age (Beamish RJ 1979, Sekigawa T, Doiuchi R), for this thesis it was decided to use only sections for age estimation.

1.4 Aims of the thesis

The “sole sanctuary” area, as underlined by the work of Santelli *et al.* (2017) presents different assemblages of mega zoobenthic invertebrates and different grain size (Fig. 6).

Indeed, the area was largely characterized by muddy-sand bottoms and dominated by holothuriansk (especially *Holothuria (Panningothuria) forskali* and *Parastichopus regalis*), bryozoans (mostly *Amathia semiconvoluta*) and

ascidians (mainly *Phallusia mammillata*). This has probably led to a lower fishing effort by trawlers (both bottom otter trawlers and *rapido* trawlers) mainly due to the fact that the sea cucumbers subjected to stress eviscerate (Mosher 1965, Tortonese 1965, Brusca & Brusca 1996), damaging the catch and reducing its commercial value, and bryozoans clogs the nets, reducing their efficiency (Salvalaggio *et al.* 2014).

This difference in fishing effort (Fig. 7), as also highlighted by several studies (Scarcella *et al.*, 2012, 2014, Ferrà *et al.*, 2018, 2020), can cause differences in the disturbance of benthic habitats leading to changes in the abundance, in biomass and diversity of communities and species (i.e., direct effects, Collie *et al.* 2000, Kaiser *et al.* 2006) and changes in sediment particle dynamics, and in the balance between predators and prey (i.e., indirect effects, Dounas *et al.* 2007, Tjensvoll *et al.* 2013, Hiddink *et al.* 2016).

Based on these differences, the main aim of the study is to verify if in the portion of the population sampled inside the so called "Sole Sanctuary" there is a different growth rate compared to the soles found in the neighboring areas and if the area is characterized by the presence of specimens belonging to the older age classes.

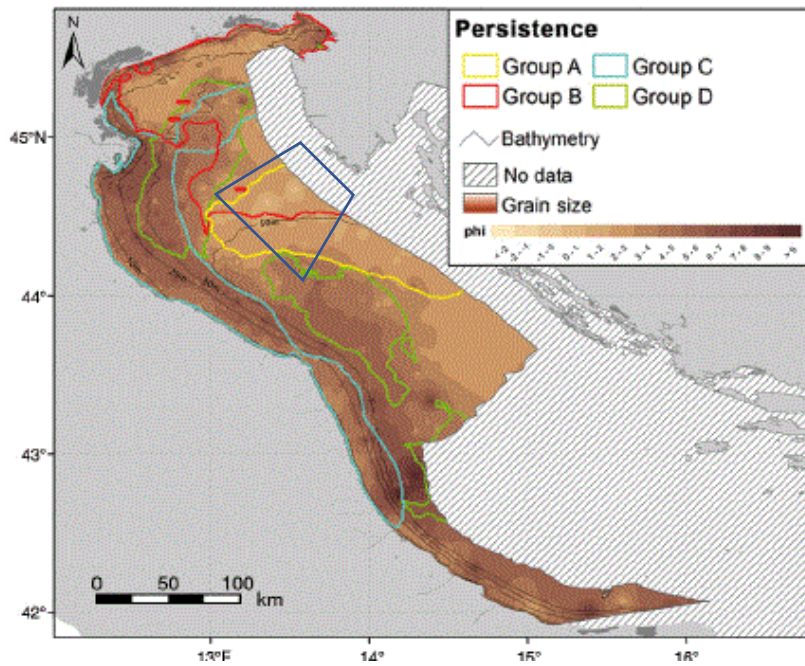


Figure.6 Grain size map and persistence of 4 megazoobenthic assemblages (modified from Santelli *et al.*, 2017); the blue trapezoid identifies the area of the “Sole sanctuary”

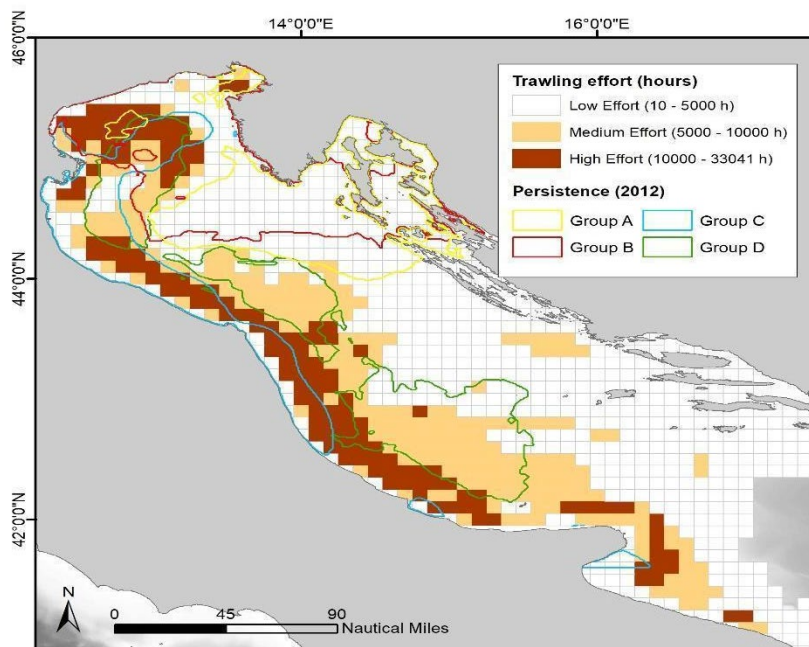


Figure. 7 Map of trawling effort (hours) based on AIS data (source Coro *et al.*, 2022) with megazoobenthic assemblage

Chapter two

MATERIAL AND METHODS

2.1. Study area

Individuals of *Solea solea* were sampled in the GFCM Geographical Sub-Area 17 (GSA 17: central and northern Adriatic Sea).

The Northern Adriatic Sea is the northernmost region of the Mediterranean Sea; it extends from the bathymetric line of 100 m, up to 45 ° 47'N in the north and is bordered by the Italian peninsula to the west and the Balkans to the east. The Adriatic Sea is characterized by an extremely shallow mean depth (about 30 m) with a very weak bathymetric gradient along the major axis, and by a strong river runoff (~3000 m³ s⁻¹) relative to the receiving basin; in fact, Po and the other northern Italian rivers are believed to be the source of about 20% of the total Mediterranean river runoff (Hopkins, 1992).

The northern Adriatic is also influenced by intense surface flows (wind stress, heat and water flows). The prevailing winds are the bora, a cold, dry and gusty north-east wind, prevalent in winter, and the scirocco, a hot and humid wind that blows from the south-east along the axis of the Adriatic basin.

The “Sole sanctuary” is characterized by a seabed with a presence of muddy-sand, while in the remaining parts of the basin we find a broad range of sediments, ranging from sandy mud, mud/sandy mud to mud (Santelli *et al.*,

2017). The northernmost part of the basin is characterized by a greater presence of sand, while the eastern portion, especially near and below the Po river, has a mostly muddy bottom.

2.2 Samples collection

The specimens analysed in the present study were sampled during the SoleMon survey. The “SoleMon” project (Solea Monitoring) started in the years 2005-2006, and was funded by the Italian Ministry for Agriculture and Forestry (D.G. Fisheries and Aquaculture) in the framework of the 6th triennial plan of marine fishery and aquaculture in marine and brackish waters (Theme C – C6), and gathered institutions involved in marine research, located along the western and eastern coasts of the Adriatic Sea.

Since 2005 the same gear with no modifications has been used. The gear is a modified beam trawl named as *rapido* trawl by the Italian fishermen. The gear was appositely planned to be fished on different types of bottom and consists in a modified beam trawl with a rigid mouth. The frame is rigged with 46 iron teeth along the lower leading edge. Joined to the iron frame there are 4 skids and a reinforced rubber diamond-mesh net in the lower part to protect the polyamide net bag (Width: 3.59 m; Height: 0.25; Weight: 225 kg; Four 120-mm wide skids). The gear is always in contact to the seabed by an inclined wooden board fitted to the front of the iron frame which acts as a spoiler and

keeps the skids and the teeth tightly pushed down to the sea floor. The drawing of this trawl is given in figure 8. This gear was estimated to be operated by a vessel with a towing power of at least of 400 HP.

On the plan in figure 9 the mesh sizes are indicated in bar length. The mesh numbers in height correspond to well finished and joined netting sections; the joining mesh are also showed. The length of the codend is 2.7 m.

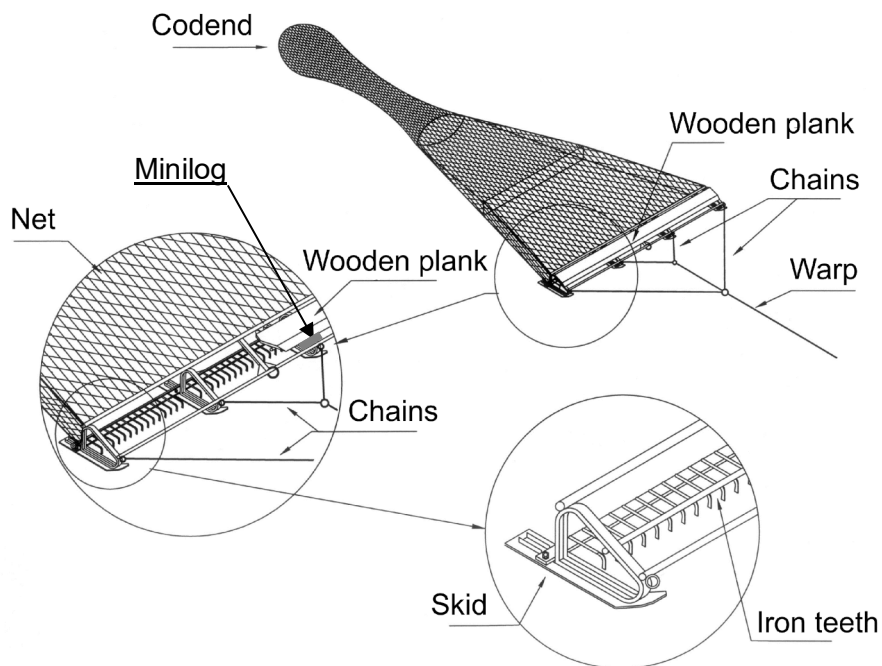


Figure 8 - Scheme of a rapido trawl

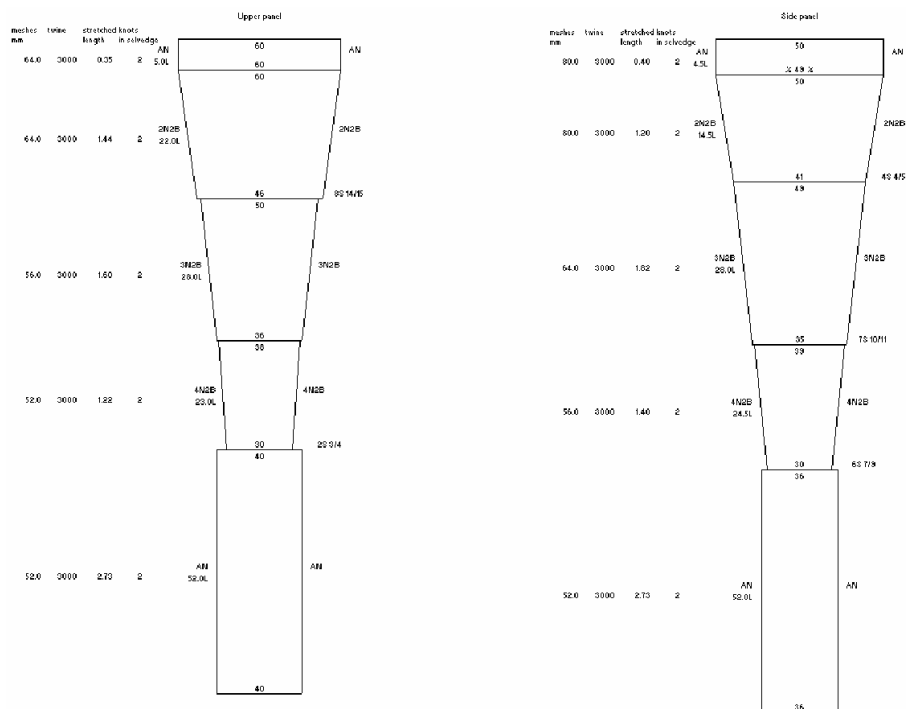


Figure 9 – Plan of the rapido trawl net.

The general drawing of the rigging is given in figure 10. The vessel utilizes two gears simultaneously, named as “RAPIDO A” in the right side and “RAPIDO D” in the left side (Fig. 10).

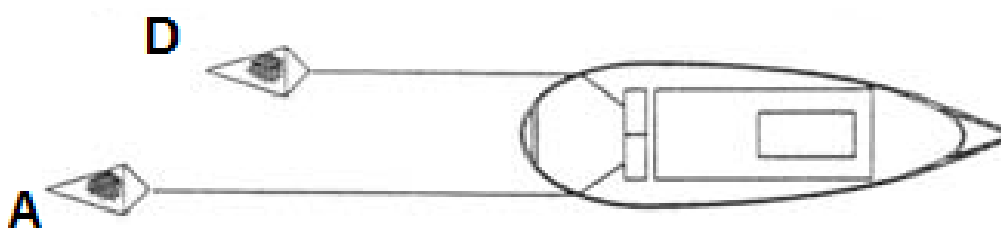


Figure 10 – Scheme of the gear position during the haul

Taking the characteristics of the gear and the rigging into account the warps should have a diameter of 14-16 mm. The length of warps to be shot is

determined by the depth. The recommended relationship between depth and warp length is given in figure 11. Although in certain peculiar circumstances some adaptations can be made to this relationship, it is recommended to respect the depth/warp length ratio as far as possible. The gear positioned in the right side of the vessel has 15 m of warp more than the other, in order to avoid possible interference between the two gears during the haul (Fig 10).

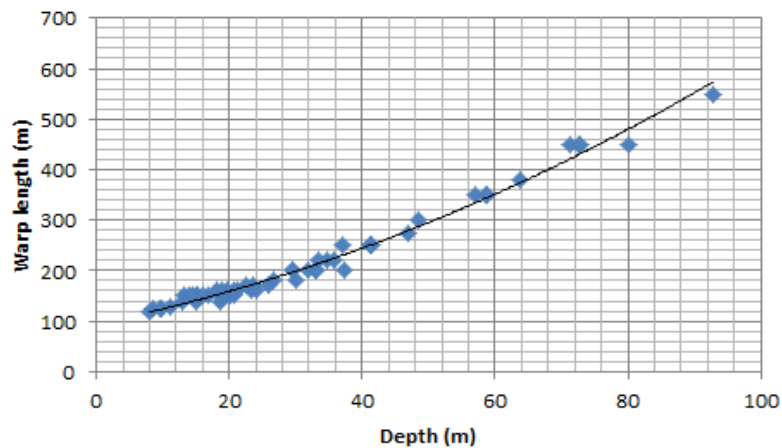


Figure 11 - Relationship between depth and warp length for the rapido trawl.

2.3 Sampling methodology

2.3.1 Vessel characteristics

The vessels used for the SoleMon surveys is the research vessel “G. Dallaporta” since 2007, when an intercalibration with the commercial vessels utilized before has been conducted. The N/O “G. Dallaporta” technical characteristics are summarized below:

Length (m):	35.3
Tonnage (TJB):	285
Year of construction:	2000
Materia:	Steel
Power (kW):	809
Warp diam (mm):	14-16
Warp length (m):	2500

The same vessel and crew have been used as much as possible every year in the area to carry out the survey series in view of reducing variations between years due to vessel effect.

2.3.2 Period of the survey

The SoleMon survey is carried out every year between November and December (Tab. 3).

Tab. 3 Haul calendar of SoleMon surveys divided by year

Haul	Year					
	2014	2015	2016	2017	2018	2019
1	25-nov	28-nov	25-nov	07-dic	02-dic	28-nov
2	26-nov	28-nov	26-nov	29-nov	03-dic	28-nov
3	26-nov	28-nov	26-nov	28-nov	03-dic	27-nov
7			03-dic	21-nov	30-nov	21-nov
10	19-nov	19-nov	02-dic	20-nov	23-nov	21-nov
11			03-dic	21-nov	30-nov	21-nov
12	24-nov	25-nov	24-nov	27-nov	01-dic	30-nov
14	27-nov	20-nov	27-nov	20-nov	23-nov	21-nov
15			03-dic	21-nov	30-nov	22-nov
17	23-nov	30-nov	23-nov	25-nov	29-nov	06-dic
19	28-nov	20-nov	04-dic	21-nov	30-nov	26-nov
20	25-nov	28-nov	04-dic	21-nov	30-nov	22-nov
21	23-nov	01-dic	22-nov	25-nov	27-nov	04-dic
22	20-nov	19-nov	02-dic	20-nov	23-nov	20-nov
23	20-nov	18-nov	01-dic	19-nov	22-nov	20-nov
31	10-nov	23-nov	15-nov	10-nov	04-dic	14-nov
32	21-nov	18-nov	01-dic	19-nov	22-nov	20-nov
36	21-nov	03-dic	05-dic	23-nov	04-dic	26-nov
44	25-nov	28-nov	25-nov	07-dic	02-dic	28-nov
45	26-nov	29-nov	26-nov	28-nov	03-dic	27-nov
47	27-nov	30-nov	23-nov	27-nov	30-nov	30-nov
49	10-nov	23-nov	15-nov	10-nov	04-dic	14-nov
51	19-nov	29-nov	26-nov	28-nov	03-dic	29-nov
53	20-nov	19-nov	02-dic	20-nov	23-nov	20-nov
58	19-nov	19-nov	02-dic	20-nov	23-nov	21-nov
59	27-nov	30-nov	23-nov	07-dic	01-dic	05-dic
60	20-nov	19-nov	02-dic	20-nov	23-nov	20-nov
65	23-nov	01-dic	22-nov	25-nov	27-nov	04-dic
66	10-nov	23-nov	15-nov	10-nov	04-dic	14-nov
77			04-dic	21-nov	30-nov	22-nov

2.3.3 Hauls localization

The hauls (Fig 12) considered within the "Sole sanctuary" in this thesis (which for convenience were defined as "inside") are positioned between the international waters edge and the Croatian coasts with a depth ranging from about from 38 to 58 m.

The stations outside the sanctuary (which for convenience were defined as "outside") include 5 stations in Croatian territorial waters, both coastal and offshore, 1 station in Slovenian territorial waters and the others distributed in Italian territorial waters, both along the coast and offshore. The depth range of these hauls is from about 16 to 73 m.

Except in few cases, due to technical problems, the hauls are made every year in the same position.

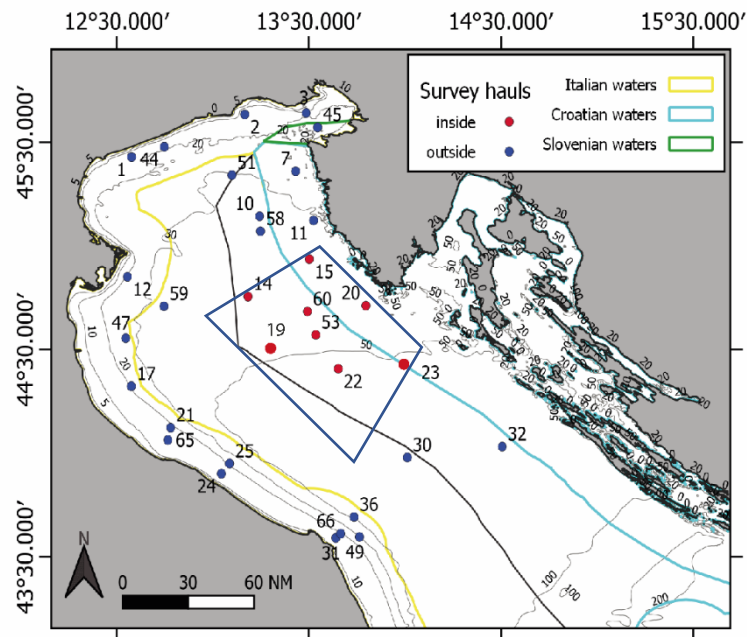


Figure.12 Hauls of SoleMon surveys considered in this thesis; the blue polygon represents the area of the “Sole sanctuary” (“Inside” area).

2.3.4 Operating the gear

The hauls are made only during daylight. The daylight period is defined as the time between 30 minutes after sunrise and 30 minutes before sunset.

The standard fishing speed is 5.5 knots on the ground. The actual speed as well as the covered distance is monitored and recorded.

A station consists of 30 minutes haul trawling two gears simultaneously during daytime, starting when the gear settles on the bottom and ending when hauling commences. Depending on the circumstances a shorter period is allowed not dropping below 10 minutes. In such cases the haul is repeated and the catches are pooled together.

In general, the hauls are made at constant depth. The depth variations during the haul should not exceed $\pm 5\%$ relatively to the initial depth. The discrepancies to this target should be recorded.

2.4 Samples processing on board

The catches of the two gears towed simultaneously are analyzed separately for “RAPIDO A” and “RAPIDO D”. To comply with ethical standards, cervical dislocation was used as a procedure, in accordance with the current European directive and national law (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010, Decree Law n. 26 of 4 March 2014),

because administration aboard an anesthetic would have prolonged the suffering of the same fishes. Length, weight, sex and maturity were recorded for all soles sampled. The measurement of length is ever the overall length, the tail being extended following its longer dimension. The measurement unit was the lower millimeter and were weighed individually per gram. The identification of the sex it was defined following four categories: male, female, undetermined (impossible to determine it by eye) and not determined (the individual has not been examined). Sexual maturity was, instead, defined macroscopically by referring to the identification keys provide by ICES (2010) and given in Annex I.

A subsample of all the soles caught, maintaining, where possible, a distribution of homogeneous size classes, were stored onboard and transported to the laboratory for the subsequent otoliths extraction for age estimation.

2.5 Samples processing in the laboratory

2.5.1 Otoliths extraction

Otoliths were extracted by practicing an incision above the ventral eye along the anteroposterior axis, and with the aid of a cutter, the skull is dissected and the brain removed (Fig. 13). Sagitta, which are easily visible in the vestibular apparatus, are removed with the aid of steel tweezers, cleaned in tap water, dried

and stored in plastic tube, with their identification code (Carbonara and Follesa, 2019).

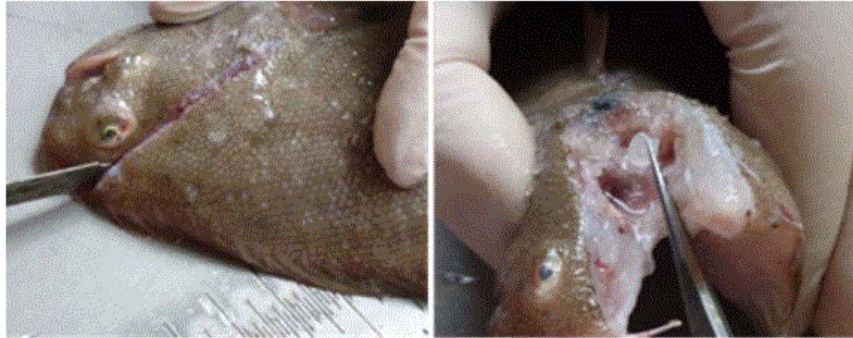


Figure. 13 Extraction of otolith in *S. solea*

2.5.2 Otolith processing

In some cases, sagitta are extremely opaque or too thick for all seasonal increases to be clearly observed on the upper surface, observation on the thin section bisecting the core in the transverse plane provide more reliable observation.

The burn technique (Panfili *et al.*, 2002) was used to increase the contrast between the growth zones. In detail, the right otolith was burned (350 ° C for 10 minutes in the stove) to darken the translucent zone so that it was clearly visible. Next, the otolith was mounted on a slide using clear epoxy resin (Crystalbond 509 AMBER) so that the back protruded from the slide just before the center of the core.

The otolith was then smoothed using lapping machine with rotating motorized plates on which an abrasive cloth of P800 was applied. The polished part of the otolith was turned upright so that the eroded portion comes into contact with the slide surface. The erosion operation was then repeated on the anterior part of the otolith until the nucleus was visible. The otolith was then polished with a micro lap polishing cloth soaked in 0.1-micron no. 3 Alumina.

The otolith section thus obtained was placed in a petri dish with a dark bottom and distilled water. The age determination on the section was performed with the use of an optical reflected light microscope with magnification of 10 and 16X connected to a digitized computer video system (Leica Application Suite 4.3.0.).

2.6 Data analysis

2.6.1 Common sole distribution and fishing effort

Considering the otoliths that were collected during the six years of survey and dividing them into five age classes (0, 1-2, 3-4, 5-8, 9+), a map was created to better understand how the soles are distributed inside the basin.

Fishing effort data was taken from previous studies based on the analysis of data from VMS (Vessel Monitoring System) and AIS (Automatic Identification System) (ISPRA, 2013; report DORY project, 2020; Russo *et al.* 2020)

2.6.2 Total length distribution, maturity and length-weight relationship

In order to characterize the common sole samples used for this thesis, graphs were created representing the distribution of the total lengths and the subdivision between mature and immature specimens. For this subdivision (between mature and immature) it was decided to subdivide the common sole on the basis of the results obtained by Scarcella et al. (2014).

The relationship between length and weight was described using the potential function (Bagenal, 1978):

$$W = aLT^b$$

where W is the weight, LT is the total length, a and b are the parameters of the relationship. These parameters can also be estimated through a linear regression of the logarithmically transformed length and weight data. In this way the relation from curvilinear becomes linear:

$$\log_{10}W = a' + b\log_{10}LT$$

where a' which is the \log_{10} of the coefficient a in the potential relation and represents the intercept of the line with the ordinate axis, while the parameter b represents the exponent of the potential form and the slope of the regression line.

If $b = 3$, the growth is isometric and it is therefore perfectly proportional in the three dimensions of the space; in this case the small specimens have the same shape and condition as the larger specimens. If the parameter b is different from 3 then the growth is allometric. In particular, if $b > 3$ the largest specimens have had a greater increase in height and width than in length (positive allometry); this can occur either because of a notable ontogenetic change in the shape of the body with increasing size, or, more commonly, because the larger specimens have a more robust shape than the smaller ones.

Conversely, if $b < 3$, the larger individuals increase more in length than the smaller ones (negative allometry).

2.6.3 *Otoliths analysis*

Each sectioned otolith was read by three readers. When readings differed by one or more years, a fourth reading was made.

To determine the accuracy of the age, estimate between the two readings, two indices were calculated: the Average Percentage Error (APE) and the Coefficient of Variation (CV).

The APE was calculated using the formula:

$$APE_j = 100 \times \frac{1}{R} \sum_{i=1}^R \frac{|x_i - x_j|}{x_j}$$

where R is the number of readings for each individual; x_{ij} is the i-th age attributed to the individual j-th; x_j is the average value of the age of the individual j-th.

The mean APE was calculated using the formula:

$$APE_{mean} = 100 \times \sum_{j=1}^n APE_j$$

The CV was calculated with the formula:

$$CV_j = 100 \times \frac{\sqrt{\sum_{i=1}^R \frac{(x_{ij} - x_j)^2}{R-1}}}{x_j}$$

where X_{ij} is the i-th age attributed to the individual j-th, X_j is the average value of the age of the j-th individual, R is the number of readings for each individual.

The mean CV was calculated with the formula:

$$CV_{medio} = 100 \times \sum_{j=1}^n CV_j$$

For each section of the otoliths, the measurement of the total length (dorsal-ventral length, AB), the dorsal length (OA) and the length of each annulus (R1, R2 Rn) were taken using the RFishBC package of R (Fig.14)

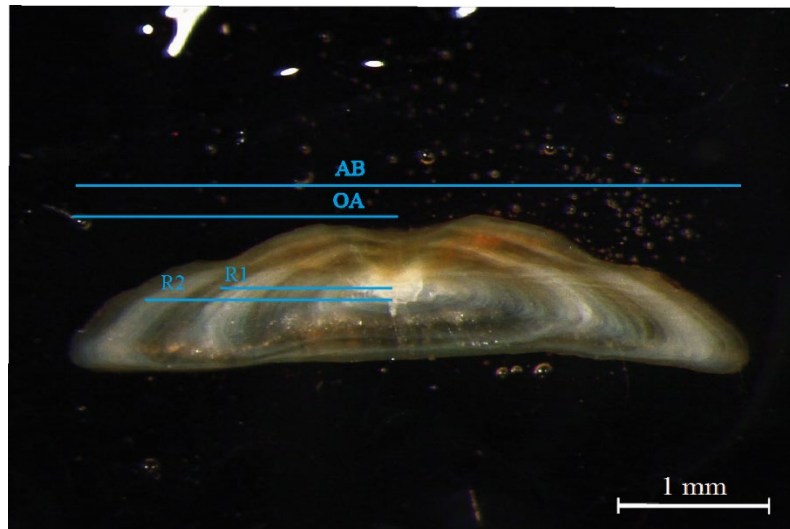


Figure.14 Scheme of the measurements taken on the thin section

These lengths were subsequently used for the back-calculation analysis (see below).

2.6.4 Back-calculation

Back-calculation of fish lengths at age is a useful tool for fishery biologists as it is based on the positive relation between body size and size of bony structures (Carlander 1969, 1977, 1997).

This analysis of daily or annual growth from otoliths requires firstly that the rate of deposition of increments in otoliths does not vary. This usually can be verified experimentally (Geffen 1992). Secondly, it assumes that these increments can be read with accuracy and precision (Campana 1992). Thirdly, all back-calculation models assume that there is a relationship between the growth of the otolith (increment width) and the somatic growth, usually length, of the fish. Evidence for this phenomenon usually is derived from

strong correlations between the size of the otolith and body size of fish (Vigliola and Meekan, 2009).

Francis (1990) defined back-calculation as “... *a technique that uses a set of measurements made on a fish at one time to infer its length at an earlier time or times. Specifically, the dimensions of one or more marks in some hard part of the fish, together with its current body length, are used to estimate its length at the time of formation of each of the marks. ... The marks are generally annual rings associated with growth checks, ...*”. Thus, back calculation is the reconstruction of the length of a fish at previous ages from measurements made on calcified structures.

Dahl (1907) and Lea (1910) proposed the first back-calculation model assuming a direct proportional relation between body and scale growth.

The Dahl-Lea model back-calculates length for individual fish as:

$$L_i = L_c(S_i/S_c);$$

L_i = back-calculated length at annulus i ;

L_c = length at capture;

S_i = scale radius to annulus i ;

S_c = total scale radius.

As measurements in this thesis, the total length of the otolith, the dorsal length and the distance from the center of the structure to the edge of an annulus have been calculated.

2.6.5 Growth curves

The von Bertalanffy growth function was fitted to the results of the back calculation for each area and a graph was created to compare the two von Bertalanffy curves.

To optimize convergence, initial values for the population parameters (fixed effect) were provided considering plausibility of life histories of the species (Froese and Pauly, 2022) and information from previous analysis (i.e. FAO-GFCM 2021): 380 mm for L_{∞} , 0.3 years⁻¹ for k_0 , -0.5 years for t_0 , 0.2 years⁻¹ for k_1 and 1.8 years for t_1 .

Growth was described according to the standard von Bertalanffy growth function:

$$L(a) = L_{\infty}(1 - \exp(-k(a - t_0)))$$

Where a is age, k in the growth coefficient, t_0 is the theoretical age when size is zero, and L_{∞} is asymptotic size (Carlson and Goldman, 2007). A non-linear least squares regression procedure was used to estimate the parameters of the von Bertalanffy growth function (VBGF) using length at age pairs and

minimizing the sum of the squared residuals between observed and expected values.

Kimura likelihood ratio test (Kimura 1980) has been used to examine the difference between the 2 VB curves.

There are four hypothesis tests in the Kimura test: H0 vs. H1, H0 vs. H2, H0 vs. H3, and H0 vs. H4. If more than one hypothesis was rejected ($p < 0.05$) the curves can be considered statistically different (Kimura,1980).

The model fitting and the Kimura test were conducted by using `growthlrt()` function in R Package `fishmethods`.

2.6.5 Additional analyses on trophic features of common sole inside and outside Sole sanctuary

In addition to the analyses already described, it was decided to take into consideration the data on stomach contents obtained from the work of Fanelli *et al.* (submitted) in order to analyze possible differences in the common sole diet in the two areas analyzed in this thesis.

The work from which the data was taken refers to samples taken during the survey SoleMon 2019, and among all the data, only those coming from the same stations used for the previous analyzes were taken into consideration.

The mean Stomach Fullness Index (SFI) was calculated for each sample as the ratios between the stomach content weights and both the gutted body weight (GW) and the total body weights (TW) as a proxy of feeding intensity. The following indices were then calculated (Hyslop *et al.*, 1980): per cent frequency of occurrence (%F), per cent numerical composition (%N), per cent gravimetric composition (%W).

In order not to incur differences in the diet due to a difference in size of the specimens used for the analyzes, it was decided to compare the size distribution of the samples in the two areas.

To analyze the similarities in terms of composition and structure of the samples, the similarity indices were calculated by calculating a matrix using the Bray-Curtis coefficient (Gorley, 2006):

$$d_{jk} = \frac{\sum_i |y_{ij} - y_{ik}|}{\sum_i y_{ij} + \sum_i y_{ik}}$$

where y_{ij} is the number of individuals of the i -th species in the j -th sample, and y_{ik} the number of individuals of the i -th species in the k -th sample.

To graphically represent the similarity results, the non-metric MDS (Multi-Dimensional Scaling) technique was applied, which allows the points to be reported on a two-dimensional plane based on the similarity values obtained with the matrix. This type of representation is obtained through a complex

algorithm (Shepard-Kruskal) which uses an iterative optimization procedure to identify the least distortion in the representation of the multi-dimensional structure of the punt on a two-dimensional plane. To more successfully configure the relationships between samples with the NMDS method, the calculated similarity matrix is transformed into a rank similarity matrix (Clarke, 1993), in which a progressive value is assigned to each value of the index obtained, to where the value one is given to the highest similarity value, two to the next, and so on. The estimate of the distortion introduced due to the geometric forcing that projects the theoretically required $n-1$ dimensions into two dimensions for n samples is indicated by the stress value (Tab. 4). This can be imagined as the average deviation between the real distances (similarities) of all mutual comparisons between possible pairs of two points in the matrix and the resulting one on the plane. The stress value is, therefore, important to evaluate the quality of the NMDS representation.

Table 4 Interpretation limits of MDS Stress values (Clarke, 1993)

Stress	Interpretation
0.00 - 0.05	Excellent representation
0.05 - 0.10	Good order, without false deductions
0.10 - 0.20	Possible errors of interpretation, especially in the details
> 0.20	Not usable, possible errors of interpretation

Finally, to calculate the contribution of each species (%) to the dissimilarity observed between the two samples, a SIMPER analysis was performed.

Chapter three

RESULTS

3.1 Distribution and fishing effort

The spatial distribution of sole, by class of age (Fig. 15), showed that juveniles (0 year) mostly concentrate along the Italian side from the coastal waters up to 30 m depth around the mouth of the Po River, which is a shallow area characterized by a high concentration of nutrients.

The common sole between 1 and 2 years old can be observed both along the entire coastal strip from Trieste to Ancona, in the central part of the basin and minimally near the Croatian coasts.

Common soles between 3 and 8 years old are distributed almost exclusively in the central part of the basin, away from the coasts and mainly inside the sanctuary. The older specimens (9+), on the other hand, were sampled exclusively within the "Inside" area.

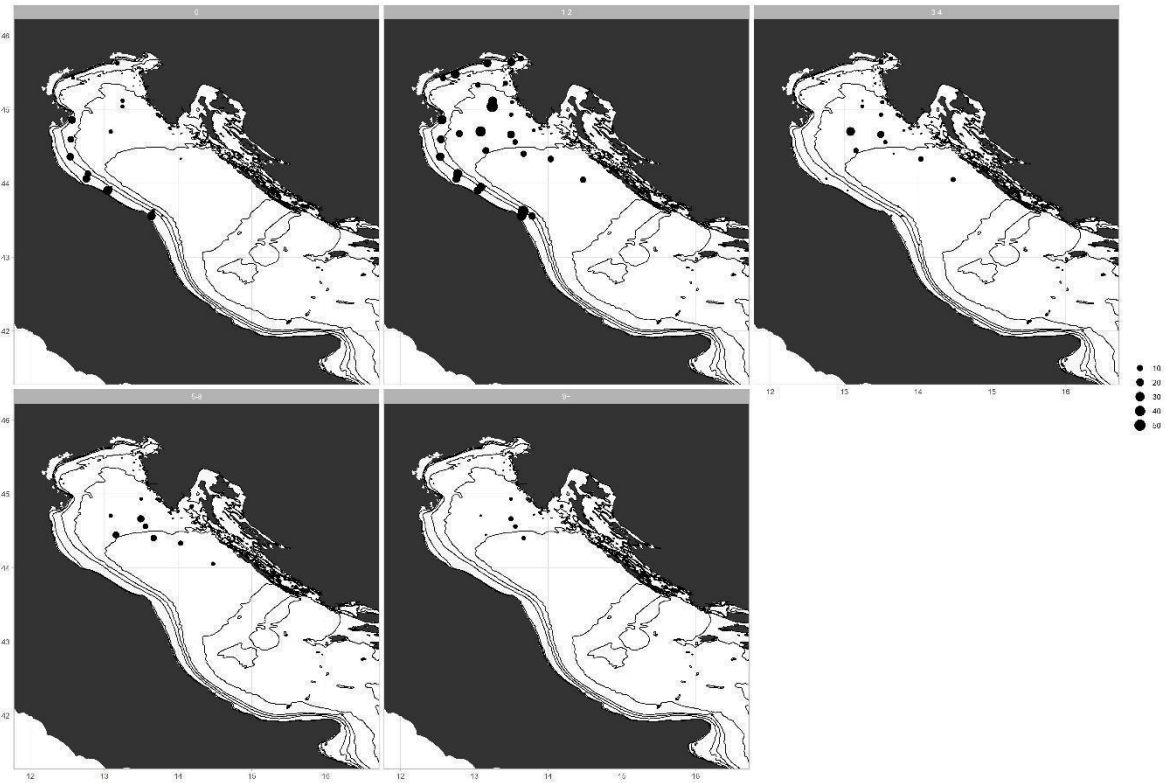


Figure.15 Spatial distribution of the sole during the six years of sampling divided into five age classes (from top left: 0, 1-2, 3-4, 5-8, 9+)

The maps concerning the fishing effort relating to OTB and TBB (Fig. 16, 17), taken in the various works, show how over time the area with the least impact is precisely the area of the "Sole sanctuary".

In recent years, however, an increase in the fishing effort by Italian gillnets has been noted in a portion of the "Sole sanctuary" (Fig. 18).

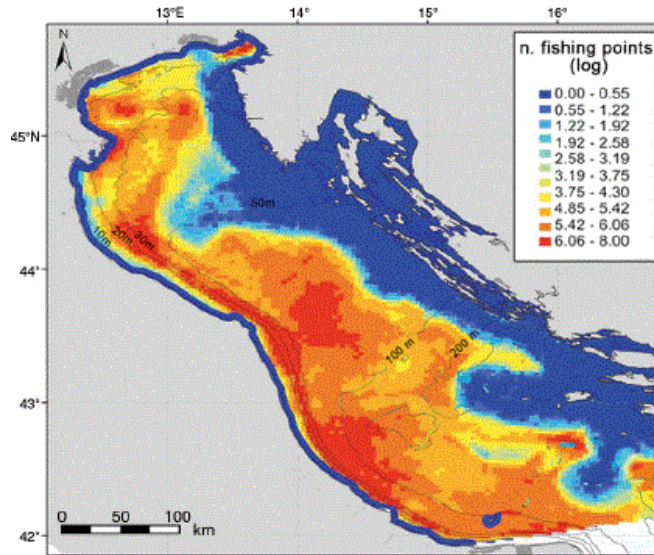


Figure. 16 Fishing effort distribution of trawling relative fishing effort based on VSM data (modified from ISPRA 2013)

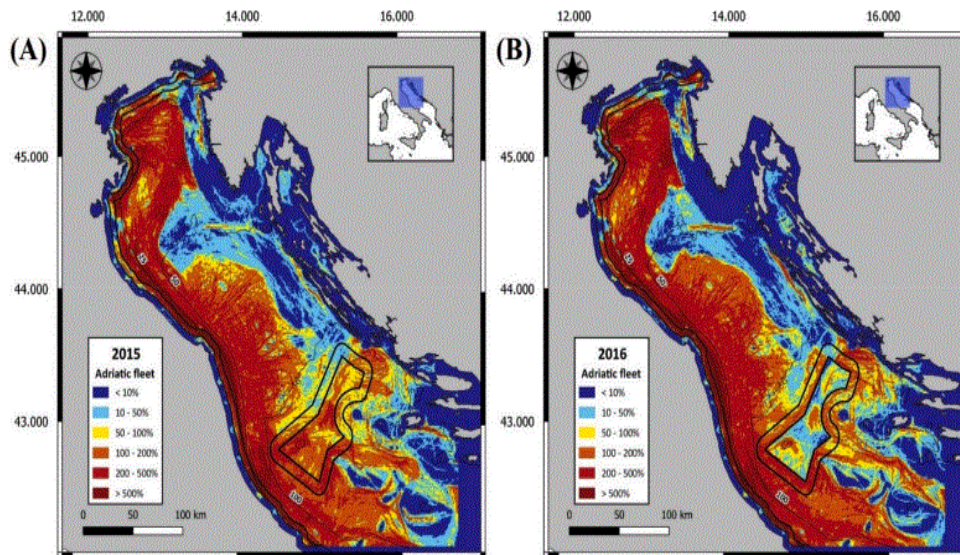


Figure. 17 Fishing effort distribution of trawling fleet based on AIS data in 2015 (A) and 2016 (B) (modified from Russo *et al.*,2020)

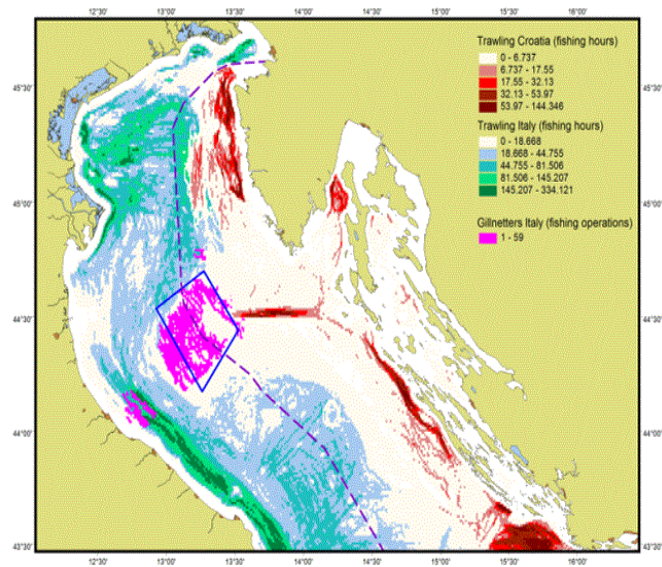


Figure. 18 Fishing effort distribution of trawling fleet base on AIS data (modified from report DORY project, 2020).

3.2 Total length distribution, maturity and length-weight relationship

During the six years considered by the SoleMon survey, the soles sampled from 31 different stations (8 “inside”, 24 “outside”, Fig. 12) had showed in the "inside" area a total length ranging from a minimum of 26.2 cm to a maximum of 36.2 cm in total length (TL), with an average of 29.61 ± 0.18 cm.

In the "outside" area, however, the soles ranged from a minimum of 24.5 cm to a maximum of 36.3 cm TL, with an average of 29.65 ± 0.15 cm, and total length distribution (Fig. 19).

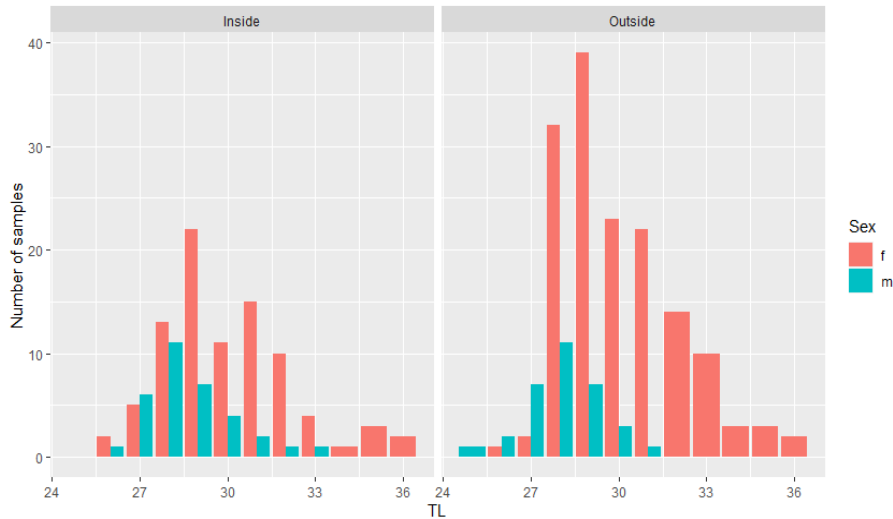


Figure.19 Total length (TL, cm) distribution, divided by sex, within the two areas

Furthermore, the common soles collected in “Sole sanctuary” area were found to have, for the same total length, a greater age than those sampled in the "outside" area (Fig. 20). The *S. solea*, included between 27-30 cm TL, included some specimens between 3 and 6 years old, reaching a maximum of 12 years in the "Inside" area. On the other hand, the sample from the "Outside" area comprised 2-year-old specimens with a maximum of 8 yrs. Taking into consideration the common sole with TL > 33 cm, specimens from the "Sole sanctuary" were estimated to have an age between 7 and 15 years, while those from the neighboring area likely have ca. 8-year-old.

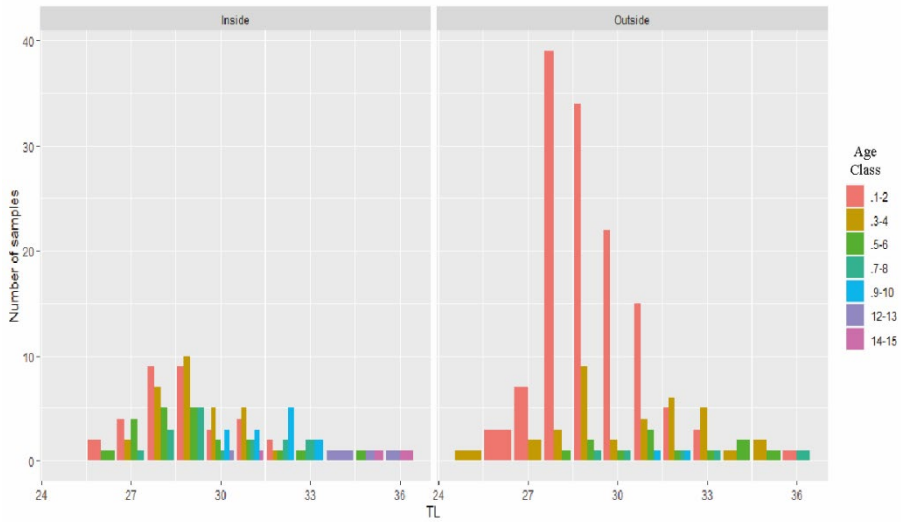


Figure. 20 Length distribution of common sole by age classes from the “inside” and “outside” area

Within the "Sole sanctuary" mature soles dominated (from 3 years upwards), while an opposite situation was observed outside (Fig. 21).

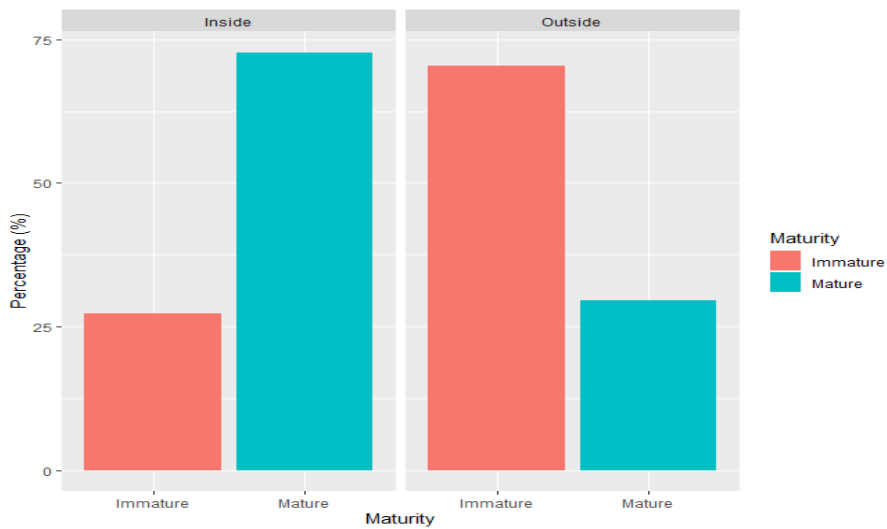


Figure. 21 Subdivision between immature (0-2 years) and mature (3+ years) divided by area

The exponents of the length-weight relationships ($b = 3.807$ for “outside” area and $b = 3.481$ for “inside” area; Fig. 22 and 23) of the *S.solea*, estimated in this thesis, are comparable with the results obtained in previous works, which were found to be between 2.78 and 3.36 (Tab. 1), and had showed positive allometric growth ($b > 3$).

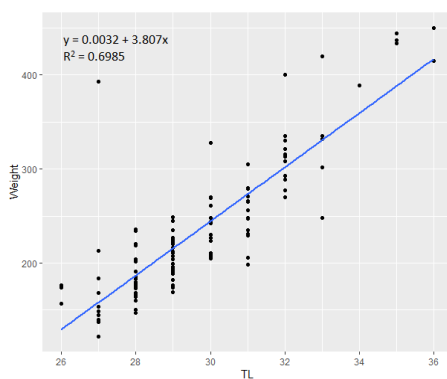


Figure. 22 Length-weight relationship in the “inside”. The blue line represents the regression line.

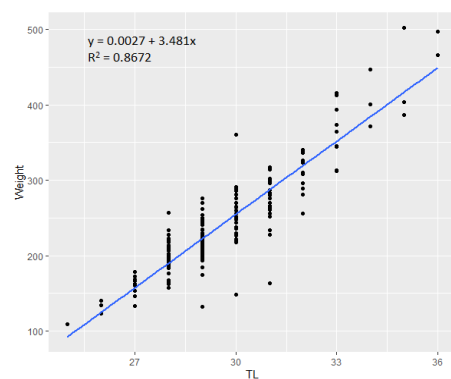


Figure. 23 Length-weight relationship in the “outside”. The blue line represents the regression line.

3.3 Otolith analysis and growth

A total of 304 otolith sections (121 “inside” and 183 “outside”) were analyzed. Variability indices, CV and APE, were both very low (6.7% and 4.98%), indicating that the ageing procedure adopted gave a reasonable level of consistency (or reproducibility) between readings.

The mean back-calculated total lengths at age of the common sole were similar in both sexes (Tab.5).

Table 5. Mean back-calculated total lengths (cm) for male and female

Estimated age	Mean	
	Female	Male
1	140.89	137.41
2	201.15	191.67
3	223.63	213.64
4	235.89	225.25
5	248.00	236.40
6	258.17	243.60
7	265.80	256.28
8	274.59	260.12
9	282.57	268.68
10	281.74	284.20
11	292.68	282.60
12	299.47	288.10
13	292.57	296.80
14	296.60	302.90
15	305.93	305.60
16	297.90	296.90
17	304.60	304.60
18	304.60	304.60
19	328.30	328.30
20	328.30	328.30
21	335.90	335.90
22	335.90	335.90
23	342.10	342.10
24	342.10	342.10
25	347.60	
26	347.60	
27	353.80	
28	353.80	

The von Bertalanffy growth parameters (Tab. 6 and Fig. 24) were estimated by applying the non-linear least square algorithm on the age readings collected in the two areas on the basis of the data sources described above.

Table 6 von Bertalanffy growth parameters calculated for each area

Von Bertalanffy growth parameters			
	L_{∞}	k	t_0
Inside	280.61	0.41	-1.13
Outside	265.36	0.61	-0.79

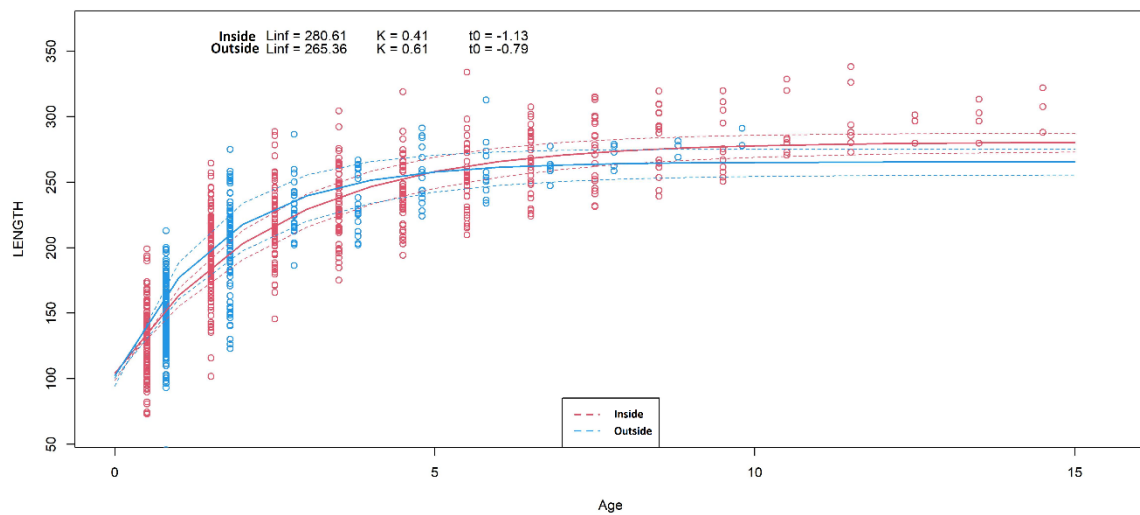


Figure. 24 von Bertalanffy growth curve and related growth parameters

The values of K and L_{∞} differed between the two areas. A lower K value in the "inside" area denotes that growth is slower in the "Sole sanctuary". Furthermore, a higher value of the L_{∞} , again in this area, suggests that the soles, which take refuge inside the sanctuary, can reach a greater total length than those of the surrounding areas.

The analysis was carried out using the Kimura Test, since p value was <0.05 for 4 out of 5 comparisons (Tab. 7). The test confirmed the significant difference in the growth of sole in the two areas.

Table 7 Kimura test results

Kimura test				
tests	hypothesis	chisq	df	p
1 Ho vs H1	$L_{inf1}=L_{inf2}$	4.66	1	0.031
2 Ho vs H2	$K1=K2$	8.18	1	0.004
3 Ho vs H3	$t_{01}=t_{02}$	3.32	1	0.068
4 Ho vs H4	$L_{inf1}=L_i=K2$ $t_{01}=t_{02}$	29.05	3	0.000

3.4 trophic features of common sole inside and outside Sole sanctuary

A total of 82 common sole stomachs were analyzed for the "Inside" area were a total of 82 (43 from females and 39 from males), while for the "Outside" area there were a total of 185 (113 from females and 72 from males; Tab.).

The SFI, calculated for the "Outside" area, was always higher than the one calculated for the "Inside" area (Tab. 8).

Table 8 The mean Stomach Fullness Index

Sex	n. stomachs analysed		Stomach Fullness index, SFI Mean \pm Err. St. (g)			
	Inside	Outside	Inside		Outside	
			on GW	on TW	on GW	on TW
Female	43	113	0.075 \pm 0.015	0.07 \pm 0.014	0.223 \pm 0.025	0.212 \pm 0.024
Male	39	72	0.05 \pm 0.009	0.049 \pm 0.009	0.231 \pm 0.039	0.226 \pm 0.038
Combined	82	185	0.063 \pm 0.009	0.060 \pm 0.008	0.225 \pm 0.021	0.217 \pm 0.020

Both the graphical (Fig. 25, 26) and statistical comparison (p value <0.05) showed differences when comparing the two groups of samples.

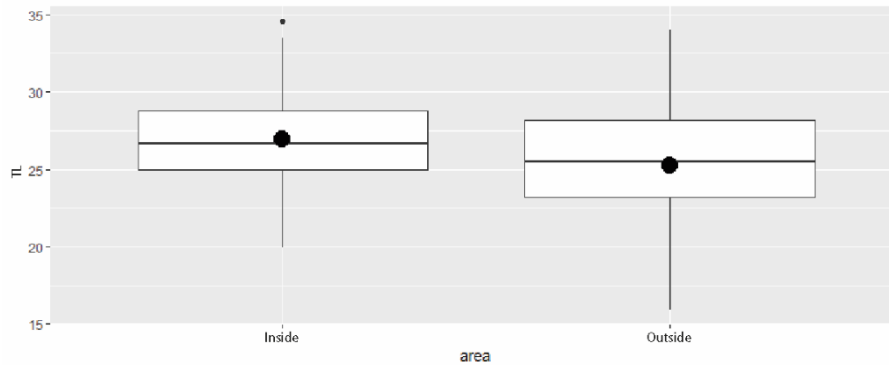


Figure. 25 Boxplot of total lengths by area. The box in the Boxplot extends from the lower quartile to the upper quartile; the horizontal line represents the median, and the point inside the inter quartile range represent the mean

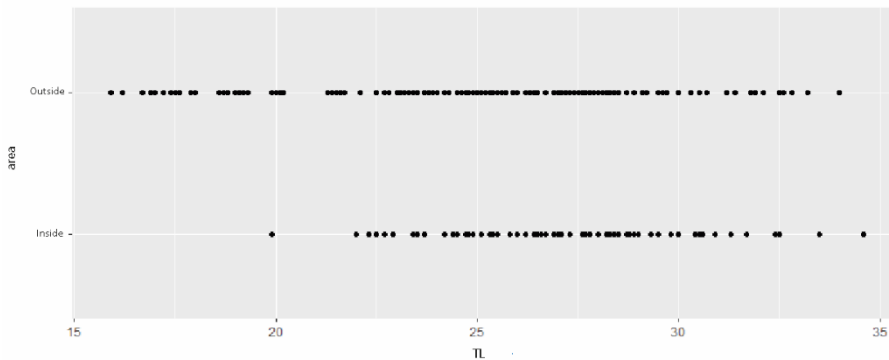


Figure. 26 Scatter plot of total lengths by area

Therefore, taking into consideration the first maturity size calculated for females by Fabi et al. (2009) (TL = 25.8 cm), it was decided to take into consideration only the samples related to the samples having a TL > 25 cm.

The NMDS plots (Fig. 27) showed how, although there was a slight overlap, the data relating to the "inside" area were always quite grouped.

The results that were obtained through the NMDS, which showed a stress value always between 0.05 and 0.10 and a linear fit greater than 0.8 (Fig. 28), were also confirmed by the statistical analysis (p value <0.05).

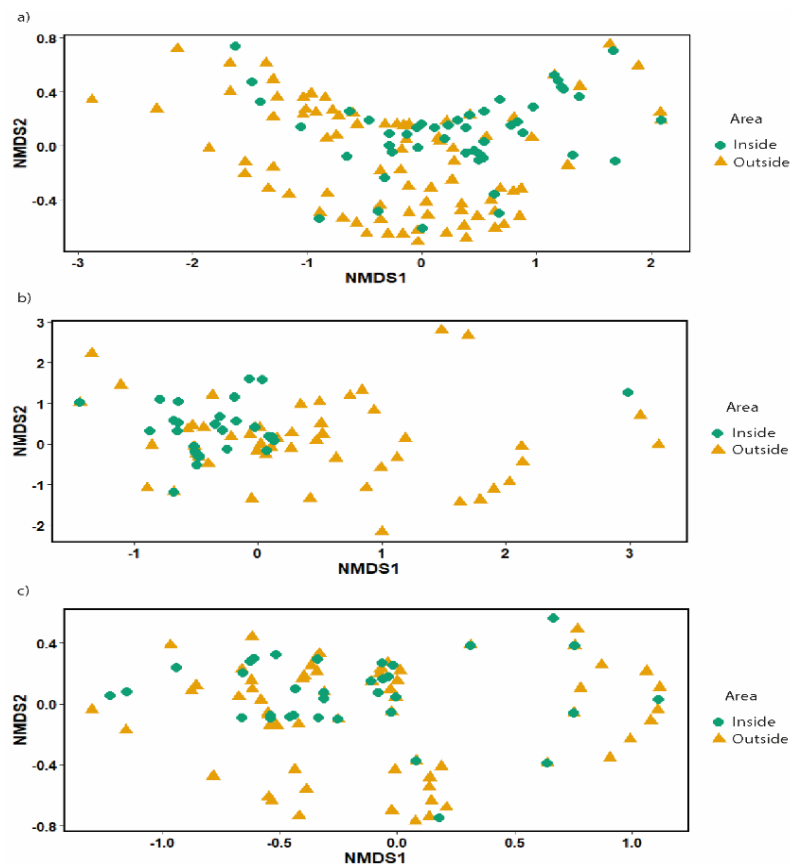


Figure. 27 NMDS obtained from the analysis, using the Bray-Curtis index, by areas on the indexes of gravimetric composition of prey (a), prey numerical composition (b) and frequency of occurrence of prey (c).

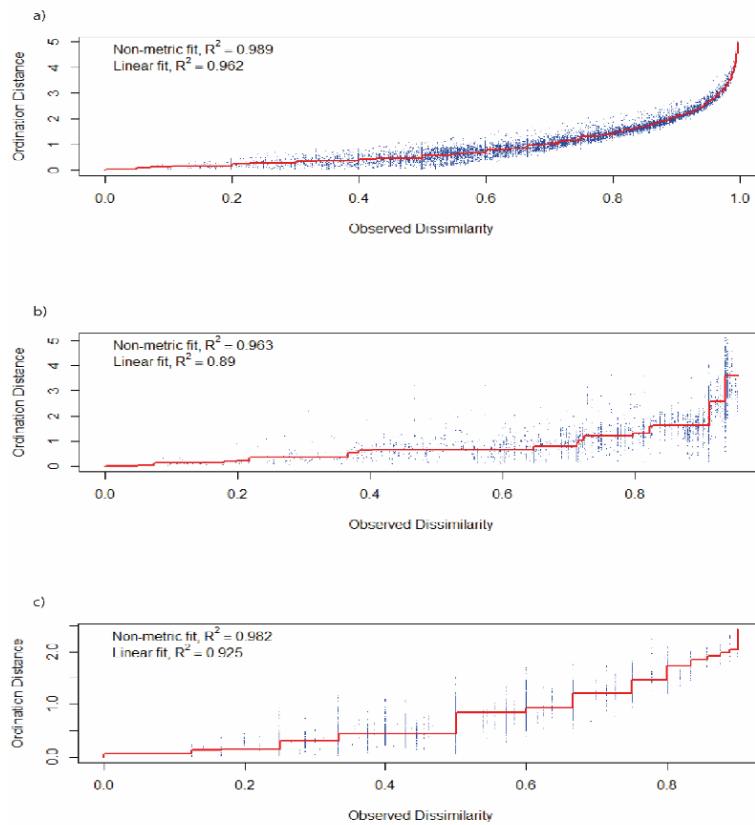


Figure. 28 Stress plots related to NMDS obtained from the analysis on the index of gravimetric composition (a), numerical composition (b) and frequency of occurrence (c).

The comparison through the SIMPER test highlighted statistical differences for some taxa found in the stomachs analyzed in the two areas, such as Echinodermata, Serpulidae, Aphroditidae (Tab. 9).

Table 9 SIMPER test results

	average	sd	ratio	ava	avb	cumsum	p
emptyness	0.245859	0.139892	1.75748748	5.837837838	9.541666667	0.383145	0.001
Parassites	0.180774	0.129308	1.398006461	1.635135135	4.541666667	0.664862	0.001
Decapoda.eggs	0.090191	0.136344	0.661493082	2.675675676	3.958333333	0.805414	0.444
Echinodermata	0.015613	0.036798	0.424296443	0.067567568	0.229166667	0.829746	0.014
Shells	0.013905	0.03167	0.439069632	0.189189189	0.125	0.851416	1
Polychaeta	0.013893	0.031455	0.441694131	0.175675676	0.145833333	0.873067	1
Ampeliscidae	0.0125	0.043289	0.288754865	0.189189189	0.041666667	0.892547	1
Crustacea	0.011323	0.036746	0.308130574	0.189189189	0.0625	0.910192	1
Gastropoda	0.005317	0.019652	0.270531525	0.013513514	0.125	0.918477	0.336
Solecurtus.strigilatus	0.00417	0.019854	0.210052798	0.067567568	0	0.924976	1
Ophiura.sp.	0.003414	0.018796	0.18161192	0.027027027	0.020833333	0.930296	0.312
Oestergrenia.digitata	0.003154	0.017152	0.183895972	0.040540541	0.020833333	0.935211	1
Processa.sp.	0.003059	0.018817	0.162545311	0.013513514	0.020833333	0.939978	0.141
Corbula.gibba	0.002283	0.017292	0.132016987	0	0.020833333	0.943535	0.001
Ampharetidae	0.002283	0.017292	0.132016987	0	0.020833333	0.947093	0.001
Fish.bones	0.001978	0.014883	0.132873271	0.027027027	0	0.950175	1
Antalis.inaequicostata	0.001886	0.00996	0.189360873	0.027027027	0.020833333	0.953114	0.973
Bivalvia	0.001727	0.010227	0.168897479	0.013513514	0.020833333	0.955806	0.665
Glycera.unicornis	0.001704	0.013862	0.122894779	0.027027027	0	0.958461	1
Serpulidae	0.001645	0.00844	0.194883534	0	0.041666667	0.961024	0.001
Pectinidae	0.001554	0.010449	0.148746381	0.027027027	0	0.963446	1
Tellina.donacina	0.001509	0.011044	0.136619052	0	0.020833333	0.965798	0.001
Tellinidae	0.001373	0.008835	0.155409296	0.013513514	0.020833333	0.967937	0.856
Elphidium.crispum	0.001358	0.00928	0.146331435	0.027027027	0	0.970054	1
Foraminifera	0.001334	0.007524	0.177256929	0.013513514	0.020833333	0.972132	0.64
Terebellides.stroemi	0.001319	0.011646	0.113281324	0.054054054	0	0.974188	0.999
Sipunculidae	0.00128	0.008922	0.143499887	0.027027027	0	0.976183	1
Stemapsis.scutata	0.001255	0.013307	0.094336804	0.013513514	0	0.97814	1
Alpheus.glaber	0.001255	0.013307	0.094336804	0.013513514	0	0.980096	1
Mollusca	0.001224	0.008698	0.140674339	0.027027027	0	0.982003	1
Algae	0.000992	0.009881	0.100353112	0.013513514	0	0.983548	1
Alvania.cimex	0.000992	0.009881	0.100353112	0.013513514	0	0.985093	1
Marphysa	0.000992	0.009881	0.100353112	0.013513514	0	0.986639	1
Philocheras.trispinosus	0.000992	0.009881	0.100353112	0.013513514	0	0.988184	1
Ampelisca.anophtalma	0.000989	0.008735	0.113281324	0.040540541	0	0.989726	0.999
Turritellidae	0.000832	0.00801	0.103886001	0.013513514	0	0.991023	1
Polynoidae	0.00066	0.005823	0.113281324	0.027027027	0	0.992051	0.999
Wood	0.000641	0.005939	0.107859662	0.013513514	0	0.993049	1
Hippolytidae	0.000627	0.004405	0.142242777	0	0.020833333	0.994025	0.001
Lucinella.divaricata	0.000627	0.004405	0.142242777	0	0.020833333	0.995002	0.001
Apseudes.latreillei	0.000577	0.00529	0.109085712	0.013513514	0	0.995901	1
Aphroditidae	0.000577	0.00529	0.109085712	0.013513514	0	0.996801	0.001
Tethys.fimbria	0.000526	0.00478	0.110032807	0.013513514	0	0.99762	1
Glyceridae	0.000448	0.004023	0.111397749	0.013513514	0.020833333	0.998318	0.025
Maldanidae	0.000418	0.003733	0.111904825	0.013513514	0	0.998969	1
Ctenophora	0.000391	0.003484	0.11233142	0.013513514	0	0.999579	1
Heterobranchia	0.000177	0.001533	0.115330958	0.013513514	0	0.999855	0.999
Glycera.lapidum	9.31	0.00064	0.145472224	0	0.020833333	1	0.025
Larvae	0	0	0	0	0	1	0.001
Owenia.fusiformis	0	0	0	0	0	1	0.001
Peronidia.albicans	0	0	0	0	0	1	0.001
Eunice.vittata	0	0	0	0	0	1	0.001
Leptopenctata.tergestina	0	0	0	0	0	1	0.001
Ampelisca.tenuicornis	0	0	0	0	0	1	0.001
Callionymus.risso	0	0	0	0	0	1	0.001
Upogebia.tipica	0	0	0	0	0	1	0.001
Nereididae	0	0	0	0	0	1	0.001
Timarete.sp.	0	0	0	0	0	1	0.001
Pista.sp.	0	0	0	0	0	1	0.001
Sabellidae	0	0	0	0	0	1	0.001
Paraonidae	0	0	0	0	0	1	0.001
Plastic	0	0	0	0	0	1	0.001
Gracilipurpura.rostrata	0	0	0	0	0	1	0.001
Turritellinella.tricarinata	0	0	0	0	0	1	0.001
Gammaridea	0	0	0	0	0	1	0.001
Glycera.capitata	0	0	0	0	0	1	0.001
Donacidae	0	0	0	0	0	1	0.001
Aricidea.Acмира.catherinae	0	0	0	0	0	1	0.001

Chapter four

DISCUSSION

4.1 General aspects

The present thesis has allowed us to analyze the differences in growth of the common sole contrasting two areas (the "sole sanctuary" vs. the surrounding area) characterized by a different fishing effort.

The results obtained, considering the six years of the SoleMon survey agree with the spatial distribution of common sole in the Adriatic Sea described by previous studies (Fabi *et al.*, 2009; Grati *et al.*, 2013, Scarcella *et al.*, 2014) and that showed the greatest concentration of juveniles along the Italian side from coastal waters up to 30 m deep, characterized by the presence of muddy bottoms.

The spatial distribution of juveniles could be the result of a combination of factors. Variations in depth result in different environmental conditions, such as light penetration, temperature, salinity, local hydrodynamics and substrate type, as well as important biological factors, such as the availability of prey and the risk of predation.

In his work Gibson (1994) points out that the availability of prey and the risk of predation together with the temperature are the most important factors in the determination of juvenile flatfish.

Several studies (Beyst *et al.*, 1999; Lagardère, 1987; Molinero and Flos, 1991) have also described how the common sole belonging to the age group between 0-1 feed mainly on polychaetes, amphipods and small bivalves, which are found in close association with sandy and muddy substrates.

In contrast, the older soles (9+) are distributed southwest of the Istrian peninsula to the midline between the western and the eastern Adriatic. This area is characterized by a great variety of habitats ranging from relict sands, inhabited by a very rich population community dominated by the cnidarian *Alcyonum palmatum* and the echinoderma *H. forskali*, at detrital bottoms, characterized by the presence of gravel and mollusc debris shells, branched bryozoans and in some cases holothurians (Grati *et al.*, 2013; Santelli *et al.*, 2017).

Previous studies, which analyzed the fishing effort in the north-central Adriatic Sea (ISPRA, 2013; report DORY project, 2020; Russo *et al.*, 2020), showed that the "sole sanctuary" is not impacted by OTB and TBB activities. This difference in fishing effort is certainly related to the difficulty of TBB and OTB in operating in this area (Scarcella *et al.*, 2014; Bastardie *et al.*, 2017; Santelli *et al.*, 2017). The main reason is due to the presence inside the sanctuary of holothurians and bryozoans (Santelli *et al.*, 2017). As reported, in fact, by Salvalaggio *et al.* (2014) evisceration by sea cucumbers damages and reduces

the commercial value of the catch, while bryozoans (e.g. *A. semiconvoluta*) clogs the nets, thus reducing their efficiency.

Furthermore, some studies (Fabi *et al.*, 2009; Grati *et al.*, 2013; Scarcella *et al.*, 2014) have observed that thanks to this migratory pattern, and probably also due to the difference in fishing effort, most of the spawners are concentrated outside the west coast of the Istrian peninsula. In accordance with these studies, our results have highlighted a greater presence of mature common sole within the "Sole sanctuary" with respect to the surrounding areas. In addition, our results showed that within the sanctuary females with a greater age than the surrounding areas occur.

As regards the exponents of the length-weight relationship, values of 3.00 in the North Sea (De Veen, 1976), 3.21 in Douarnenez Bay (Deniel, 1984), 3.31 in the North Atlantic (Coull *et al.*, 1989) and 2.71-2.83 in Izmir Bay (Hossucu *et al.*, 1999) were recorded. Our results instead are comparable with values found for specimens from the GSA 17, which reported, collectively between males and females over several years, values of 3.08 (Piccinetti & Giovanardi, 1984), 3.24 (Froglia & Giannetti, 1986), 3.04 (Fabi *et al.* 2009), 3.36 (Santojanni *et al.*, 2012), 3.057 (Bolognini *et al.*, 2013), and, in agreement with Bolognini *et al.* (2013), the growth was found to be allometrically positive.

4.2 S. solea growth

Comparing the growth parameters of the von Bertalanffy growth curve for the species from literature and those obtained in the present study (Tab. 6; Fig. 24), significant differences between the two areas were observed, with soles inside the sanctuary showing a slower growth and the possibility of reaching a higher LT (L inf. "Inside" = 28.06 cm vs. L inf. "Outside" = 26.53 cm).

Differences between our results and those previously obtained for the Adriatic Sea (i.e. L inf. = 40.1 cm, $k = 0.68$ by Piccinetti and Giovanardi, 1983) may be attributable to the smaller sample size analysed in this study which is therefore not representative of the entire population. However, here our main goal was to compare the area of the "Sole sanctuary" vs the surrounding areas, and thus our sampling strategy was set for this objective.

The results of this work are extremely important for improving fishery management and stock assessment.

As for fishing, indeed, in 2019, 56% of the catches were made by Italian fast trawling fleets (TBB), 21% by Italian, Slovenian and Croatian fishing boats (GNS and GTR) operating mainly within three nautical miles from the coast, 20% by trawl-fishing vessels (OTB) and the remaining 3% from fishing for the Croatian rampon (Scientific, Technical and Economic Committee for Fisheries, STECF, 2021).

However, as observed from the analysis of the fishing footprint in the area, there is a certain amount of fishing effort related to the Italian gillnets inside the "Sole sanctuary". Furthermore, the catches by Croatian GTR and GNS, in terms of biomass, are dominated by larger specimens and growing over the years, with a higher catch in fall and early winter and a peak in December (Matic-Skoko *et al.*, 2018).

Here we observed that an important portion of spawners inhabit the "Sole sanctuary" for shelter, and that most likely are stationed here reaching considerable ages. This information is crucial for managing and protecting such large part of the spawner population in the North-central Adriatic Sea.

Population, characterized by an advanced age structure with a substantial abundance of "older" specimens are to be considered more stable and less prone to the collapse due to overfishing (Hixon *et al.*, 2014).

Marine fish stocks were indeed vulnerable to the loss of genetic variability, potentially leading to reduced adaptability, population productivity and persistence when the older members of the fish population were removed (Hauser *et al.*, 2008).

Additionally, the FAO-GFCM working group on stock assessment of demersal species (WGSAD) recommended reducing mortality due to the low spawning biomass of the stock.

Furthermore, for the researchers working on the stock assessment, the data relating to the presence of common soles aged between 9 and 15 years will be useful to be able to set the parameters of the predictive models to the best.

Certainly, in the future other studies will be necessary to better understand what are the other factors that influence the difference in the growth here found between the two areas and to modify the present evaluation and management plan of *S. solea* within the GSA 17.

4.2 S. solea trophic features

The results obtained from the stomach content analysis (SCA), showed marked differences in the diet of common soles from the "sole sanctuary" vs. those from the neighboring areas.

Furthermore, SCA highlighted the presence of invertebrates (i.e., polychaetes, molluscs, small crustaceans and echinoderms), in accordance with other studies (Ramos, 1981; Lagardère, 1987; Henderson, James and Holmes, 1992). The diet of specimens from the "sole sanctuary" is rich in polychaetes, especially

carnivores such as the *Glycera* genus or the Aphroditidae family, generally more abundant in non-trawled areas, as observed by Romano *et al.* (2016).

Chapter five

5 Concluding remarks

In conclusion, the present thesis showed that:

- The "sole sanctuary" represents a real refuge for the common sole spawners, especially females;
- The sanctuary hosts specimens that reach higher ages than those from the surrounding areas;
- Growth rates differ between specimens from the "sole sanctuary" vs. those from neighboring areas;
- Similarly, also the trophic features of the species differ between "sole sanctuary" vs. surrounding areas;
- The application of some conservation measures to the "sole sanctuary" could play a fundamental role in the management of this fish stock.

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ANNEX I. CODES OF SEXUAL MATURITY FOR *S. SOLEA*

Reference pictures for female sole



Stage1: Primary: Translucent => line test

Secondary: Pale in color, gonad less than ½ body length, blood vessels are threadlike



Stage2: Blood vessels are taut, tissue is healthy and recovered in comparison to stage5; Eggs can be from grains through to non-hydrated fully developed eggs



Stage3-4: Hyaline/ hydrated eggs are distinguishable. Stage4 shows and higher quantity of eggs.

Stage5: Blood vessels are no longer taut, the tissue does not look healthy yet, the gonads already shorter than stage 4 (no picture available)

Reference pictures for male sole



Stage1: Primary: semi-skimmed milk color, kidney shape.



Stage2: Primary: 3D and more filled out. Secondary: cream color, from little to maximum size.

Stage3: Primary: large and full gonad; miltis running, either when squished or when cut. Secondary: Coffee with cream color. The spermatoducts well developed, wide, big and round (no picture available).



Stage4: Primary: flabby, thinly elongated, (lobbed). Secondary: opacified.