PHD COURSE IN LIFE AND ENVIRONMENTAL SCIENCES

Report Form for PhD student annual evaluation (XXXVI and XXXVII cycles)

Name of PhD student: Laura Sabatini

Title of PhD research: Comparative investigation of the biology and ecology of Egyptian sole (*Solea aegyptiaca*, Chabanaud, 1927) and common sole (*Solea solea*, Linnaeus, 1758) in the Adriatic Sea

Name of PhD supervisor: Emanuela Fanelli
Researchlab name: Lab of Marine Biology and Ecology

Cycle:
[X]XXXVI
[ ]XXXVII

PhD Curriculum:
[X]Marine biology and ecology
[ ] Biomolecular Sciences
[ ]Civil and environmental protection

DISVA instrumentation labs/infrastructureeventually involved in the project:
[ ]Actea Mobile Laboratory
[ ] Advanced Instrumentation lab
[ ] Aquarium
[ ]MassSpec lab
[ ] MaSBiC
[ ] Simulation/informatics lab
[ ]Other. Please, indicate: .....

ABSTRACT:
In the Adriatic Sea, common sole, one of the most valuable fishery resources, coexists with a rarer and cryptic species, morphologically very similar, the Egyptian sole. The PhD project aims to deeply analyse and compare several aspects of the life cycle and ecology of these two sibling species. During the second year, the sampling activities have been completed. Overall, 645 common soles and 398 Egyptian soles have been sampled. Different research lines have been developed and are still in progress. So far, 74 different *taxa* have been identified by stomach and intestine analyses (44 at species or genus level). Both species feed mainly on polychaete worms. The two soles seem to be batch spawners: the eggs progressively mature and are released in batches. Moreover, they show a similar growing trend: a fast growth during the first two years of life and the achievement of the asymptotic length starting from the third year. The analysis of sectioned otoliths has revealed the actual longevity of these species, more than 10 years.

Part 1. Scientific case of the PhD Research

- BACKGROUND

As in other areas of the Mediterranean, in the Adriatic Sea common and Egyptian sole are sympatric and share the same habitats: sandy-muddy bottoms in marine and brackish waters. They are considered two cryptic

species because of their external similarity, but they are genetically separated and reproductively isolated (Borsa and Quignard, 2001; Boukouvala *et al.*, 2012).
These two species are valuable fishery resources in the Mediterranean Sea:

- Mediterranean Sea provides 15% of the overall common sole's world landings, the 23% of these landings come from the north and the central Adriatic Sea (FAO-FISHSTAT source);
- Egyptian sole is an important fishery resource in the Eastern Mediterranean Sea (Mehanna, 2007; Gabr, 2015).

Fishermen in the Adriatic Sea do not distinguish the two species, as was reported in other Mediterranean areas (Boukouvala *et al*., 2012), and the Egyptian sole does not appear in the Mediterranean fisheries statistics, except for Egypt. The SoleMon scientific surveys carried out annually in the GSA17 showed that the occurrence of Egyptian sole in scientific catches was about 1% of the common sole occurrence, and the species was characterized by a coastal distribution up to 30 m of depth, with positive hauls mainly in the northern part of the basin (Sabatini *et al.*, 2018). However, during fishery data collection activities in the northern Adriatic, it was observed that, in some cases, sole landings of Chioggia's fishing vessels were composed mostly by Egyptian soles (Sabatini, personal observation). Species misidentification could occur during the collection of fishery data and, consequently, the evaluation of stock status of common sole in the GSA17 (Grati *et al.*, 2013) could be questioned due to a potential bias in species identification. Moreover, the current knowledge on biology and many aspects of the ecology of these two species in the Adriatic Sea is still scarce.

- SCIENTIFIC AIMS

The PhD project aims to deeply analyze and compare several aspects of the life history traits and ecology of Egyptian and common sole in the Adriatic Sea. Information extremely important to support the implementation of an ecosystem approach in fishery research and management actions. In detail, the project focuses on investigating:

- the feeding habits and the trophic level;
- the reproductive biology and females' fecundity;
- age and growth;
- the ecological connection among the different life stages and their habitat use (in particular as nursery areas and spawning grounds).

- WORKPLAN AND RESEARCH ACTIVITIES

Over the three years of research, the activities are carried out following this roadmap:

1. First year: fishery-dependent and fishery-independent sampling activities;
2. Second year: conclusion of sampling activities; laboratory activities: stomach content and stable isotope analyses; histological analysis of gonads; analysis of age and growth;
3. Third year: conclusion of stomach content and stable isotope analyses; chemical composition of the otoliths analyses; data elaborations and writing.

SECOND YEAR ACTIVITIES

1. Objective: conclusion of the sampling activities

Methods

Fishery-dependent sampling
At the beginning of the second PhD year, fishery-dependent sampling activities were concluded. The samples were collected from the landings of coastal otter trawlers, gillnetters and *rapido* trawlers at Chioggia harbour in November and December 2021. Information about the fishing areas or the geographic coordinates were provided by fishermen. Four length classes have been defined based on previous information about age, growth, and length at first maturity of both species: ≤ 20 cm, 21-25 cm, 26-29 cm, ≥ 30 cm, for each class 15

specimens have been sampled. The aim was to try to cover the widest length range possible to intercept the ontogenetic shifts in the variables studied.

Fishery-independent sampling

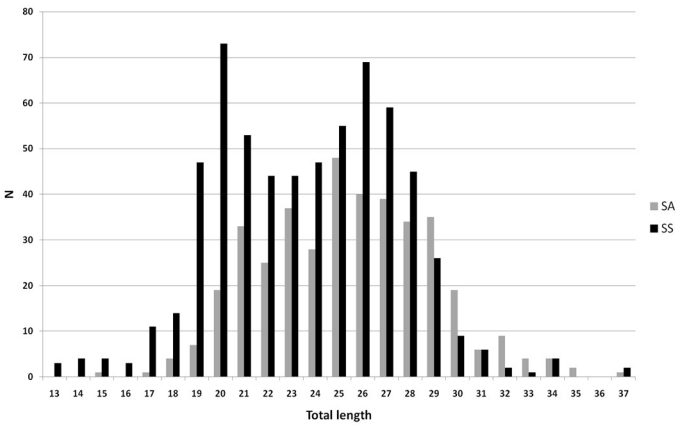
Fishery-independent sampling was conducted during the SoleMon *rapido* trawl survey (28th November - 19th December 2021). The survey was carried out at 61 stations distributed over the central and northern Adriatic Sea according to a randomly depth-stratified sampling scheme (Grati et al., 2013). The *rapido* gear was provided with a DST Logic Recorder, that allows recording data of bottom temperature and salinity. The samples were collected at the stations where both species were caught. Some benthic species were put in labelled containers and stored in the freezer (-20°C) for the analyses on the stable isotopes.

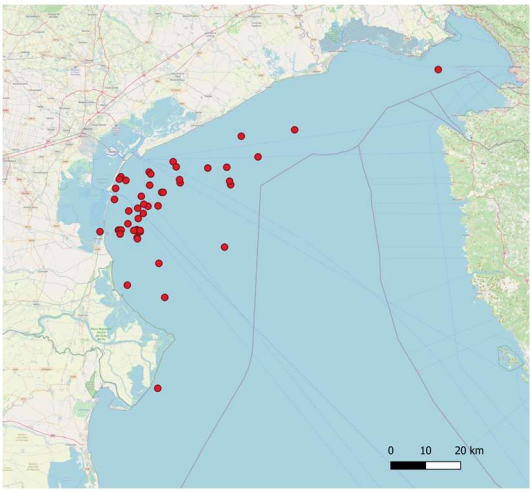
Specimens analysis

For each specimen, standard measurements and biological parameters (total length, weight, sex, and macroscopic stage of maturity) were recorded. The stomach and the intestine were extracted, weighed and put in labelled tubes filled with 70% alcohol. The liver was removed and weighed. A slice of muscle was cut and stored in labelled tubes in the freezer (-20°C) for the following analyses on the stable isotopes. The gonads were removed, weighed, and preserved in labelled tubes filled with Dietrich solution for the histological analyses. Then, sagittal otoliths were extracted, cleaned, and stored dry in labelled tubes.

Expected/Obtained Results

Overall, 645 common soles (369 females, 275 males and 1 indeterminate), and 398 Egyptian soles (215 females, 182 males and 1 indeterminate) were caught. The common sole showed a wider length distribution due to the presence of the smallest specimens (Fig. 1). The red points in the map (Fig. 2) indicate where the samples of both species were caught, they are concentrated in the Venice gulf. Moreover, during SoleMon survey, Egyptian soles were captured along the Veneto and Friuli coastal waters, between 1.5 and 3 nautical miles. The data collected confirm the previews observations about the spatial distribution of the Egyptian sole, the species inhabits shallow waters (Chabanaud, 1927), while common sole has a wider distribution from the coast to deeper waters (Quéro et al., 1986).





Figures 1 and 2. 1) On the left, length frequency distributions of samples caught. 2) On the right, map of the study area and the fishing points.

2. Objective: stomach content analysis and stable isotope analysis

Methods

Stomach contents and stable isotope analysis

Stomachs are weighed before and after dissection. Then, each prey is identified to the lowest taxonomic level possible under a stereomicroscope, with the support of classification manuals. Pictures of different prey are taken. The organisms belonging to the same taxonomic group and level are counted and weighed (Amundsen

and Sánchez-Hernández, 2019). For each open stomach, the percentage of filling and the digestive state of the prey are assessed. Similarly, the intestine contents are evaluated.
The samples of muscle are dried to constant weight at 60° C, then ground to a fine powder, and, finally, 1 mg of powder is weighed and stored in tin analysis cups (Fanelli et al., 2011). The $\delta^{15}N$ and $\delta^{13}C$ isotopes composition of tissue are analyzed through isotope ratio mass spectrometry to estimate the trophic level of the two species.

Expected/Obtained Results.

Stomach content analysis is still underway. Both species feed mainly on polychaete worms, but also crustaceans and echinoderms (Fisher et al., 1987). So far, 74 different *taxa* have been identified, among them 44 have been classified at the species or genus level. This wide range of prey has also been obtained by the intestine investigation. Species other than those in the stomach and in a good state of conservation have often been found (Fig. 3). Soleidae have a very small stomach (about 10% of the whole digestive tract length), a well-developed intestine (80%), and a high digestive rate (De Groot, 1971). So, the intestine appears to play an active role not only in nutrient assimilation but also in the digestion process. The data analysis will allow me to investigate and compare the feeding strategy and niche width and to identify variations due to intrinsic or extrinsic factors.
Stable isotopes analysis is still underway. Samples selection and preparation will be carried out in the next months. Combined measurements of $\delta^{15}N$ and $\delta^{13}C$ stable isotopes will provide information on source material and trophic relationships.

Figure 3. Prey found in the intestine tract of an Egyptian sole (9 different species have been identified).

3. Objective: histological analysis of gonads and fecundity estimation

Methods

For histological analysis, a subsample of 3-4 gonads per sex and maturity stage have been selected for each species. Gonads have been prepared according to the following protocol: dehydration (increasing concentration of ethanol); clearing with Histolemon; embedding in paraffin (Paraplast); slicing with the microtome; staining with Hematoxylin-Eosin (Mazzi, 1977) (Fig. 4).

Figure 4. Histological analysis: samples preparation and image analysis of gonadal tissues.

Fecundity has been evaluated on mature ovaries. Fixed gonads have been weighed and a subsample has been selected from one ovary (2% of the total weight of that ovary). Pictures of all eggs found in the subsample have been taken. Eggs diameter have been measured.

Expected/Obtained Results

Due to microscopy laboratory access restrictions and malfunctions, the analyses are still underway. The histological samples of common sole are ready to be analysed under microscope and classified; the samples of Egyptian sole are in progress. From each species, 25 mature ovaries have been selected for fecundity estimation, the data analysis is underway. Both species seems to be batch spawners: "the eggs are released in batches usually over a protracted spawning period (weeks or months). Only a portion of the yolked oocytes is selected to be spawned and hydrated in each batch." (Murua et al., 2003). With this information, it will be possible to identify and describe the different stages of ovary and testis development and to validate the macroscopic maturity classification that is usually used during monitoring activities. The length at first maturity, the reproductive period, the energetic investment in the reproductive event, and the strategy adopted in terms of the trade-off between egg size and number will be assessed. Moreover, the comparison between the two sibling species may give some possible explanations of their reproductive isolation (different spawning periods and/or spatial segregation).

4. Objective: analysis of age and growth

Methods

At the laboratory, both whole *sagittae* are immersed in ethanol with the distal surface up (*sulcus acusticus* downwards) and read under a stereomicroscope using reflected light against a black background (5x and 10x magnification). The right *sagittae* are selected to be transversely sectioned down to the core. The otoliths are burned at 350° C for 10 minutes in a muffle furnace. Then, burned otoliths are included in resin (Crystalbond 509 Amber), ground on abrasive paper and polished with alumina powder. The sections are immersed in fresh water and read under a stereomicroscope with reflected light against a black background (10x magnification). Burning and sectioning techniques are used to improve the quality of observations, enhancing the growth rings contrast, and compare the age estimations between the two reading approaches (Panfili et al., 2002).

Expected/Obtained Results

The two species have a similar trend of growth: fast growth during the first two years of life and the achievement of the asymptotic length starting from the third year (Fig. 5). The two age estimation approaches show comparable results. However, the analysis of sectioned otoliths reveals the actual longevity of these species in the north and the central Adriatic Sea. The presence of very old individuals is remarkable because they improve the population resilience against natural and anthropic perturbations, so they play an important role in the long-term population dynamics (Froese, 2004, and the references therein). Further investigations are in progress.

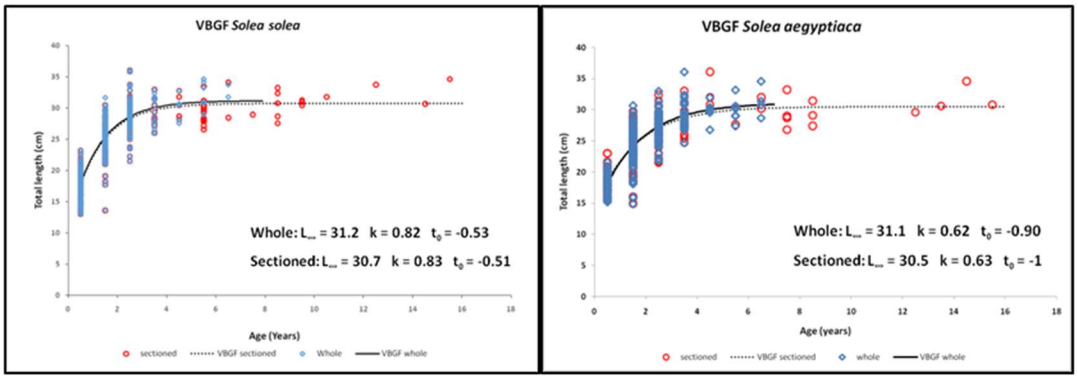


Figure 5. Comparison of von Bertalanffy growth functions fitted to the age estimations made from whole and sectioned otoliths.

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Part 2. PhD student information on the overall year activity (courses/seminars/schools, mobility periods, participation to conferences)

List of attended courses/seminars/schools

Courses:

Organized by DISVA

Getting Started with R: Environmental Computing, Prof. Giuseppe d'Errico, June 2022.

Ultrastructural analyses of taxonomic characters in Porifera and Cnidaria, Profs Barbara Calcinai, Stefania Puce and Martina Coppari, June-July 2022.

Organized by FAO-GFCM

Virtual training course on the identification and handling of seabirds incidentally caught during fishing operations –9th February 2022.

Virtual training course on the identification of macrobenthic invertebrates incidentally caught during fishing operations – 11th February 2022.

Seminars:

A "Shot of Science" seminars at DiSVA

Wastewater and marine quality: are they connected? Speaker: Federico Girolametti, 24th May 2022.

PhD week seminars

The resolution revolution in Cryo-electron-microscopy, in Structural Biology and in Life Sciences. Speaker Martino Bolognesi, Department of Biosciences, University of Milan, 7th June 2022.

Current threats to research ethics and how to cope with them. Speaker Marco Seeber, Department of Political Science and Management, University of Agder, Norway, 9th June 2022.

External activities

Participation in the SoleMon *rapido* trawl survey (CNR-IRBIM project) carried out in the northern and central Adriatic Sea from November 28th to December 19th, 2021. From 16th to 19th December 2022, I held the position of chief scientist. Organization and planning of the next SoleMon campaign (November 21st - December 18th, 2022) as chief scientist.

List of conferences/workshops attended and of contributions eventually presented

51st SIBM Congress, 14th – 17th June 2022 (online).

1st Cross-border training lab and exchange of experiences between Croatian and Italian operators in Italy. Ancona, 11th October 2022. ARGOS PROJECT (INTERREG Italy – Croatia Programme 2014/2020). Contribution presented: "Data collection for demersal species and the SoleMon project".

Supplementary didactic activities

Guided visit of the Museum of Adriatic Zoology G. Olivi and the Chioggia fishery market for students of the course "Biologia della Pesca" held by Prof. Emanuela Fanelli.

Tutoring at IRBIM-CNR of the UNIVPM student Marco Lupacchini, 1st – 22nd December 2021.

Participation in the European Researchers' Night - Sharper event with the "Fai la scelta giusta!" didactic activity, Ancona, 30th September 2022.

Participation in the KumLab event with the "Fai la scelta giusta!" didactic activity, Ancona, 12th October 2022.

Part 3. PhD student information on publications

No publications yet

14/10/2022

Student signature

Supervisor signature